REPRODUCTIVE RESPONSE OF RABBITS FED SUPPLEMENTAL*Moringaoleifera* (Lam) LEAF MEAL

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ABSTRACT

Reproductive inefficiency is a major challenge in rabbit production. Leaf meals such as *Moringaoleifera*rich in phytochemicals have been used to enhance reproductive performance in cattle and catfish. Information on the use of *Moringaoleifera*Leaf Meal (MoLM) to enhance reproductive efficiency in rabbits and its effect on organs has not been adequately documented. Therefore, the reproductive response of rabbits to supplemental MoLM was assessed.

In a 24-week trial, sixty rabbits were allotted to four diets containing supplemental MoLM : 0 (control), 2.5, 5.0 and 7.5%with 10 does and 5 bucks per treatment. Dry Matter Intake (DMI, g) and Daily Weight Gain (DWG, g) were determined. Blood (5.0 mL) was sampled from bucks and does for Haematology (Erythrocytes, $x10^{3}mm^{-3}$, Packed Cell Volume (PCV, %), haemoglobin, g/100 ml), Alanine amino Transferase (ALT, IU/L) and hormonal (testosterone, oestrogen) assay using standard procedures.Bucks were assessed for semen characteristics and then assigned to does on each treatment at 2 does/buck for mating trial using standard procedures. Blood (3mL) was sampled from pregnant does at third trimester for progesterone determination using standard procedures. Conception Rate (CR), Gestation Length (GL), Litter Size (LS) and Litter Weight (LW) were determined using standard procedures. Testes and epididymides were removed, weighed and processed for sperm reserves ($x10^{6}$sperm cells/ml). Histopathology (no visible lesion, mild, moderate, severe and very severe) of liver, kidney and ileum was assessed using standard procedures. Data were analysed using descriptive statistics, polynomial regression and ANOVA at $\alpha_{0.05}$.

The DMI was not significantly different among the treatments. The DWG of rabbits on control (6.3±0.8), 2.5% (5.2±0.4) and 5.0% (5.2±0.5) MoLM were higher than those fed 7.5% (4.4±0.4). Erythrocytes (6.8±0.2), PCV (40.2±1.6) and Hb (13.0±0.5) were higher in rabbits fed 2.5% MoLM. The ALT of rabbits on control (19.2±2.0) was significantly lower than those on MoLM diets (38.7±4.2 – 48.2±2.7). Oestrogen values ranged from 16.5±1.2 to 25.8±2.0 ng/L and was higher in rabbits fed MoLMthan those on control (10.0±0.8ng/L). Progesterone levels were higher in rabbits on MoLM diets ranging from 1.8±0.4 to 2.8±0.4 ng/L than those on the control (0.63±0.1ng/L). Serum testosterone level was not significantly different among the treatments. Sperm motility (90.3±0.2%), mass activity (3.0±0.01), live sperm cells (88.5±3.4%) and CR (100.0%) of rabbits fed 2.5% MoLM were higher than those rabbits on other treatments. The GL, LS, and LW were not significantly different among the treatments. Testicular sperm reserves ranged from 14.0±1.5 to 53.0±11.1,while epididymal sperm reserves were not significantly different among the treatments. Histopathology of liver, kidney and ileum indicated moderate necrotic lesions at 5.0 and 7.5 % MoLM. Regression of sperm motility on MoLM levels in bucks indicated an optimum inclusion level of 2.7% (R^2=0.56) while regression of conception rate on MoLM levels in does showed an optimum inclusion level of 2.5% (R^2=0.61).

Moringaoleifera leaf meal at 2.5% inclusion in the diet of rabbits enhanced growth, semen quality and fertility rate without adverse effect on blood profile.

Keywords: Rabbit semen quality, Hormonal assay, *Moringaoleifera* leaf meal, Haematology

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DEDICATION

This work is dedicated to THE ALMIGHTY GOD.

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CERTIFICATION

This is to certify that this work was carried out by MrsAdenikeAbiodun ADEYEMIwith Matriculation Number 118461 in Animal Physiology and Bioclimatology Unit of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

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TABLE OF CONTENTS **PAGES**

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

Rabbits can be considered as one of the several species of farm animal suitable for meat production. Rabbits are prolific i.e with high number of young per kindling, providing meat with high nutritive value which can be an excellent economic source of animal protein for human consumption thus making rabbit production attractive. Some outstanding attributes that promote rabbit production include good growth rate and ability to utilize forages to turn out high quality meat. They have a short gestation length, early sexual maturity and ability to rebreed several times within a year generation interval (Sharp *et al*., 2007).

Despite these numerous advantages, rabbit production has not achieved its potential as cheap animal protein source in the tropics (Herbert and Adejumo, 1995). The survivability of the weaners and reproductive inefficiency act as major source of setback to efficient rabbit production in the tropics (Gbadamosi and Egbunike, 1999). The efficiency of sperm production, libido and quality of sperm tend to remain uniform throughout the reproductive life of an animal but may be significantly altered by age, nutrition, environment, health status, drugs, and chemicals (Togun and Egbunike, 2006).

Apart from exogenous hormone injection (Abdel-Azeem, 2010), nutritional treatment has also been used for improving reproductive efficiency of livestock including the use of selenium supplementation in rabbits (El-Masry *et al*., 1994, Abdel-Azeem, 2010), retinol supplementation in cows (Chew, 1993) and vitamin E (El-Masry *et al*., 1994) and various plants leaf meal (Ogbuewu *et al*., 2012) in rabbits. Recently, there has been interest in the

utilization of *Moringa oleifera* commonly called drumstick tree, as a protein source for livestock (Makkar and Becker, 1997). Moringa leaves have quality attributes that make it a potential ingredient in partial replacement for soyabean meal or fish meal in non-ruminant diets. The *Moringa oleifera* plant has been reported to have high nutritional, medicinal and therapeutic qualities (Fahey, 2005). *Moringa oleifera* can easily be established in the field, has good coppicing ability, as well as good potential for forage production. Different parts of the moringa plant contain a profile of important minerals, and are good source of protein, vitamins (beta-carotene, tocopherol) and various phenolics (Anwar *et al*., 2007), most of which can enhance reproductive performance.

A number of research findings on *Moringa oleifera* had been documented which includes its chemical composition and nutritional qualities (Yamego *et al*., 2011; Ewuola *et al*., 2012). It has been reported to be relatively rich in nutrients, energy and vitamins (Makkar and Becker, 1996, Ayssiwede *et al.*, 2011). It contains some phytochemicals such as β carotene, α & β tocopherol, six phenolic acids detected as analogs of chlorogenic acid (Coppins, 2008) and its cholesterol reducing activity in wistar rats (Ghasi *et al*., 1999), which is influenced by β-sitosterol (Rajanandh and Kavitha, 2010). The presence of phytochemicals indicates possible preventive and curative properties of *M. oleifera* leaves as reported by Kasolo *et al*. (2010).

Moringa leaf extract has been reported to lower blood glucose in rat (Ndong *et al.*, 2007) and enhanced spermatogenesis in rats (Cajuday and Pocsidio, 2010). It is low in anti-nutritional factors (Kasolo *et al*., 2010, Ogbe and Affiku, 2012) and it has been used in broilers (Onu and Aniebo, 2011), laying hens (Abou-Elezz *et al*., 2011), monogastrics (Adeniji and Lawal, 2012; Ewuola *et al*., 2012) and ruminant production (Mendieta-Araica

et al., 2010). It has been used to replace groundnut cake (Adeniji and Lawal, 2012), soyabean (Ewuola *et al*., 2012) and forages (Kakengi *et al.*, 2003) in livestock production without adverse effect on growth indices.

Despite the good growth attributes rabbits possess, there is a need to boost their nutrition for optimum performance. Plants such as *Moringa oleifera* contain several essential nutrients that can enhance growth and reproduction. *Moringa oleifera* leaf contains substances that influence steriodogenesis (Chew, 1993) which could have some effects on reproductive hormones such oestrogen, testosterone and progesterone. Histopathological evaluation of some of the visceral organs may help to evaluate the effects of *Moringa oleifera* consumption on organ integrity. This study aimed at assessing the reproductive response of growing rabbits to varied dietary inclusion levels of *Moringa oleifera* leaf meal.

1.1 OBJECTIVES

The objectives of this study are to:

1. Evaluate the effects of *Moringa oleifera* leaf meal on feed intake, weight gain, feed conversion efficiency, some haematological and biochemical parameters.
2. Assess hormonal response of rabbits to varied levels *of Moringa oleifera* leaf meal at attainment of maturity and at gestation.
3. Evaluate the effects of *Moringa oleifera* leaf meal on fertility rate and reproductive performance of rabbits; and
4. Investigate the effects of *Moringa oleifera* leaf meal supplementation on some visceral organs of rabbits.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 RABBIT PRODUCTION AND MANAGEMENT

The domestic rabbit (*Oryctolagus cuniculus)* originated from the wild rabbits of Southern Europe and North Africa where they were seen as pest damaging their crops. In their natural environment, rabbits are outgoing, completely herbivorous and most active in the twilight or in the dark (Lebas *et al*., 1997). Rabbits are small livestock that are easy to manage, clean and relatively odourless. They can be managed on backyard farm or on large scale production. They produce white palatable meat, high in protein (about 21%), low in fat, low in cholesterol, and well flavoured like chicken. High quality rabbit skins are used in fur garments and significantly used in the research fields. Rabbits are also raised for show or as pets. Some other advantages of keeping rabbits over other livestock species include limited cost of the housing structures, efficient reproductive ability in terms of offspring (kg/year/doe) and their all year round breeding ability if well-managed and early age of sexual maturity (4-5 months). Rabbits require little space than large livestock especially in areas where there is shortage of agricultural land. Rabbits are easy to transport and market (Lebas *et al*., 1997). Its small size means it has a correspondingly high metabolic rate (which limits its ability to exist on a low energy concentration diet), and makes it a highly sought prey (which needs to be agile and athletic to outrun predators). This small size also makes it easy to manage them within a small space and handled by both young and old).

2.1.1 Breeds of rabbits

Rabbits are generally classified according to size, weight and type of pelt. Small rabbits weigh about 1.4-1.8 kg at maturity, medium breeds 4.1-5.4 kg, and large breeds 6.4-7.3 kg.

The two most popular breeds for meat production are the New Zealand and the Californian. These breeds are most popular because they combine white fur and good growth characteristics. The New Zealand rabbit has a completely white, red or black body, whereas the Californian is white with colored nose, ears and feet. The two most popular rabbits for fur production are the Rex and the American Chinchilla. The Rex is slightly smaller (3.2 kg) than the American Chinchilla (4.5 kg). The most available breeds in Nigeria are crosses of New Zealand white and Chinchilla (Abu *et al*., 2008)

New Zealand white: The most popular breed of rabbit for meat production is the New Zealand White. In fact, it is estimated that 90% of rabbits raised for meat worldwide are of this breed. This breed was originally developed in 1916 by W.S. Preshaw for the purpose of optimal meat production and fur trade. They have a well-rounded body and small bone structure, resulting in a larger portion of their weight being meat. They have pink eyes and a great disposition. The adult female is usually between 4 to 5.4kg and the male between 3.6 to 4.5kg. They reach maturity when they are 6 months of age (Macari and Machado, 1978). They produce large litters of 8 to 12 kits, which has fast growth rate to fryer size in 8 to 12 weeks. The New Zealand White is widely used as foundation stock because of its great qualities. However, most all hybrids are unable to continue their strengths from generation to generation, often losing it after just one generation. But with a strong New Zealand White stock, you can continue to breed strong, healthy, productive rabbits for generations.

Chinchilla: The Chinchilla rabbit is one of the few rabbit breeds that was created in America descended from the English breed known as the Chinchilla Giaganta, which incidentally corresponds to the so called "Heavy-Weight" or American Chinchilla. The Giant Chinchilla has been purebred for over 45years. The ideal Giant Chinchilla should

weigh, when fully mature, 5.9 to 6.4 kg for bucks. Does at maturity should weigh 6.4 to 6.8 kg. It is a proven fact that rabbits that weigh from 5.4 to 6.4 kg at maturity have generally been accepted as the ideal meat producing rabbit.

2.1.2 Management of rabbits

Raising rabbits requires consistent good management practices, failure in any phase will affect other areas of management. Rabbit house must protect them from extreme heat, rain, sun, wind and must be well naturally ventilated to allow good air circulation. The hutches should be raised from the floor and properly fitted to prevent entry of predators. Wire cages are recommended over wooden ones because they are durable and are easier to clean and disinfect. Adequate space of at least a 60 to 70 cm × 80 to 100 cm floor space and are 50 to 60 cm high must be provided for a doe and its litter especially if the kits are left with the doe until 8 weeks of age (Lebas *et al*., 1997). The housing must provide enough space for drinkers and feeders. Drinkers should be regularly cleaned and periodically checked for leaks.

The domestic rabbits are primarily herbivorous and consume most types of grains, forages and hay. Their diets, either grown or commercially prepared consist of ingredients from plant sources. Since rabbits can utilize a certain amount of forage, they have a place in food production by making use of some non-competitive feeds. Taiwo *et al*. (2004) reported higher weight gain and better feed conversion efficiency in rabbits fed a mixed feeding regime of *Tridax procubens* with concentrate than those on sole concentrate diet. Rabbits fed *Verano stylo* or Guinea grass only had lower dry matter intake and consistent losses in weight, thus concentrate supplementation of forage diets is necessary for rabbits (Bamikole and Ezenwa, 1999). Forage can contribute up to 50% of rabbit diets, although there is

improvement in performance of rabbit fed concentrate and forage compared to feeding forage or pellets alone. The concentrate, grass and legume combination showed promising ability to reduce the cost of production and improve the performance of grower rabbits (Iyeghe-Erakpotobor *et al*., 2006). Sanni *et al*. (2005) observed that feeding rabbits a combinations of concentrate and Stylosanthes at 50:75 (g : g; weight supplied per day) gave the highest return on investment, thus the cost of feeding grower rabbits could be lowered by supplementing concentrate diet with Stylosanthes. Forage free diet is not ideal for optimal performance of rabbits. It induces diarrhea, reduces feed conversion ratio, average weight gain and dry matter intake. The level of crude fibre in the diet affects the digestibility of nutrients by rabbits (Okonkwo *et al*., 2010).

Forages such as Sunflower, *Tridax procubens, Aspilla africana, Panicum maximum, Gliricidia sepium, Moringa spp* and many other plant materials are relished by rabbits. Performance of weaner rabbits could be improved by supplementing concentrate diet with up to 50% *Syndrella nodifloran* forage without any alteration to their blood profiles (Omoikhoje *et al*., 2006). Rabbits are nocturnal, and eat more at night. Rabbits like fresh food and plenty of water. Rabbits should be fed twice a day. A nursing doe will need more feed than a pregnant doe or young buck. The easiest way to know when rabbits are adequately fed is that there will be a small amount of leftover feed in the morning. Rabbit feed should contain 15 to 18 % protein and at least 10% crude fibre.

The rabbit's gastrointestinal physiology is a complex system that centers round the separation of digestible and indigestible components of the diet in the proximal colon. The clinical importance of this system is the need for a consistent diet high in long particle length (>0.5 mm) indigestible fiber to maintain the motility of the caecum and colon (Davies

et al., 2003). Most of the common gastro-intestinal problems seen in captive/domestic rabbits are related to inappropriate diets (low fiber, high protein, high carbohydrate) and in frequent feeding of treats to which the rabbit is not accustomed. In modern production systems, the animals are given balanced pelleted feed and fed as required except bucks which are fed *ad libitum*. Under less intensive regimes, does receive the same feed ration from the weaning of one litter to the birth of the next. The ration is normally 3 to 35 g DM per kg of live weight per day (Lebas *et al*., 1997). Growing rabbits raised in a group are always fed *ad lib*. Total daily feed consumption for different age groups of rabbits are: 110 to 130 g for young fattening rabbits (4 to 11 weeks old); 350 to 380 g for lactating does with litters (weaning at four weeks); 120g for adult rabbits (maintenance). Rabbits are sensitive to mould therefore the hygiene of the fodder and by-products to be fed must be ascertained (Lebas *et al*., 1997).

Selective breeding is the standard for obtaining a good rabbit stock. It is necessary that one should always breed from good stock and continually improve your stock. Selecting the rabbit breed that suits your purpose of production is recommended. Breed for good size, excellent mothering traits, large litters and overall great stock. In establishing a rabbit enterprise, it is advisable to purchase animals with records. Some factors to consider when purchasing foundation stock include breed, breeding efficiency, milk production, growth efficiency, longevity and disease resistance. Bucks can be mated at 5 - 6 months and does at 6 – 7 months. Bucks and does must be housed separately. For mating, the doe should always be taken to the buck's cage and not vice versa. If they failed to mate within a few minutes, the doe should be given to a different buck or returned later. Normally a buck should be used once daily because it has been observed that smaller litters result from too

frequent use of a buck. The buck should not be used more than three times a week to give room for adequate sperm cell formation and quality semen output (Lebas *et al*., 1997).

The doe will usually eat less 2 or 3 days before kindling. The normal gestation period of a rabbit is between 30 and 32 days. The nest box should be placed in the hutch on the 28th or 29th day of pregnancy to avoid contamination by the doe. Nest boxes should give the doe seclusion, provide adequate ventilation and protect litter from cold. A nest box with one side cut down should be insulated and filled with straw. Kindling usually takes place during the night (Rashwan *et al*., 2003) and it is necessary that the doe is not disturbed while kindling to prevent her from destroying the litter. The mortality of suckling rabbits is in connection with the nest quality (Szendro, 2008). Just before or as soon as kindling has been completed, the doe pulls more fur from her body to prepare a nest. The kindling nest should be checked regularly to remove dead kits. The average litter size is about 6 in the tropics, but it can range from 4 to 12 as an average doe is equipped to nurse up to 8 kits. It is better to breed several does simultaneously, to foster kits from the large litter to the small ones 2 or 3 days after kindling to even out the milk supply.

Reproductive efficiency of rabbits is important in determining the profitability of commercial rabbitries. The level of production in rabbit directly depends on the rate of reproduction. Generally, the rate of reproduction in livestock is important but age at first mating is more important because, if the unproductive period before the first litter can be shortened, rabbit productivity will naturally be increased (Lebas *et al*., 1997). Rabbits can produce 4 to 6 litters in a year (Lebas *et al*., 1997).

Mmereole (2009) investigated the effect of rebreeding intervals on the performance characteristics of doe, testing three rebreeding intervals – 7 day, 14 day and 21 day intervals

with a view to identifying the optimum breeding interval with respect to the following economic traits – body weight changes during pregnancy, litter weight, litter size, percentage stillbirth and pregnancy rates. Result obtained indicated that the weekly mean weights of doe during pregnancy were significantly higher in TI (7 d rebreeding intervals) than in all other treatment groups, the mean litter weights, mean litter size, percentage stillbirths and pregnancy rates were significantly higher in does placed on treatment 1 (7 d intervals). The author concluded that 7 days can be adopted if efforts are made to reduce the high percentage stillbirths associated with this rebreeding interval.

Weaning is a period when the young rabbit gradually give up milk for solid feed and the young is separated from the doe. This is usually done when the young one is 4 – 6 weeks old and sexing should be done at this time. Weaning is advisable when the young rabbit's live weight is 500g and above as this has been observed to increase post-weaning survival rate (Lebas *et al*., 1997; Szendro, 2008). The young rabbits begin to eat solid feed at 18 to 20 days and at 30 days the doe's milk provides not more than 20 % of the daily dry-matter intake. In practice, young rabbits benefit from the late weaning until the age of six weeks.

Yu and Chiou (1997) in a study aimed at understanding of digestive function from the development of the digestive tract from suckling to maturity in rabbits. The relative weights of the digestive tract (in relation to body weight) in different segments increase linearly during the rapid growth period between 2 and 8 weeks of age; thereafter intestinal weight gain is slower. An underdeveloped mucosal histology was observed in the hindgut of suckling rabbits at 2 weeks compared with 4 weeks of age but after weaning as hindgut fermentation becomes significant, the mucosa increased in surface area.

Rabbits are susceptible to several diseases that reduce production to unprofitable levels. The common diseases of rabbits are scours also known as bloat, coccidiosis, ear mange, sore eyes, sore hocks and vent disease. The respiratory disease caused by *Pasturella multocida* is responsible for decreased productivity and a high mortality rate in rabbits. This can be managed by removing environmental irritants, humidifying the environment to mobilize nasal discharge and ensuring that the rabbits eat well to prevent other infections such as gastrointestinal diseases (Barbara, 2011). In Nigeria, it is estimated that the overall mortality between birth and marketing was 30 - 40 % with the highest record in the young ones especially weaners (Abu *et al*., 2008).

2.2 HAEMATOLOGY

Haematology is the study of blood and its components. Blood is the fluid present in the body that consists of liquid containing numerous cells and protein suspended in it (William, 2003). It transports substances to and from tissue cells, nutrients such as proteins, glucose, vitamins, minerals, lipids into cells, transports oxygen by red blood corpuscles (oxyhaemoglobin), removes wastes products such as urea and CO_2 from cells. Blood is important in regulating temperature by altering the blood flow through the skin and provides immunity against infections. It is involved in the distribution of hormones to all parts of the body and contains substances that enhance clotting following a wound (William, 2003). It is composed of plasma which is the pale yellow sticky liquid portion of the blood which makes up 55% of the total blood volume with the red and white cells forming the remain portion. Plasma components are water (92%), dissolved protein (8%), glucose, amino acids, vitamins, minerals (mainly NaCl), urea, CO_2, hormones and antibodies (Weiss and Wardrop, 2010). Haematological values may be influenced by sex, age and nutrition among others.

Dietary components have been reported to have measurable effects on blood constituents (Animashahun *et al*., 2006).

Burnett *et al*. (2006) examined the effects of gender, maturity (juveniles vs. adults) and breed (New Zealand White vs. Mixed) on rabbit haematology. Comparison was made between values for haemoglobin, packed cell volume, mean corpuscular haemoglobin concentration and white blood cell count using manual and automated methods. Significant differences were observed between the automated and manual values for haemoglobin (Hb), packed cell volume (PCV), and mean corpuscular haemoglobin concentration (MCHC), with values for automated methods being higher for Hb and MCHC and lower for PCV (Burnett *et al*. 2006). The authors observed that husbandry practices had an effect on neutrophil, eosinophil, basophil, lymphocyte, and platelet values while maturity influenced neutrophil and lymphocyte counts. The automated white blood cell (WBCa) count was affected by breed. Husbandry practices affected automated PCV and MCHC values and red blood cell counts (RBC). Maturity influenced automated Hb, PCV and RBC values. Mixed breeds had higher automated Hb, PCV and RBC values than New Zealand White rabbits. Male rabbits had higher values than females for manual and automated Hb, PCV, and RBC in the Caribbean.

Blood collection procedures related to duration preceding fasting, time of sampling, time of samples to stand, haemolysis, use of plasma instead of serum, storage until time of measurement, method of analysis, are factors that can affect blood parameters measured (Wolford *et al*., 1986). Etim *et al.* (2014) assessed the effects of nutrition on haematology of rabbits and stated that the physiology of farm animals is influenced by several factors, one of which is nutrition. The recommended maximum volume of blood that can be collected

safely during one bleeding is 7.7 ml/kg of body weight (Mitruka and Rawnsley, 1981). Haematological values for normal male New Zealand White rabbits are as shown in Table 1.

2.2.1 Packed Cell Volume (PCV)

Packed Cell Volume also known as Haematocrit is the proportion of blood volume that is occupied by erythrocytes (the ratio of red blood cells to the whole blood volume) expressed as a percentage. When blood is collected in an anticoagulant and centrifuged for 15 min at 3000 revolutions/min, erythrocytes are submitted in the lower part of the vessel, white blood cells form a thin film on the surface of red blood cells and yellow plasma occupy the top. The PCV is an easily obtained measure for detecting anaemia or polycythemia and can be useful in estimating changes in haemodilution or haemoconcentration (Brian *et al*., 2000). The PCV is used together with the red blood cell count in calculating the mean cell volume (MCV) and together with the haemoglobin content in calculating the mean corpuscular haemoglobin concentration (MCHC) (Jain 1986).

2.2.2 Haemoglobin

Haemoglobin is an iron-porphyrin-protein complex. It has a central role in physiology by binding, transporting, and delivering oxygen to tissues. Haemoglobin is synthesized within developing RBCs and its synthesis is coordinated with the developmental stages of the erythroid precursors. Haemoglobin is a respiratory pigment found in red blood corpuscles. Haemoglobin is a conjugated protein, synthesized inside immature erythrocyte in the red bone marrow. It consists of two components Haeme and Globin. Haem, an Iron and

Table 1: Haematological values of normal male New Zealand White rabbits

PARAMETERS	UNIT	MEAN	S.D	RANGE
Haematocrit (PCV)	ml%	41.5	4.25	30.0-50.0
Erthrocytes	x $10^{6}/mm^{3}$	6.70	0.62	5.46-7.94
Haemoglobin	g/dl	13.9	1.75	10.4-17.4
Mean Cell Volume	µm3	62.5	2.00	58.5-66.5
MCH	µµg	20.7	1.00	18.7-22.7
MCHC	%	33.5	1.85	30.0-37.0
Sedimentation Rate	mm/hr	2.00	0.50	1.00-3.00
Platelet	x $10^{3}/mm^{3}$	480	88.0	304-656
Leukocyte	x $10^{3}/mm^{3}$	9.00	1.75	5.50-12.5
Neutrophil	x $10^{3}/mm^{3}$	4.14	0.82	2.53-5.75
	%	46.0	4.0	38.0-54.0
Eosinophil	x $10^{3}/mm^{3}$	0.18	0.04	0.11-0.25
	%	2.00	0.75	0.50-3.50
Basophil	x $10^{3}/mm^{3}$	0.45	0.08	0.28-0.62
	%	5.00	1.25	2.50-7.50
Lymphoctyes	x $10^{3}/mm^{3}$	3.51	0.68	2.14-4.88
	%	39	5.50	28.0-50.0
Monocytes	x $10^{3}/mm^{3}$	0.72	0.14	0.44-1.00
	%	8.00	2.00	4.00-12.0

Source : Mitruka and Rawnsley (1977)

MCH-Mean Cell Haemoglobin , MCHC-Mean Cell Haemoglobin Concentration

porphyrin compound make up 4% and Globin (an amino acid) forms 96%. Haemoglobin gives red colour to the blood (Wajcman and Moradkhani, 2010).

2.2.3 Erythrocytes

These are the red blood cells (RBCs). It carries oxygen to tissues. All energy in RBCs that is devoted to maintaining cell shape, membrane structure, enzymatic functions, reduced iron in haemoglobin and other functions does so to optimize oxygen delivery to tissues. Erythrocytes have no nuclei and no organelles, and thereby no ability to synthesize proteins. They are cells full of haemoglobin and made in the bone marrow. They live for about 120 days. The life span of rabbit erythrocytes was estimated to range between 45 to 70 days (Jain, 1986). After which they are destroyed and recycled by the liver. Comparative studies reveal that RBC lifespan correlates positively with species longevity (years) and with body mass. That is, larger mammals tend to live longer and have longer RBC lifespan when compared to smaller species (Christian, 2010). The full complement of functional proteins must be present by the time the reticulocyte matures.

RBCs are composed of about 61% water, 32% protein (mostly haemoglobin), 7% carbohydrates, and 0.4% lipids. Isolated RBC membranes in most animals are composed of approximately 20% water, 40% protein, 35% lipid and 6% carbohydrate. Several factors have been reported to influence erythrocyte and haemoglobin concentrations in the blood which include nutrition, health status, among others. Achuba and Awhin (2008) reported that the red blood cell counts and haemoglobin concentration decreased significantly in the blood of petroleum-fed rabbits.

2.2.4 Leukocytes

These are also known as White Blood Cells (WBC). They are colourless cells and possess a nucleus. Their function is to defend the body against pathogens. It is made in bone marrow and lymphatic tissue. After centrifuging whole blood, a yellowish-white layer of leukocytes and platelets forms on top of the column of red blood cells, this is known as buffy coat. The white blood cell differential count determines the number of each type of white blood cell present in the blood. It can be expressed as a percentage (relative numbers of each type of WBC to the total WBC) or as an absolute value (percentage x total WBC). The higher the value of leucocyte count, the better the ability of the animal to fight diseases (Robert *et al*., 2003). Of these, the absolute value is much more important than the relative value. There are five basic white blood cell types: Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes (Teske, 2010). Each WBC cell type has its own unique features.

Neutrophils: These are the most common of the WBCs and serve as the primary defense against infection. Neutrophils circulate in the blood for only a few hours. In healthy animals, neutrophils randomly leave the circulation migrating into the gut, lungs, and skin. This process is essential in preventing bacterial infection. When neutrophil number in the blood reaches a critically low level, animals are highly susceptible to bacterial infection (Weiss *et al*., 2010). The typical response to infection or serious injury is an increased production of neutrophil. Early in the response to infection, immature forms of neutrophils will be seen. These are called Stabcells. The presence of these immature cells can be the earliest sign of a WBC response, even before the WBC becomes elevated.

Eosinophils: These cells play a role in allergic disorders and in combating parasitic infections. Eosinophils differentiate and mature in bone marrow over 2 – 6 days depending

on the species and they are tissue-dwelling cells that reside predominantly in the skin, respiratory tract and digestive tract (Young and Meadow, 2010). Elevation in eosinophil counts is associated with allergic reactions, parasite infections, chronic skin infections, and some cancers. Decreases in eosinophil counts are associated with stress, steroid exposure, or anything that may suppress WBC production generally.

Basophils: These cells can digest bacteria and other foreign bodies (phagocytosis) and also have some roles in allergic reactions. Its process of maturation takes about 2 - 5 days which is completed in the bone marrow. Basophils accumulate at sites of inflammation (Pohlman, 2010). Its elevation is associated with some cancers, some allergic reactions, some infections, radiation exposure. Reduced count is associated with stress reactions, some allergic reactions, hyperthyroidism and prolonged steroid exposure.

Monocytes: These cells respond to inflammation, infection and foreign bodies by ingesting and digesting the foreign material. A major function of monocytes is to restrict replication of intracellular microorganisms (Stafford *et al*., 2002). Recovery from an acute infection, viral illness and parasitic infection, collagen disease, some cancers increase monocyte counts while its decrease is associated with infection, rheumatoid arthritis, steroid exposure and some cancers.

Lymphocytes: These cells play both an immediate and delayed role in response to infection or inflammation. Increased numbers of lymphocytes are seen in most viral infections, some bacterial infections, some cancers while decreased numbers of lymphocytes are seen in steroid exposure, some cancers, immunodeficiency and renal failure. An increase in WBC count and differentials suggest anaemia, infection and leukemia (Holland, 2013). Both the WBC count and WBC differential can provide clues as to why you have elevated or low

white blood cell numbers. Both tests can also be used when a condition is getting worse or not improving. WBC counts that return to normal indicates an improving condition. WBC counts that do not change or worsen indicate a condition that is not being treated properly.

2.2.5 Thrombocyte

This is also known as Platelets. The principal function of platelets is to prevent bleeding. It is involved in the repair of damaged tissue and releasing the hormone platelet growth factor. It has a short life (less than 7 days). It is also made in bone marrow. Platelets are the smallest of the three major types of blood cells. The size is about 20% of the diameter of red blood cells, the most numerous cell of the blood. The platelet count in normal New Zealand rabbit is estimated at 304 - 656 x $10^3/mm^3$ (Mitruka and Rawnsley, 1977) but since platelets are so small, they make up just a tiny fraction of the blood volume. They contain proteins on their surface that allow them to stick to breaks in the blood vessel wall and also to stick to each other. They contain granules that can secrete other proteins required for creating a firm plug to seal blood vessel breaks. Also, platelets contain proteins similar to muscle proteins that allow them to change shape when they become sticky. They are shaped like a plate, like the name implies. When platelets are stimulated by a break in the blood vessel wall they change shape. They become round and extended long filaments. They may even look like an octopus, with long tentacles reaching out to make contact with the broken blood vessel wall or with other platelets. With these long filaments, platelets then form a plug to seal the broken blood vessel (Boudreaux and Catalfamo, 2010).

However, when there is an injury or cut, and the endothelial layer is broken, the tough fibers that surround a blood vessel are exposed to the liquid flowing blood. It is the platelets that react first to injury. The tough fibers surrounding the vessel wall like an envelope, attract

platelets like a magnet, stimulate the shape change and platelets then clump onto these fibers, providing the initial seal to prevent bleeding, the leak of red blood cells and plasma through the vessel injury. Thrombocytopenia is the medical term for a low blood platelet count. Platelets stop blood loss by clumping and forming plugs in blood vessel holes. Thrombocytopenia often occurs as a result of disorders such as leukemia or an immune system problem, or as a medication side effect. Thrombocytopenia may be mild and cause few signs or symptoms. In rare cases, the number of platelets may be so low that dangerous internal bleeding can occur. Thrombocytopenia usually improves when the underlying cause is treated (Prasad, 2008).

2.3 SERUM BIOCHEMISTRY

Serum biochemistry is used to monitor progression of disease before final evaluation such as pathology of arteries and organs (Marinou *et al*., 2010). The intracellular enzymes have a wide distribution in the body, but are present in variable concentrations in individual tissues (Fishman, 1960). Following tissue injury, serum enzyme levels do not necessarily change in proportion to their activities in the damaged tissue. The increased permeability of the damaged cell membrane may affect the release of one enzyme more than another, and other factors such as differently altered rates of excretion may affect the serum levels (Leppelmann, 1958). Changes in physiological, biochemical and haematological values can also be used as indicators of welfare in rabbit breeding (Hoy and Verga, 2006).

Enzymes are proteins which have catalytic properties and activate substrates. They are the catalysts of all biological and metabolic reactions in the body. Enzymes are globular proteins. Their folded conformation creates an area known as the active site. The nature and arrangement of amino acids in the active site of the enzyme make it specific for only one

type of substrate (Srivastava and Chosdol, 2007). The measurement of the serum levels of numerous enzymes has been shown to be of diagnostic significance. This is because the presence of these enzymes at a high level in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood. Standard values for some serum parameters of rabbits as complied by Mitruka and Rawnsley (1981) as presented on Table 2. Transferases are present in most of the tissues of the body. The activities of both Asparte amino Transferase (AST) and Alanine amino Transferase (ALT) are high in tissues especially liver, heart, and muscles. Any damage or injury to the cells of these tissues may cause release of these enzymes along with other intracellular proteins/enzymes into the circulation leading to increase activities of these enzymes in the blood (Srivastava and Chosdol, 2007).

Alanine aminotransferase (ALT) is found in particularly large amounts in the liver and plays an important role in metabolism, the process that converts food into energy. Normally, ALT is found inside liver cells. If the liver is inflamed or injured, ALT is released into the bloodstream. Measuring blood levels of ALT gives information about how well the liver is functioning and whether a disease, drug, or other problem is affecting it. Assay for liver enzymes will ascertain the potential for liver cell damage. An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. Low levels of AST are normally found in the blood. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in the blood is directly related to the extent of the tissue damage. After severe damage, AST levels rise in 6 to 10 hours and remain high for about 4 days. The AST test may be done at

Table 2: Standard Values For Some Serum parameters of rabbits

PARAMETERS	UNIT	RANGE	MEAN
Glucose	mg/dl	78 – 155	132
Blood urea nitrogen	mg/dl	9 – 32	18.2
Cholesterol	mg/dl	20 -83	26.0
Total protein	g/dl	5 – 8	6.8
Albumin	g/dl	2.8 – 4.0	3.3
Globulin	g/dl	2.2 – 4.0	3.4
Alanine Amino Transferase	i.u/l	49 -79	65
Aspatate Amino Transferase	i.u/l	42 – 98	71

Source: Mitruka and Rawnsley (1981).

the same time as a test for ALT. The ratio of AST to ALT sometimes can help determine whether the liver or another organ has been damaged. Both ALT and AST levels can test for liver damage.

Findings by Burnett *et al.* (2006) from a study carried out in the Caribbean showed that husbandry practices had effect on potassium (K), phosphorus (P), creatinine (C), bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase and calcium. Phosphorus, AST, cholesterol and glucose were higher for juveniles; while chloride was higher for adult rabbits. Only C, P, and K were affected by breed, with the mixed breed having higher values than New Zealand White rabbits. Males had higher values for potassium, total protein, and albumin, while females had higher values for cholesterol.

2. 4 REPRODUCTIVE PHYSIOLOGY OF RABBITS

2.4.1 Reproductive physiology of male rabbits (bucks)

The male gonads begin to differentiate on the 16th day after fertilization. After birth the testes develop less quickly than the rest of the body. From the age of five weeks they begin to grow very rapidly. Accessory glands undergo a similar development, but at a more even rate and are less precocious (Lebas *et al*., 1997). The structure of a male rabbits' reproductive organ is presented in Figure 1. Puberty generally is considered as the time when spematogenesis starts in males (Bearden and Fuguay, 1980).

Spermatogenesis begins between days 40 and 50. The testicular tubes become active at about 84 days. The first spermatozoa are present in the ejaculate at about 110 days. Bucks reach maturity when their testes become androgenically active (Skinner, 1967). Sexual maturity which is the moment when daily sperm production ceases to increase is reached at

32 weeks by New Zealand White rabbits in temperate climates (Lebas *et al*., 1997) and at about 35 weeks in the tropics (Ewuola and Egbunike, 2010). However, a young buck in these same conditions can be used for reproduction from the age of 20 weeks. The physical and chemical characteristics of the semen are fundamental factors in the evaluation of puberty and sexual maturity (Macari and Machado 1978). Indeed the first manifestations of sexual behaviour appear at days 60 to 70 when the rabbit makes it first attempt at riding. Coitus may occur for the first time at about 100 days, but the viability of the sperm cells is very weak or nil in the first ejaculate. So first mating should be timed for age 135 to 140 days (Lebas *et al*., 1997). The onset of puberty varies from breed to breed, but conditions in the rabbitry also play an essential role, particularly feeding, which is even more important than climate. Ewuola and Egbunike (2010) reported delayed puberty, poor semen quality, impaired spermatogenesis, induced embryo mortality in male rabbits fed diets containing a mycotoxin - fumonisin.

Fielding (1991) gave the average volume of rabbit semen as 0.6 ml. Sperm concentration is evaluated at 150 to 500 ($\times 10^6$) spermatozoa per ml but both volume and concentration are liable to vary. False mountings, one or two minutes before copulation, increase the concentration of the ejaculate. In two successive services, the first acts as a preparation for the second, which is less voluminous but more concentrated. During subsequent matings the volume of the ejaculate decreases, while concentration increases between the first and the second ejaculate and then diminishes (Lebas *et al*., 1997). The total number of spermatozoa per ejaculate follows the same trend. Maximum spermatozoa production is obtained by using the buck regularly once in two - three days. If the buck is used regularly twice a day, each ejaculate has only half of the concentration of spermatozoa. On the other hand, if bucks service several times a day, the three or four ejaculates may be concentrated enough to effect

fertilization. Further ejaculates contain very few spermatozoa and cannot effect fertilization. Daily spermatozoa production is roughly 150 to 300 million, independent of the rate of ejaculation (Lebas *et al*., 1997).

2.4.2 Reproductive physiology of female rabbits (does)

Sexual differentiation takes place on the 16th day after fertilization. Ovogonial division begins on the 21st day of foetal life and continues until birth. The first follicles appear on the 13th day after birth and the first antrum follicles at about 65 to 70 days (Lebas *et al*., 1997). Puberty generally is considered as the time when spematogenesis starts in males and is defined as the age at first oestrus with ovulation in female (Bearden and Fuguay, 1980). Does are able to mate first at 10 to 12 weeks, but as a rule this will not produce ovulation. The onset of puberty varies greatly with the breed and body development. Does generally reach puberty when they have grown to 70 to 75 percent of their mature weight. However, it is usually preferable to wait until they reach 80 percent of their mature weight before breeding them. These relative weights should not be considered absolute thresholds for all rabbits, but rather limits applicable to the population as a whole. Sexual behaviour (acceptance of mating) appears long before the ability to ovulate and bear a litter. Such behaviour should not be regarded by the breeder as a sign of puberty, but as pre-puberty play (Lebas *et al*., 1997; Sharp *et al*., 2007).

Ovulation takes place at regular intervals when the female is in heat or oestrus. Female rabbit do not have oestrus cycle with regular periods of heat during which ovulation will occur spontaneously. Does are considered to be in oestrus more or less permanently. Ovulation occurs only after mating. A female rabbit is therefore considered to be in heat when she accepts service and in dioestrus when she refuses. There are many observations

which denote the alternating periods of oestrus during which the doe accepts mating and dioestrus in which she refuses. But the present state of knowledge does not make it possible to predict either the respective lengths of oestrus and dioestrus or the environmental or hormonal factors determining them. It has been noted that 90 percent of the time when a doe has a red vulva she will accept mating and ovulate, whereas when the vulva is not red the doe will accept service and become fertilized only 10 percent of the time. A red vulva is therefore a strong indication, though not a proof of oestrus.

A doe in heat assumes a characteristic pose, called lordosis, with the back arched downwards and hindquarters raised. A doe in diestrus tends to crouch in a corner of the cage or exhibit aggression towards the buck. The sexual behaviour of a female rabbit is thus very special, have no cycle and can stay in heat for several days running. On the ovary, follicles not having evolved to the ovulation stage through lack of stimulation undergo regression and are replaced by new follicles, which remain for a few days in the pre-ovulating state and may then in turn regress. The primary follicles form more than 80% of relative volume of ovary (Zitny *et al*., 2004). All growing follicles possess the potential to develop into mature follicles, follicle growth is a continuous process (Kranzfelder *et al*., 1984). In most mammals the progesterone secreted during gestation inhibits oestrus and the pregnant female refuses to mate, but a pregnant doe may accept mating throughout the gestation period which is a common behavior in the second half of pregnancy. A breeder cannot therefore use the sexual behaviour of does as an indication of pregnancy. Ovulation is normally induced by the stimuli associated with coitus and occurs 10 to 12 hours after mating. Exposure of young female rabbits to male presence and/or photoperiod may help to induce early puberty, amplify behavioural oetrus and improve kindling rates (Berepubo *et al*., 1993).

The moment the ovary follicles are ruptured, the infundibulum covers the ovary. The ovocytes are in fact fertilizable from the moment they are liberated, but they are not actually fertilized until about an hour and a half after release. The sperm is deposited by the male in the upper part of the vagina. The spermatozoa make their way upwards rapidly. They can reach the fertilization area (in the distal ampulla, near the isthmus) 30 minutes after coitus. During their journey the spermatozoa undergo a maturing process which enables them to fertilize the ovocytes. Of the 150 to 200 million spermatozoa ejaculated, only two million (1 percent) will reach the uterus. The rest are defeated by obstacles at the cervix and uterotubal junction (Lebas *et al*., 1997). The structure of a male rabbits' reproductive organ is presented in Figure 2.

2.5 REPRODUCTIVE HORMONES AND THEIR SYNTHESIS

2.5.1 Gonadotropins

In vertebrates, reproduction is primarily controlled by the Hypothalamic-Pituitary-Gonad (HPG) axis. This axis is illustrated as shown in Figure 3. The onset of puberty is marked by increase in the secretion of sex steroids in response to central activation of the hypothalamic-pituitary-gonadal axis. The hypothalamic neuroendocrine system regulates synthesis and release of the gonadotropins (follicle-stimulating-hormone (FSH) and Luitenising Hormone (LH) from the pituitary, which in turn stimulates gonadal development, in particular via the induction of sex steroid synthesis. Sex steroids send feedback to the hypothalamus and the pituitary, thereby regulating gonadotropin synthesis and release (Thackray *et al*., 2010; Kanda *et al*., 2011).

The primary function of the HPG axis is to facilitate the production of germ cells and to coordinate reproductive events in relation to body condition and environment. Androgens

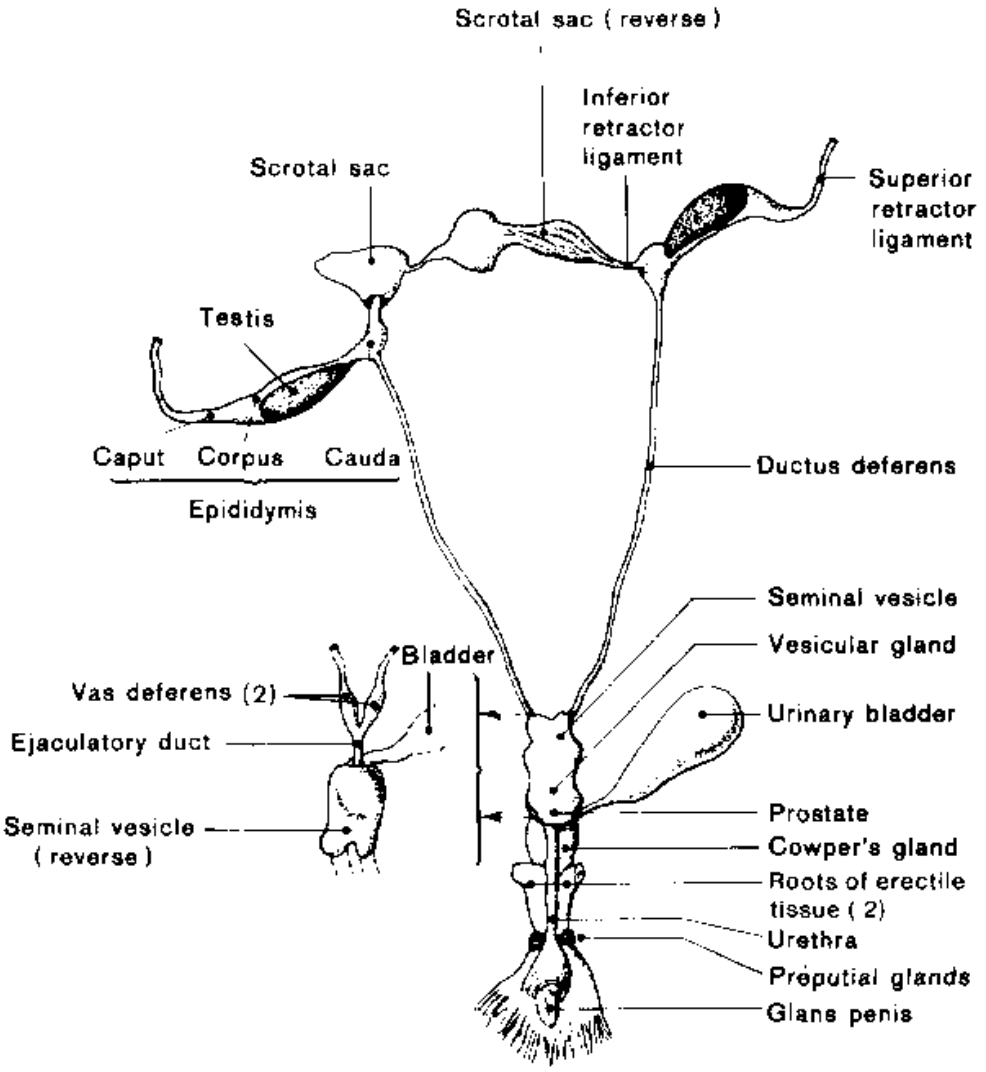


Figure 1: Reproductive organ of a male rabbit

Source: Anna and Lance (2000)

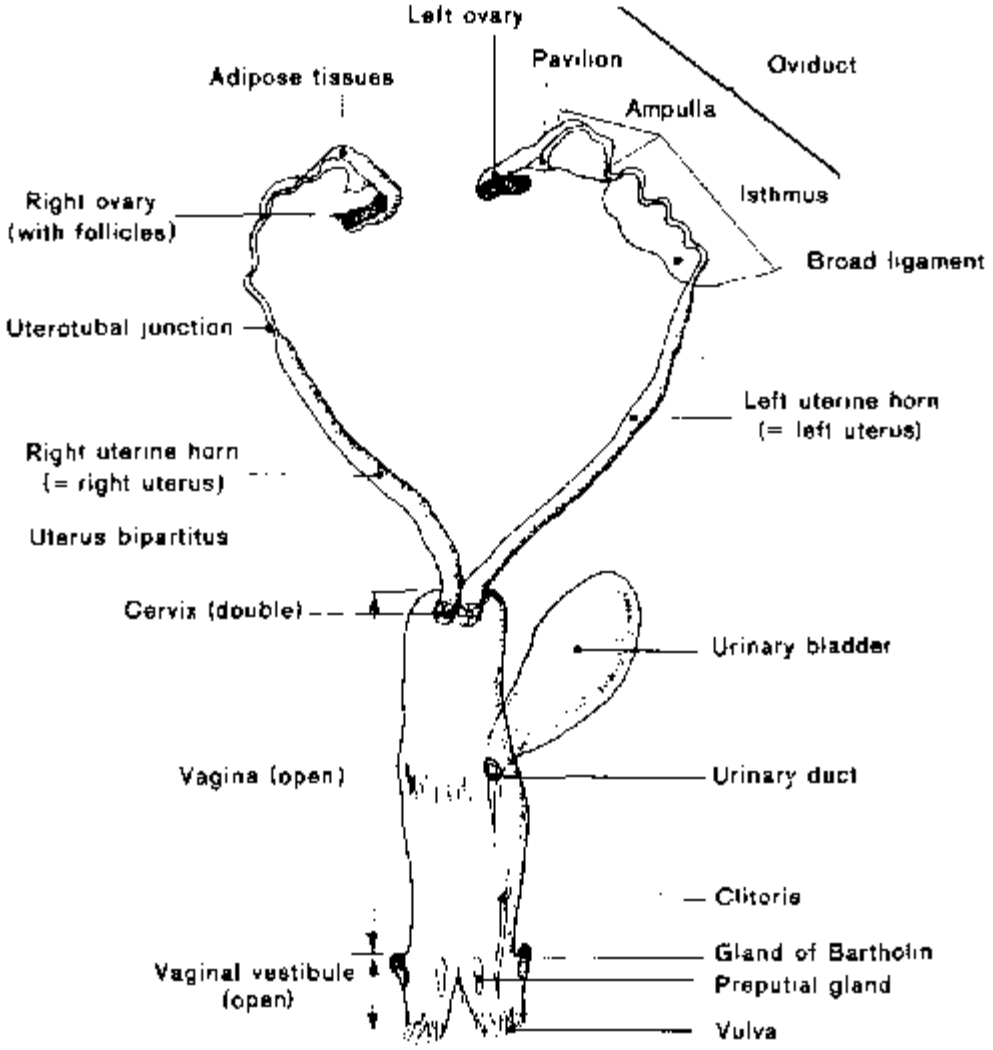


Figure 2: Reproductive organ of a female rabbit

Source: Anna and Lance (2000)

(testosterone and androsterone) play a pivotal role in the development of the reproductive system, phenotypic sex and are crucial for testicular spermatogenesis/spermiogenesis, as well as for the expression of male sexual behavior (Akingbemi, 2005; Wang *et al*., 2009). It is well known that sex steroids, in particular estrogens, play a pivotal role for brain differentiation during early development and that disruption of these processes can result in persistent changes leading to altered timing of puberty or behavioral changes (Dickerson and Gore, 2007).

Gonadotropin Releasing hormone (GnRH) stimulates secretion of LH, which in turn stimulates gonadal secretion of the sex steroids testosterone, estrogen and progesterone. Sex steroids inhibit secretion of GnRH and also appear to have direct negative effects on gonadotrophs. The gonads also secrete at least two additional hormones - inhibin and activin- which selectively inhibit and activate FSH secretion from the pituitary. Diminished secretion of LH or FSH can result in failure of gonadal function (hypogonadism). This condition is typically manifested in males as failure in production of normal numbers of sperm. In females, cessation of reproductive cycles is commonly observed. Elevated blood levels of gonadotropins usually reflect lack of steroid negative feedback. Removal of the gonads from either males or females, as is commonly done to animals, leads to persistent elevation in LH and FSH. In general, elevated levels of gonadotropins may not have any biological effect. FSH activates proliferation and inhibits apoptosis in rabbit ovarian cells (Laukova and Sirotkin, 2007).

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are called gonadotropins because they stimulate the gonads - in males, the testes and in females, the ovaries. They are not necessary for life, but are essential for reproduction. These two hormones are secreted

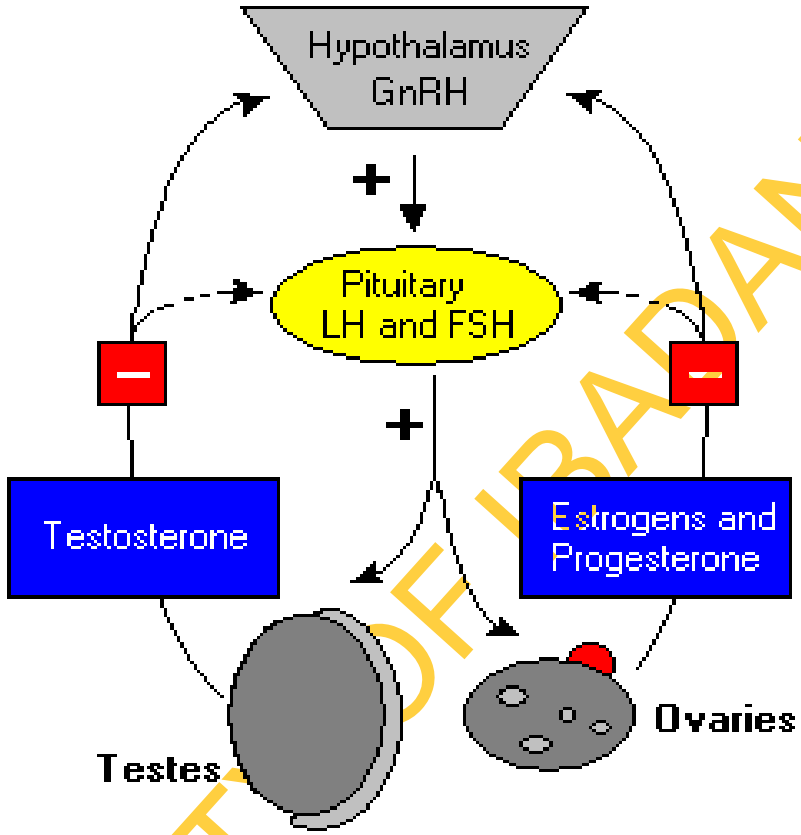


Figure 3: Hypothalamic-pituitary-gonadal pathway

Source: Bowen, 2004

from cells in the anterior pituitary called gonadotrophs. Most gonadotrophs secrete only LH or FSH, but some appear to secrete both hormones. LH and FSH are large glycoproteins composed of alpha and beta subunits. The alpha subunit is identical in all three of these anterior pituitary hormones, while the beta subunit is unique and endows each hormone with the ability to bind its own receptor. Physiologic effects of the gonadotropins are known only in the ovaries and testes. Together, they regulate many aspects of gonadal function in both males and females (Bowen, 2004). Luteinizing Hormone in both sexes stimulates secretion of sex steroids from the gonads. In the testes, LH binds to receptors on Leydig cells, stimulating synthesis and secretion of testosterone. Theca cells in the ovary respond to LH stimulation by secretion of testosterone, which is converted into estrogen by adjacent granulosa cells (Williams and Stancel, 1996).

In females, ovulation of mature follicles on the ovary is induced by a large burst of LH secretion known as the preovulatory LH surge. Residual cells within ovulated follicles proliferate to form corpora lutea, which secrete the steroid hormones: progesterone and estradiol. Progesterone is necessary for maintenance of pregnancy (Al-Asmakh, 2007). LH is required for continued development and function of corpora lutea. The name luteinizing hormone derives from this effect of inducing luteinization of ovarian follicles. Follicle-Stimulating Hormone stimulates the maturation of ovarian follicles. Administration of FSH in mammal induces "superovulation" or development of more than the usual number of mature follicles and hence an increased number of mature gametes. FSH is also critical for sperm production. It supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation. The process by which gametes are formed in gonads is called Gametogenesis. The specific type of meiosis that forms sperm is called Spermatogenesis while that of egg cells or ova is called Oogenesis. Starting at puberty, a

male will produce literally millions of sperm every single day for the rest of his life. The production of one egg cell via oogenesis normally occurs only once a month, from puberty.

2.5.2 Steroidogenesis

Steroidogenesis is the process by which steroid hormones are synthesized from cholesterol. Cholesterol is a sterol which is a natural product derived from the steroid nucleus. Besides being the building block for steroid hormones, cholesterol is also a component of the cell membrane. It is thought that the cholesterol present in the cell membrane is responsible for allowing steroid hormones to enter the cell, bind to the hormone receptor, and ultimately to a specific site on the chromatin, in turn activating a gene. Cholesterol is very important in the production of steroid hormones, in fact it is the precursor for bile acids (bile acids aid in fat digestion), steroid hormones, and provitamin *D*. This conversion takes place in the gonads. Progesterone is the steroid hormone that ultimately converts to the other steroid hormones through a variety of reactions. Therefore, the regulation of steroid hormones can be indirectly tied to the inhibition of this enzyme, known as cytochrome P-450. Leydig cells from animals deprived of LH had diminished capacity to convert pregnenolone to testosterone and reduced P450 C17-hydroxylase/C17, 20-lyase content (Klinefelter *et al*., 1987). It involves the synthesis of pregnenolone from cholesterol and the subsequent conversion to progesterone and successively to C19 androgens, which can be further aromatized by P-450 aromatase to estrogens.

Cytochrome P-450 is an enzyme that oxidizes cholesterol, which is a necessary step if it is to become progesterone. The steriodogenic pathway is presented in figure 4. Wu *et al*. (1977) monitored ovarian secretion of hormones hourly for a period of 9 h following the administration of human chorionic gonadotropin (hCG) to estrous rabbits. It was observed

from the study that ovarian vein plasma concentration and secretion rates of estradiol-17 3 (E2) and testosterone (T) rose sharply at the 2 h period. By 4 h, when ovarian blood flow reached its maximum, the secretion rate of E2 and T reached a second peak. Secretion rates of estrone (E1), androstenedione (A), progesterone (P) and 20o-dihydroprogesterone (20a-OH-P) showed only a single peak at the 4 h period. The findings from their study also suggest that the significant but brief rise in E2 and T production serves an intraovarian function rather than a systemic function. The endocrine glands (the adrenal gland, the ovary and the testes) are responsible for the production of steroid hormones. Many but not all steroids are hormones however, all reproductive hormones are steroids. They are target organ hormones meaning they exert a direct effect in peripheral tissue. Some examples of steroid hormones are estrogens, androgens (androsterone), progesterone, (Sandabe and Timothy, 2007), Corticoids {Glucocorticoids and Mineral corticoids}. The pathway for steriodogenesis is as illustrated in Figure 4.

Steroid hormones are crucial substances for the proper function of the body. They mediate a wide variety of vital physiological functions ranging from anti-inflammatory agents to regulating events during pregnancy. They are synthesized and secreted into the bloodstream by endocrine glands such as the adrenal cortex and the gonads (ovary and testis). Androgens and estrogens play a major role in the development of both sexes secondary characteristics. Androgens, or testosterone and androsterone give the male its sex characteristics during puberty and for promoting tissue and muscle growth. Estrogens, or estrone and estradiol are forms of testosterone synthesized in the ovaries, which control female secondary characteristics and regulation of the menstrual or oestrus cycle (Gulliver, 2013). Another sex hormone needed for preparing the uterus for implantation of the ovum is progesterone. Hormones are needed throughout the body for various functions, however, just as important

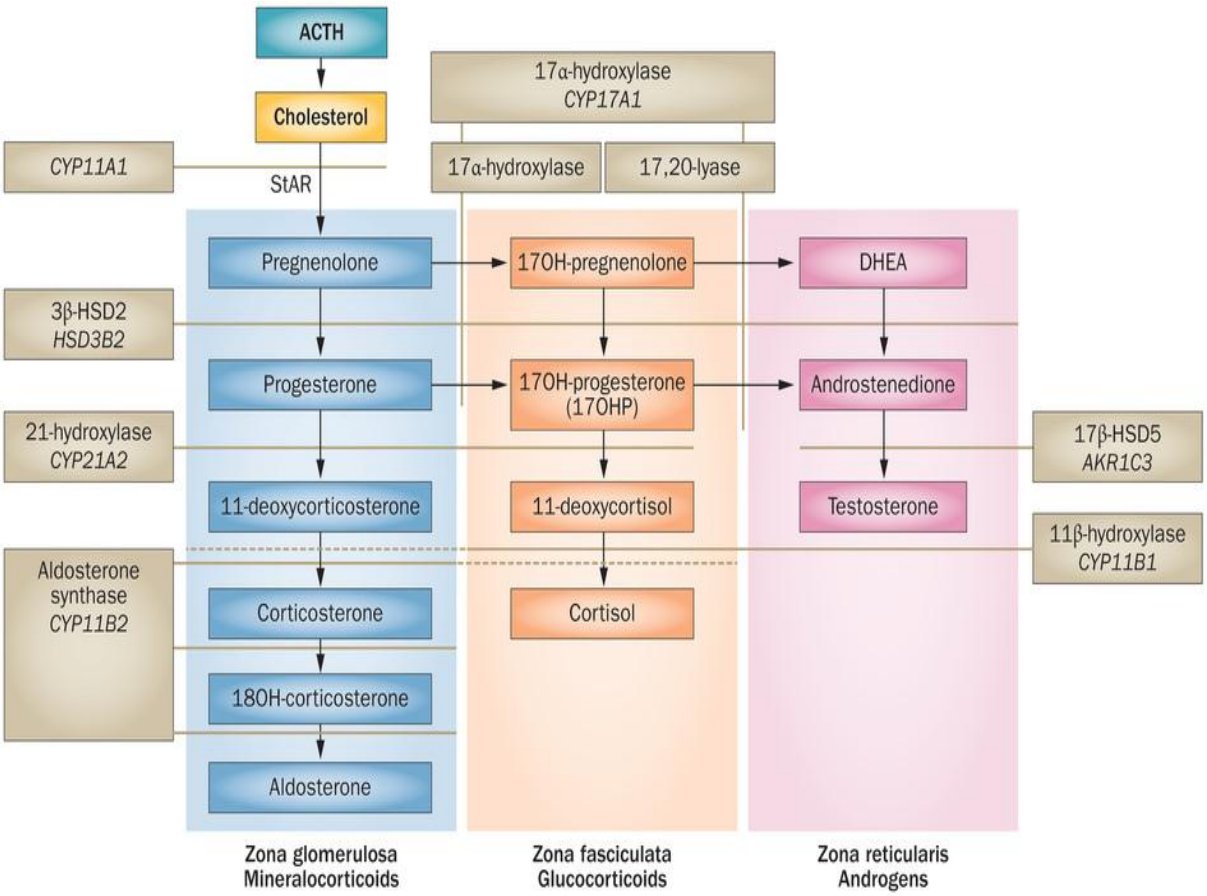


Figure 4: Pathway for steriodogenesis

Source: Ham *et al*., 2014

as their function is, they must be regulated. High levels of progesterone at the moment of insemination, which are particularly frequent in primiparous lactating rabbit does (36.5%), have a deleterious effect on receptivity and fertility, leading to a 33% productivity decrease (Theau-Clement *et al*., 2008).

Steroid hormones are made on a basis of need. Whenever the body needs a certain process done or needs a certain protein synthesized, the brain releases a signal to produce a certain type of hormone and the signals are transmitted through intermediary hormones. According to Gonzalez-Mariscal *et al*. (1996), maternal nest-building in rabbits which is expressed towards the end of third trimester of pregnancy consisting of: digging a burrow, collecting straw and shaping it into a nest inside the burrow, plucking body hair and lining the straw nest with it. The sequential expression of these activities is correlated with specific changes in the plasma concentration of estradiol, progesterone (P) and prolactin (PRL). The authors further substantiated the participation of these hormones in the control of maternal nest-building. Digging was maximally expressed when circulating levels of both estradiol and progesterone were equal. Progesterone is essential for the development of decidual tissues, and if fertilization occurs, high circulating progesterone levels are important not only for facilitating implantation, but also for maintaining pregnancy by stimulating uterine growth and opposing the actions of factors involved in myometrial contraction (Al-Asmakh, 2007).

2.5.3 Morphology of the Sperm cell

Normal sperm cells have oval head with a long tail. Abnormal sperms have head or tail defects like large head, misshapen head, crooked tail or double tail and cytoplasmic droplet. These defects may affect the ability of the sperm to reach or penetrate an egg. The fertilizing ability of rabbit spermatozoa is acquired in passage through the epididymis, during which as

certain changes take place in the spermatozoa. Significant diminution in acrosome width and length occurs during passage of spermatozoa from caput to cauda epididymis (Bedford, 1963). The speed at which the sperms ascend the female tract is apparently not affected by the presence in them of male and female sex-determining elements (Hammond, 1934). Adequate nutrition with high protein will enhance sperm motility and concentration (Oyeyemi *et al*., 1998) and reduce the number of abnormal sperm cells (Oyeyemi and Okediran, 2007).

2.6 THE MORINGA PLANT

Moringa oleifera (Lam) belongs to onogeneric family of shrubs and tree (Moringaceae) and it is considered to have its origin in the northwest region of India, south of the Himalayan Mountains. *Moringa oleifera* is commonly known as "Drumstick". It is a small or medium sized tree, about 10m height, found in the sub-Himalayan tract (Rastogi *et al*., 2009). There are more than 12 varieties of Moringa species. It is native to the Indian sub-continent and naturalized in tropical and sub-tropical areas around the world. It is a deciduous tree or shrub that is fast-growing and resistant to drought. Its common names include Benzolive, Drumstick Tree, Kelor, Marango, Mlonge, Mulangay, Saijhan and Sajna. *Moringa oleifera* is the best known of the thirteen species of the genus Moringacae. Moringa was highly valued in the ancient world. The Romans, Greeks and Egyptians extracted edible oil from the seeds and used it for perfume and skin lotion (Fuglie, 1999).

In 19th century, plantations of Moringa in the West Indies exported the oil to Europe for perfumes and lubricants for machinery. People in the Indian sub-continent have long used Moringa pods for food (Rudappa, 2009). The edible leaves are eaten throughout West Africa and parts of Asia. This tree can be found growing naturally at elevations of up to 1,000 m

above sea level. It can grow well on hillsides but is more frequently found growing on pastureland or in river basins. It is a fast growing tree and has been found to grow to 6 – 7 m in one year in areas receiving less than 400mm mean annual rainfall (Fahey, 2005). The plant possesses many valuable properties which make it of great scientific interest. These include the high protein content of the leaves, twigs and stems, the high protein and oil contents of the seeds, the large number of unique polypeptides in seeds that can bind to many moieties, the presence of growth factors in the leaves, and the high sugar and starch contents of the entire plant (Jaya *et al*., 2013). It must also be noted that few parts of the tree contain some toxins that might decrease its potential as a source of food for animals or humans (Makkar and Bekker, 1997).

Moringa is one of the most useful tropical trees (Mbikay, 2012). The relative ease with which it propagates through both sexual and asexual means and its low demand for soil nutrients and water after being planted makes its production and management easy. Introduction of this plant into a farm which has a biodiverse environment can be beneficial for both the owner of the farm and the surrounding eco-system. The stem is normally straight but occasionally is poorly formed. The tree grows with a short, straight stem that reaches a height of 1.5 – 2.0 m before it begins branching but can reach up to 3.0 m. The extended branches grow in a disorganized manner and the canopy is umbrella shaped. The alternate, twice or thrice pinnate leaves grow mostly at the branch tips. They are 20 - 70 cm long, grayish-downy when young, long petiole with 8 - 10 pairs of pinnae each bearing two pairs of opposite, elliptic or obovate leaflets and one at the apex, all 1 - 2 cm long; with glands at the bases of the petioles and pinnae. The flowers are pleasantly fragrant, and 2.5 cm wide are produced profusely in auxillary, drooping panicles 10 to 25 cm long. They are white or cream colored and yellow-dotted at the base. The five reflexed sepals are linear-

lanceolate. The five petals are slender-spatulate. They surround the five stamens and five staminodes and are reflexed except for the lowest (Morton, 1991). The fruits are three lobed pods which hang down from the branches and are 20 - 60 cm in length. When they are dry they open into 3 parts. Each pod contains between 12 and 35 seeds. The seeds are round with a brownish semi-permeable seed hull. The hull itself has three white wings that run from top to bottom at 120-degree intervals. Each tree can produce between 15,000 and 25,000 seeds/year. The average weight per seed is 0.3 g and the kernel to hull ratio is 75 : 25 (Makkar and Becker, 1997).

2.6.1 Importance of *Moringa oleifera*

Researches on the moringa plant have revealed its exceptional nutritional qualities. The leaves are rich in protein with the 8 essential amino acids, which vary in other green leafy vegetables and many of which that are staples and low in the sulphur bearing amino acids (methionine and cysteine), whereas the moringa leaf is an extremely rich source in comparison to other greens and vegetables (Fahey, 2005). The calcium content is very high at 297mg per 100g of leaves. Leaves can be eaten fresh, steamed, added to salads, as curries, and in soups. Sliced, young green pods can be used in savoury and meat dishes. Seeds can be fried or roasted and taste like peanuts. When seeds are abundant they can be sprouted like wheat grass, eaten as tender nutritious greens. Leaves and pods of *M. oleifera* can be an extremely valuable source of nutrition for man and animals. Booth and Wickens (1988) reported the nutritional profile of the pod, fresh raw leaves and the dried leaf powder of *Moringa oleifera* as presented on Table 3.

Moringa leaves can be dried and made into a powder by rubbing them over a sieve. A study by Joshi and Mehta (2010) found the protein content (27.9%) of Moringa leaf powder to be

Table 3: Nutritional Analysis of Moringa

Nutrients	Pods (per 100 grams)	Fresh Raw Leaves (Per 100 grams)	Dried Leaf Powder (Per 100 grams)
Moisture (%)	86.9	75	7.5
Calories	26.0	92.0	205.0
Protein (g)	2.5	6.7	27.1
Fat (g)	0.1	1.7	2.3
Carbohydrate (g)	3.7	13.4	38.2\
Fiber (g)	4.8	0.9	19.2
Minerals (g)	2.0	2.3	-
Calcium (mg)	30.0	440.0	2003.0
Magnesium (mg)	24.0	24.0	368.0
Phosphorous (mg)	110.0	70.0	204.0
Potassium (mg)	259.0	259.0	1324.0
Copper (mg)	3.1	1.1	0.6
Iron (mg)	5.3	0.7	28.2
Oxalic acid (mg)	10.0	101.0	0.0
Sulphur	137	137	870
VITAMINS CONTENTS			
Vitamin A – β carotene (mg)	0.1	6.8	16.3
Vitamin B – Choline (mg)		423.0	423.0
Vitamin B1 – Thiamin (mg)	0.05	0.21	2.6
Vitamin B2 – Riboflavin (mg)	0.07	0.05	20.5
Vitamin B3 – Nicotinic Acid (mg)	0.2	0.8	8.2
Vitamin C Ascorbic Acid (Mg)	120	220	17.3
Vitamin E- Tocopherols Acetate (mg)	-	-	113.0

Source: Booth and Wickens (1988)

higher than many of the commonly consumed green leafy vegetables, spinach (2%), mint (4.8%) but an equivalent of the protein content of many pulses such as moth beans which contain (22 – 24 %) protein. Drying should be done indoors and the leaf powder stored in opaque, well-sealed plastic container since sunlight will destroy its Vitamin A content. It is estimated that only 20 - 40 % of Vitamin A content will be retained if leaves are dried under direct sunlight, but that 50 - 70 % will be retained if leaves are dried in the shade. Both fresh and dehydrated leaves of *M. oleifera* produced an increase in food intake and weight gain in vitamin A deficient rat (Nambiar, 2001). The protein content is of high quality having significant quantities of all the essential amino acids (Foidl and Paull, 2008). This powder can be used in place of fresh leaves to make lead sauces, or few spoonfuls of the powder can be added to other sauces just before serving. Addition of small amounts of leaf powder will have no discernible effect on the taste of a sauce. In this way, Moringa leaves will be readily available to improve nutritional intake on a daily basis (Fahey, 2005; Mbikay, 2012). Some reported values for the *Moringa oleifera* leaf meal are presented on Table 4.

Some uses of Moringa include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice extracted from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds). Moringa seed oil (yield 30-40 % by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication, in the manufacture of perfume and hair care products. In the West, one

Table 4: Proximate, energy and some mineral compositions of *Moringa oleifera* Leaf Meal.

COMPONENT	ADAPTED FROM		
	Oduro *et al.*, 2008	Yameogo *et al.*, 2011	Ewuola *et al.*, 2012
MOISTURE (%)	N .A.	5.9 ± 1.8	9.28
CRUDE PROTEIN (%)	27.51± 0.00	27. 2 ± 0.8	27.53
FAT (%)	2.23 ± 0.03	17.1 ± 4.5	9.93
CRUDE FIBER (%)	19.25 ± 0.07	19.4 ± 3.3	14.05
ASH (%)	7.13 ± 0.03	11.1 ± 4.3	7.98
ENERGY (Kcal/kg)	1296.09 ± 1.31	1339.1 ± 22.7	N.A
IRON (mg)	28.29 ± 0.047	N.A.	N.A
CALCIUM (mg)	2009.79 ± 0.02	N.A.	N.A

N.A.- Not Available

of the best known uses for Moringa is the use of powdered seeds to flocculate contaminants and purify drinking water, but the seeds are also eaten green, roasted, powdered and steeped for tea or used in curries. This tree has in recent times been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids suitable for utilization in many of the so-called "developing" regions of the world where undernourishment is a major concern (Fuglie, 2001).

Makkar and Becker (1997) evaluated the potential of different morphological parts of the Moringa tree as animal feed and reported the crude protein (CP) content of leaves, soft twigs and stems has 260, 70 and 60 g kg^{-1} respectively. About 87% of the total CP was in the form of true protein in the leaves (60 and 53 % in twigs and stems respectively). Data derived from nutrient characterization of *M. oleifera* leaves clearly indicates a rich nutrient profile of important minerals, a good source of protein and amino acids, vitamins, β -carotene and various phenolics with multiple feed additive purposes (Moyo *et al*., 2011).

2.6.2 Phytochemicals of moringa and their uses

Phytochemicals are dependable sources for the treatment of different health problems (Farhat *et al*., 2011). Plants and their products are potential sources of phytochemicals that have been found to counteract free radicals due to their antioxidant activity (Khalafalla *et al*., 2010). Coppins (2008) reported that Moringa leaves were found to provide low amounts of vitamin C (0.351 ± 0.046 to 0.749 ± 0.014 mg/100g dry weight (DW) as determined using UV spectrophotometry. Using LC/MS, α- and β- tocopherols, α- and β- carotenes, six analogues of chlorogenic acid including 4 caffeoylquinic acids and 2 coumaroylquinic acids (structural and/or spatial isomers) were identified. Additionally, 12 flavonoids including quercetin and kaempferol glycosides with malonyl, acetyl and succinoyl acylations, among

which quercetin and kaempferol glucosides and glucoside malonates were dectected as the major constituents based on analysis of their UV and MS data. Chlorogenic acid isomers analogs (0.181 to 0.414 mg/100 g DW), tocopherols (7.1 to 116 mg/100 g DW), carotenoids (4.49 to 45.94 mg/100 g DW) and flavonoids (0.179 to 1.643 % g DW) were obtained. The concentrations of these phytochemicals varied according to the environment, country of collection, genetics and variety of *M. oleifera* (Coppins, 2008).

Quercetin is actually the molecular backbone for the citrus bioflavonoids rutin, quercetin and hesperidin. Quercetin has also been found to inhibit the growth of human prostate cancer cells and human breast cancer cells. Quercetin has antiviral activity against several types of viruses, revealing that maximum polyphenols were identified in the drumstick leaves, which further enhances its role as an important functional food (Nambiar *et al*., 2005). The leaves had negligible amounts of tannins (12 g kg^{-1}), trypsin, amylase inhibitors, lectins, cyanogenic glucosides and glucosinolates were not detected. The saponin content of the leaves was 80 g kg^{-1} as diosgenin equivalent, which did not show any haemolytic activity. The phytate content of the leaves was 21 g/kg. Tannins, saponins, cyanogenic glucosides and glucosinolates were detected in twigs and stems but the concentrations were negligible (Makkar and Becker, 1997). Nambiar *et al*. (2005) identified flavone, acacetin and a glycoflavone 4-OMe Vitexin, phenolic acids (melilotic acid, p-coumaric acid and vanillic acid). Duke (1983) reported that moringa root-bark yields two alkaloids: moringine and moringinine. Saponins are known to possess both beneficial cholesterol lowering properties and deleterious to intestinal cell toxicity and permeability (Bamishaiye *et al*., 2011).

The methanol extract of the leaves of *M. oleifera* was investigated for some psychopharmacological actions in animals. The extract was found to produce a significant alteration in general behavioural pattern of animals (Mukherjee *et al.*, 1996). The extract also potentiated pentobarbitone induced sleeping time and lowered body temperature in experimental animals. The ethanol extracted leaves were virtually free of tannins, lectins, trypsin inhibitors, saponins, and phytate content was 2.5% (Makkar and Becker, 1996). Coppins (2008) identified and quantified the following phytochemicals in *M. oleifera*, β-carotene 4.49 – 45 94 mg/100g DW, α and β tocopherol 7.1 – 116 mg/100g DW, flavonoids 0.179 – 1.643 %/g DW and chlorogenic acid 0.181 – 0.414 %/g DW. Rajanandh *et al.* (2010) reported that *M. oleifera* leaf contain β-sitosterol (90.00 mg/g), a plant sterol that has the ability to inhibit the absorption of cholesterol in the small intestine thus inducing its hypocholestemic activity.

Chung *et al*. (1998) in a review to summarize and analyze the vast and sometimes conflicting literature on tannins and its overall effects on human health, reported that tannin is responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value. Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances. Tannin has been reported to interfere with the biological utilization of protein and to a less extent available carbohydrate and lipids (Esonu, 2001). Incidences of certain cancers such as oesophageal cancer have been reported by Chung *et al*. (1998) to be related to consumption of tannins-rich foods such as betel nuts and herbal teas,

suggesting that tannins might be carcinogenic. However, other reports indicated that the carcinogenic activity of tannins might be related to components associated with tannins rather than tannins themselves. Many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses. The dosage and kind of tannins are critical to these effects (Chung *et al*., 1998; Coppins, 2008).

Anwar *et al.* (2007) reported that *M. oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics. The Moringa plant provides a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeoylquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional value, *M. oleifera* is very important for its medicinal value. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess anti-tumor, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer, anti-spasmodic, diuretic, anti-hypertensive, cholesterol lowering, antioxidant, anti-diabetic, hepatoprotective, anti-bacterial and anti-fungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine particularly in South Asia (Anwar *et al*. 2007; Fahey, 2005).

Vinoth *et al*. (2012) evaluated the antibacterial activity of *M. oleifera* leaf extracts against four microorganisms viz; *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus and Salmonella typhii.* The ethanolic extract was active against *Salmonella typhii* and

Staphylococcus aureus whereas the aqueous extract exhibited an inhibitory effect on *Staphylococcus aureus* only. The phytochemical screening carried out indicated the presence of phenolics, flavonoids, tannins, glycosides, etc, in the extracts indicating that the active components responsible for the bactericidal activity are more soluble in organic solvents. Petruska *et al*. (2013) in an experiment on the intramuscular application of quercetin caused slight changes in some haematological parameters of rabbits in both sexes. The values of other haematological parameters were not influenced after quercetin treatment and no significant influence on gender of rabbits.

2.7 REVIEWS ON THE EFFECTS OF LEAF MEALS IN ANIMAL PRODUCTION

2.7.1 Effects of leaf meal on performance of farm animals

Leaf meal has been used in livestock production resulting in various performance responses by the farm animals. Growth performance of farm animals can be monitored using indices such as intake of feed, weight and feed efficiency. Rabbits are generally attributed to have high growth rate. This is not peculiar to rabbit production in Nigeria because the available breed are mixed breeds that their genetic make-up has been altered due to cross breeding compared to the pure breeds. In rabbit breeding, diseases of the digestive system are common in young rabbit (Lebas *et al*., 1997) and this is responsible for economic losses recorded in terms of treatment costs and mortality rates. Digestive disorders occur mainly with change in the diet of the young rabbit from milk to feed consumed by the doe.

Davies *et al*. (2003) reported that lack of enough fiber is the most common cause of gastrointestinal disturbance in rabbits. Development of good health prophylaxis and good management practices such as housing, hygiene and nutrition play a significant role among other factors. The health status of rabbit especially during the growing phase can be

enhanced by a well balanced feed. The fundamental function of feed is to provide the body with necessary nutrients for its maintenance, growth and production needs such as pregnancy, lactation, etc. The need to improve the performance of farm animals necessitated the research into introduction of various plant leaf meal or extracts in their feed. Mukherjee *et al.* (1996) investigated some psychopharmacological actions of methanolic extract of the leaves of *M. oleifera* in animals. The extract was found to produce a significant alteration in general behavioural pattern by head dip test, Y-maze test, evasion test and reduction in muscle relaxant activity by rotarod test, chimney test and traction test. Beside these, the extract also potentiated pentobarbitone induced sleeping time and lowered body temperature in experimental animals.

In a study evaluating the suitability of freeze-dried moringa leaf meal as an alternative protein source for Nile tilapia, reported no feed-related mortality during the experimental period. Diets with higher inclusion levels (15%) of moringa leaves significantly depressed growth performance of fishes compared to those fed diets 1(5%) and 2 (10%). Thus suggest that moringa leaf meal can be used to substitute up to 10% of dietary protein in Nile tilapia without significant reduction in growth (Richter *et al*., 2003).

In a study carried out by Ibom and Isika (2004), fryer rabbits were fed diets amended with macerated bitter-leaf meal (BLM) for thirty days to determine its effect on growth performance. Diet A served as control while Diets B, C and D were respectively amended with 250, 500 and 750 g of macerated BLM. The BLM had no significant effect on the total feed intake, weight gain, feed conversion ratio and feed efficiency of rabbits fed the different diets.

Kakengi *et al.* (2007) carried out an experiment to investigate the effect of substituting *Moringa oleifera* leaf meal (MOLM) for sunflower seed meal (SSM) as a protein source of egg strain commercial chickens. The egg weight was significantly highest in MOLM-0 and lowest in MOLM-10. Laying percentage and egg mass showed a significant progressive decreasing trend as MOLM proportion increased in the diet, at 10 and 20% MOLM levels. Dry matter intake and daily feed intake significantly increased progressively at 10 and 20% MOLM levels. Also, kg feed/kg eggs were significantly higher in birds fed 20% MOLM levels. The results therefore suggested that MOLM could replace SSC up to 20% as a feed ingredient without any detrimental effect in laying chickens. However, for better efficiency 10% inclusion level is optimal and feeding MOLM above 10% will require high energy based feeds for better utilization. Also, feeding MoLM as a sole source of animal protein in layers diet showed highest performance in egg production in comparison with other leaf meals already studied by the authors. In areas where MOLM can be obtained for free and quality of eggs fetch higher, substitution up to 20% with MOLM is highly recommended (Kakengi *et al.*, 2007).

Ndong *et al.* (2007) reported that medicinal plants constitute an important source of potential therapeutic agents for diabetes. The authors investigated the effects of *Moringa oleifera* (MO) on glucose tolerance in Wistar rats and Goto-Kakizaki (GK) rats, modeled type 2 diabetes. Major polyphenols were identified in MO powder which include quercetin glucosides, rutin, kaempferol glycosides and chlorogenic acids by HPLC analysis. The result of glucose tolerance test reported by the authors showed that MO significantly decreased the blood glucose of Wistar rats compared to the control after glucose administration. MO significantly decreased stomach emptying in GK rats. From the findings, it was concluded that MO has an ameliorating effect for glucose intolerance and the effect might be mediated

by quercetin-3-glucoside and fiber contents in MO leaf powder the action of MO was greater in GK rats than in Wistar rats.

Garduno-Lugo and Olvera-Novea (2008) evaluated the effects of replacing the protein from ash meal with peanut (*Arachis hypogaea*) leaf meal (PLM) in diets for male tilapia (*Oreochromis niloticus*), a high survival ratio was observed in all dietary groups suggesting that PLM can be used in *O. niloticus* feed for long periods without affecting growth performance or health.

Oduro *et al.* (2008) reported the levels of some nutrients in *M. oleifera* leaves as crude protein was 27.51%, crude fibre (19.25%), crude fat (2.23%), ash content (7.13%), moisture content (76.53%), carbohydrate content (43.88%) and 1296.00 kJ/g (305.62 cal/g) energy. The study involved a comparism between *M. oleifera* and varieties of sweet potato (*Ipomoea batatas)* leaves. Elemental analysis of the leaves indicated that the varieties of sweet potato leaves contained appreciable levels of calcium (1310.52-1402.27) and iron (9.62-23.02) mg/100g dry matter (DM) which was lower than that of *M. oleifera* (2,009.00 and 28.29 mg/100 g DM) respectively. It was concluded that relatively *Moringa* leaves contain higher levels of calcium, iron and protein making it a very rich source of dietary nutrient compared to the *I. batatas* leaves.

Nworgu and Fasogbon (2010) in an experiment to determine the optimum dietary inclusion rate of *Centrosema pubescens* Leaf Meal reported that dietary inclusion of 2 - 6% CLM for the pullet chicks significantly and progressively reduced the weight gain averagely by 31.82% compared with the control while its inclusion at same levels for the growing pullet increased weight gain averagely by 4.61% compared with the control. Thus, it is not

advisable to include *Centrosema* Leaf Meal in the diet of pullet chicks while 2% *Centrosema pubescens* Leaf Meal is recommended for the growing pullet.

Nuhu (2010) reported that final body weight, daily weight gain and total weight gain of weaner rabbits fed inclusion levels of dietary *M. oleifera* Leaf Meal (MOLM) increased with increasing levels of MOLM. The control animals performed poorer than those fed the MOLM diets. The daily feed intake and the feed conversion ratio values similarly improved with increasing level of MOLM. The MOLM increased the unit cost of the concentrate feed used and no animal died in the course of the experiment.

In a 49-day trial, Olugbemi *et al.* (2010) reported the suitability of including *M. oleifera* leaf meal as a feed ingredient in cassava (CC) based broiler diets. A reduction in performance was observed with increasing inclusion level of MOLM beyond 5% and concluded that the inclusion of MOLM in cassava based broiler diets up to 5% is possible without negatively affecting productivity or haematological indices. Onu and Aniebo (2011) reported that dietary treatment effect of *M. oleifera* on average final body weight, average daily gain, average daily feed intake and feed conversion ratio were significant. Birds fed MOLM gained significantly higher weight with superior feed conversion ratio than birds fed the control diet. However, birds fed 2.5 and 5.0 % diets recorded significantly higher body weight gain.

Okonkwo *et al*., 2010 assessed the replacement value of concentrate with Cassava Leaf Meal (CLM) in the diet of growing rabbits on the feed quality and growth parameters - the Voluntary Feed Intake (VFI), Average Daily Weight Gain (ADWG), Feed Conversion Ratio (FCR) and digestibility of various nutrients. The authors reported that ADWG decreased with increasing level of cassava leaf meal except that 15% inclusion was better than 0%

inclusion. The FCR range of 3.13 - 5.29 obtained in the study showed a very good performance of the rabbits. It was observed that higher levels of CLM inclusion resulted in a significant decrease in the values of all the parameters studied.

Ayssiwede *et al.* (2011) assessed the effects of *M. oleifera* leaf meal inclusion in diets on growth performances, carcass and organs characteristics and economic result of growing indigenous Senegal chickens using four (4) dietary treatments containing respectively 0, 8, 16 and 24 % of *Moringa* leaves meal in substitution of groundnut cake meal. At the end of the 12 weeks trial, it was reported that the *Moringa* leaves meal inclusion in the diets up to 24% had no negative impact on live body weight, average daily weight gain, feed conversion ratio, carcass and organs characteristics, health and mortality rate in birds compared to their control. There was however significant decrease in daily feed intake obtained for birds on MO16 and MO24 treatments, significantly better growth performances, feed costs and economic margins were recorded in birds fed MO8 and MO16 diets. Thus, these two dietary treatments were the only most economically profitable compared to the control, affirmed that these leguminous leaf in the diets of village chickens is a real opportunity for traditional stockholders to improve at lower cost, not only the productivity and nutritional status of their birds but also their income.

Djakalia *et al.* (2011) studied the effect of *M. oleifera* on the growth performance and health status of young post-weaning rabbits and used three feeding supplements (Moringa, mixed-M and standard feeding SF formulations) different in their composition. The best results were obtained with Moringa supplement. The highest rabbits' weight average was given by the Moringa supplement 820.62 g compared to 658.78 and 632.75 g from other supplements. The growth rates were 126.19, 69.85 and 68.66 g week^{-1} respectively. The apparent faecal

digestibility (85%) compared to the mixed and standard feeding formulation that were respectively 80 and 81 %. This index showed a high protein digestibility. However, the digestibility of different fiber components such as Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were low regardless of the feeding formulation used. In terms of mortality, the mixed feeding formulation gave the highest mortality rate (12%) compared to the moringa and the standard fed rabbit groups (4%). No difference was observed in the feed consumption index.

Increasing the level of *Zornia glochidiata* in rabbit diet beyond 10% decreased feed intake, live weight gain and influenced blood parameters (Ogunsan *et al*., 2011) Abalaka *et al.* (2012) evaluated the antibacterial activity of leaf extracts of *M. oleifera* and reported that the extract was active against *E. coli* and *S. typhi*. The result of phytochemical screening of samples revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins and tannins. Abdu *et al*. (2012) evaluated the effect of inclusion levels of carrot leaf meal in concentrate diet on the performance of growing rabbits and reported its significant effect on weight gains, feed intake and feed to gain ratios.

Onyekaba *et al*. (2013) investigated the ethanol leaves extract of *M. oleifera* for antibacterial activity using phytochemical analysis, solvent extraction and TLC analysis. The results of the preliminary phytochemical screening revealed the presence of saponins, condensed tannins, flavonoids, terpenoids, steroids, phenolics, alkaloids, phlobatannins, cardiac glycosides and reducing sugars. The TLC separation of the phytoconstituents using chloroform-methanol solvent system resolved the fractionated extract into compounds. The antibacterial assay results portrayed broad activity spectrum against the test microbes with

comparable inhibitory zones by standard antibiotics. The research showed the antibacterial potentials of M. oleifera implying that the extract could serve as a chemotherapeutic agent.

Rabbits fed 10% Bamboo Leaf Meal (BLM) had improved final live weight, weight gain and apparent ether extract digestibility while those fed 20 and 30 % BLM had reduced final live weight, weight gain and ash digestibility. Idowu *et al*. (2013) concluded that feeding rabbit 10% BLM will improve growth and nutrient digestibility of weaner rabbits. Teteh *et al*. (2013) in search of an alternative for antibiotic as growth promoter utilized plants like *M. oleifera* in chicks diet. Chicks on moringa diet grew better with higher feed conversion ratio than chicks on the control diet. The same trend was observed with relative organ weights, thus concluded that *M. oleifera* leaves incorporated at 1 and 2 % in feed can improve growth. The lack of significant difference observed in chicks fed 1 and 2 % moringa compared to diet 3 could be attributed to the high anti-nutrients content especially saponins in diet 3 which may impair the digestion and absorption of nutrients especially lipids.

Weight gain, feed intake, feed conversion and protein efficiency ratio of broilers fed 0, 2.5, 5.0 and 7.5 % *M. oleifera* leaf meal were not significantly different among the treatments. Also, carcass characteristics were not altered among the treatments in a study reported by Onu *et al*. (2014).

2.7.2 Effect of leaf meal on haematology and serum biochemistry of farm animals.

Blood consist of several components that can be used to monitor health status and metabolic activities of farm animals. It reflects the effects of dietary treatments on the animals in terms of the type and amount of feed ingested and available for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola *et al.*, 2004). This is relevant since changes in blood constituents relate to the physiological conditions of the animals.

Haematological studies are important because the blood is the major transport system in the body, and evaluation of the haematological profile usually furnishes vital information on the body's response to injury of all forms, including toxicity (Ihedioha *et al*., 2004). Serum biochemical analysis is used to determine the level of heart, liver and kidney functions as well as to evaluate protein quality utilization and amino acid levels in animals. Normally, Alanine amino Transferase (ALT) is found inside liver cells. However, if the liver is inflamed or injured, ALT is released into the extracellular matrix. Measuring ALT level in the blood gives information about the functional state of the liver and whether a disease, drug, or other problem is affecting it.

Ara *et al*. (2008) investigated the effects of *M. oleifera* on serum cholesterol level, serum triglyceride level, blood glucose level, heart weight and body weight of adrenaline induced rats in a crossover design. The *M. oleifera* leaf extract had significant effect on cardiovascular parameters. The study revealed that the leaf extract of *M. oleifera* with atenolol had profound hypolipidemic activity. Lowering of blood glucose, heart weight, and body weight in adrenaline induced rats was significant. The lowering of serum triglyceride level and serum cholesterol level between leaves extract of *M. oleifera* and atenolol in adrenaline induced rats was very significant.

Nuhu (2010) reported from a study on effect of the moringa leaf meal (MOLM) on nutrient digestibility, growth, carcass characteristics and haematological and biochemical indices of weaner rabbits. All the blood parameters studied did not vary between treatments. The result obtained also showed that the MOLM could be used as a partial or total replacement for SBM without any adverse effect on the productive performance and blood indices of weaner rabbits. Onu and Aniebo (2011) reported significant differences in packed cell volume, red blood cell and total protein of the broiler chicken fed dietary *M. oleifera*. The haemoglobin

(Hb), MCV, MCH and MCHC counts and serum albumin and globulin showed no significant difference among the treatments.

Ewuola *et al*. (2012) assessed the haematological and serum biochemical response of crossbred rabbits fed graded level of *M. oleifera* leaf meal (MOLM) in replacement of soybean meal. Results obtained showed that haematological parameters were similar among their four experimental diets, except neutrophils which was significantly higher in control rabbits than others. The serum total protein, albumin and globulin values of rabbits fed MOLM-based diets were not significantly different from those fed the control diet. Enzyme activities examined in rabbits fed 5, 10 and 15 % MOLM were not significantly different from those rabbits fed the control diet. However, the values obtained from the study were within the normal range of healthy rabbits and concluded that feeding MOLM up to 15% inclusion in rabbit diet will not have adverse effect on the biochemical response of the growing rabbits.

Zanu (2012) in a six-week feeding trial involving 180, 2-week old Cobb broiler chicks assessed the effects of partial replacement of fishmeal with *M. oleifera* leaf meal on broiler chickens. Final weight, weight gain and feed conversion efficiency significantly declined with increasing level of MoLM. Mean Corpuscular Haemoglobin (MCH) was the only haematological parameter that showed significance difference in treatment groups. The author also reported that triglycerides, Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) differed significantly. Incorporation of MoLM significantly affected the moisture, crude protein and crude fat content of the meat of experimental birds. Based on findings from the study it was concluded that *M. oleifera* leaf meal when partially

used to replace fishmeal may hamper growth rate of broiler chickens, addition of MoLM does not adversely affect mortality, carcass traits and blood variables.

Garba and Abubakar (2012) investigated the effect of graded levels of tamarind leaves (*Tamarindus indica*) based diets on the haematological and serum biochemical parameters of Yankasa rams. The results revealed no significant difference in packed cell volume, red blood cell, haemoglobin, urea, total proteins, creatinine and the serum electrolytes between control and study animals. However, significant increases were obtained in the globulin and cholesterol levels with increase in *T. indica* leaves.

Ajayi and Raji (2012) assessed the effects of feeding graded levels of blood/wild sunflower forage meal mixture (BWSFM) on haematological and serum biochemical parameters in rabbits. The results showed that though the final weight of the rabbits in the different groups were not significantly affected by the inclusion of the test ingredient, the packed cell volume (PCV), haemoglobin content (Hb), red blood cell (RBC) count, white blood cell (WBC) count, as well as the lymphocytes and serum alanine amino transferase (ALT) were significantly affected by the treatments. It was concluded that inclusion of blood/wild sunflower forage meal mixture up to 15% was well tolerated by pre-pubertal male rabbits without any adverse health condition.

Ozovehe (2013) examined the utilization, haematological and biochemical enzymes of *Clarias gariepinus* juveniles fed varying levels of *M. oleifera* leaf meal diets for a period of 8 weeks, where fish meal was substituted at 0 (control), 10, 20, 30, 40 and 50 % in the six different diets. Fishes fed the control diet significantly different from fishes fed 10 and 20 % *M. oleifera* leaf meal diet in terms of mean weight gain, specific growth rate and feed conversion ratio. The haematological parameters results showed that the mean values of

packed cell volume, red blood cell and haemoglobin decreased as M. oleifera leaf meal increased in the diet. Serum ALT, AST and Alkaline phosphatase (ALP) in the fishes fed diets containing 0, 10 and 20 % *M. oleifera* leaf meal were not statistically significant. The study showed that *M. oleifera* leaf meal has good potential for use as fish meal substitute in *C. gariepinus* diet up to 10% level without compromising growth. The toxicological investigation indicated that at above 20% *M. oleifera* leaf meal in the diet increased serum enzymes suggesting cellular damage.

Odetola *et al*. (2012) conducted an experiment to assess the performance, haematology, serum biochemistry, carcass and organ weights of growing rabbits fed graded levels of dried *Moringa oleifera* leaf meal (MOLM) of 0, 5,10, and 15 %, as a replacement for soya bean meal (SBM) in a 10-week feeding trial. Results showed that there were significant differences in the values obtained for feed conversion ratio and white blood cell counts. The values obtained for loin, hind limb, spleen, lungs and heart weights were significantly different among the treatments while there was no significant difference among the blood constants (MCV, MCH and MCHC). Among the leukocyte differential counts examined, lymphocytes, monocytes and eosinophils were not significantly different among the dietary treatments. Serum proteins were also not significantly affected by the dietary treatments. Therefore, results of the study suggested that MOLM possesses good dietary protein quality for optimal growth of rabbits and can be incorporated in rabbit's diets up to 15% inclusion level without any detrimental effects on the performance, haematology, serum biochemistry, carcass and organ weights of growing rabbits.

Aderinola *et al*. (2012) evaluated the use of *M. oleifera* leaf meal (MOLM) as a feed supplement at five varying inclusion levels (0, 0.5, 1.0, 1.5 and 2.0 %) in broiler chicken.

Broilers fed the control diet had higher total weight gain and feed conversion ratio than those on MOLM based diets. Haematological parameters were significantly reduced, though still within the normal ranges recommended. Among serum parameters, only triglyceride and cholesterol significantly decreased as the inclusion level increased. Organoleptic properties and proximate compositions of the meat samples were significant but did not follow a definite trend except fat content which decreased as the inclusion levels increased. The utilization of MOLM in broiler diet as a supplement could be adopted when the motive is production of broiler meat with low fat content or deposit.

Obun *et al*. (2013) assessed the effects of feeding graded levels (0, 5, 10, 15 and 20 %) of Neem leaf meals (NM) to broiler chicks on live weights, carcass and organ weights and blood constituents. The values of the live weights, carcass and organ weights decreased with increased NM inclusion in the diets. Packed cell volume was significantly altered across the treatment while only the serum biochemical indices of birds fed 20 % NM diets decreased compared with those fed control, 5, 10 and 15 % NM diets. Inclusion of 15% NM in broiler chicks' diet had no adverse effects on live, carcass and organ weights and immunity responses. The study suggested that neem needs further treatment to improve inclusion levels beyond 15% in broilers diets.

Ologhobo *et al.* (2013) assessed the effects of *M. oleifera* leaf meal on the haematological parameters and serum biochemical profile of broiler finishers in comparison with oxytetracycline. The experimental diets contained 250g of oxytetracycline per 100kg of feed for treatment 1 (T1), 200, 400 and 600 g of *M. oleifera* leaf meal per 100kg of feed for treatments 2 (T2), 3 (T3) and 4 (T4) respectively. Their results revealed that there was no significant difference across the treatments for most of the parameters measured. Red blood

cell (RBC) counts for birds fed T4 were significantly higher than those fed the control diet and also had the highest aspartate amino transferase (AST) mean value (72.18%) which was significantly higher than the mean value of those fed the control diet (52.26%). It was concluded from the result of the study that Moringa could be used as alternative antibiotic in place of oxytetracycline, suggesting that *M. oleifera* leaf has antimicrobial properties.

In an experiment to investigate the effect of *M. oleifera* leaf meal (MOLM) on haematology and serum biochemical parameters of weaner rabbits, Ahemen *et al*. (2013) reported significant effect of diet on only haemoglobin (Hb) concentration whose values were however within the normal range for healthy rabbits. No significant influence of diet was observed on all the serum biochemical parameters studied. The authors concluded that inclusion of *M. oleifera* leaf meal in the diets of weaner rabbits up to 15% had no adverse effect on blood profile of rabbits.

Ugwu *et al*. (2013) reported the ameliorative properties of ethanol leaf extract of *M. oleifera* (MO) on the malaria infected liver and kidney injuries of mice. Groups 1 (positive control) and 6 (negative control) were treated with 5mg/kg body weight of distilled water, group 5 (standard control) was treated with 5mg/kg body weight of artesunate while groups 2 ,3 and 4 were treated with 45, 90 and 180 mg/kg body weight of ethanolic extract of MO leaf. The results showed that serum creatinine increased significantly in group 1 compared to group 6 and other groups. Group 6 showed a non-significant difference in serum urea levels compared to group 1 and other groups. Total bilirubin (TB) increased significantly in group 1 and 2 compared to group 6 and other groups. Also, ALT significantly increased in group 1 and group 2 when compared to group 6. Group 6 showed no significant difference in aspartate aminotransferase compared to group 1 and other groups. Alkaline phosphatase

activity of mice significantly increased in groups 1 and 4 compared to group 6 and other groups.

Etim *et al.* (2014) reviewed the effects of nutrition on haematology of rabbits and stated that the physiology of farm animals is influenced by several factors, one of which is nutrition. Changes in haematological parameters are often used to determine stresses due to nutrition and emphasised that reports by different researchers indicated that different diets fed to rabbits had different effects on haematological parameters some of which were detrimental while others improved their haematological indices as they remained within the normal ranges of values for rabbits.

2.7.3 Effect of leaf meal on hormones and reproductive performance of farm animals

Reproductive efficiency in rabbit, like other livestock, is determined by a number of factors such as ovulation rate, conception rate, embryonic mortality and rebreeding intervals (Patridge *et al*., 1984). In bucks (male rabbits), poor reproductive performance in terms of low sperm production can be linked to poor health status, low libido, reduced spermatogenesis and bad semen qualities (Farrell *et al*., 1993). In does (female rabbit), poor reproductive performance can result from reduced ovulation, low conception rate, low embryo survival, poor mothering ability and short rebreeding interval (Patridge *et al*. 1984; Mmereole, 2009).

Umesiobi *et al*. (2000) reported that parameters for measuring reproductive performance in female animals include conception rate, litter size, milk production and fecundity and that of the males could be measured using the sperm concentration, sperm motility, live/dead spermatozoa and proportion of morphologically deformed sperms. Semen is a mixture of spermatozoa produced by testicles and seminal plasma secreted at different sites by

accessories glands and by the epididymis. Semen with reduced or abnormal sperm cells is not beneficial to reproduction.

The interval between parturition and re-mating determines the number of offspring per doe per annum and it makes a great difference in the total profit a farmer makes per annum. Factors such as season, parity, age and weight of females also influence the reproductive efficiency of rabbits. The influence of nutrition on reproductive performance cannot be overemphasized. El- Masry *et al*. (2012) reported that selenium and vitamin E supplementation increased blood testosterone, total sperm concentration, semen volume, total live sperm concentration and sperm motility.

Cajuday and Poscidio (2010) reported that administration of the hexane fraction of *Moringa oleifera* leaf enhanced development of seminiferous tubule, epididymides, testis and seminal vesicle in rats. Testosterone is essential for the development of male sexual characteristics and spermatogenesis. Levels of the hormone above or below normal will influence sperm cells production. Estrogen concentrations are commonly used to assess follicular growth and steroidogenic capacity. The lower levels of hormone in the animals may reveal impaired ovarian activity, which could result in reduced reproductive efficiency (Marongui and Dimauro, 2013).

Among several factors, there is a relationship between nutrition and reproduction. Nutrition may influence reproductive performance by a number of mechanisms, including central effects on gonadotropin secretion (Booth *et al.,* 1994) and local effects on ovarian and testicular functions. Gary and Kristen (1969) reported that copulation and HCG injection produced significant increase in plasma testosterone while ACTH administration failed to do

so. Rabbits treated with either chloropromazine or fluoxymestrone before copulation did not increase plasma levels of testosterone following coitus (Prabsattroo *et al*., 2012).

Inclusion of *Moringa oleifera* foliage as a protein supplement to low quality diets improved dry matter intake from 8.5 to 10.2 and 11.0 kgDM/day and digestibility of the diet and increased milk production from 3.1 to 4.9 and 5.1 kg/day but did not affect milk composition (Sanchez *et al*. 2005). Herbert *et al*. (2005) placed rabbit bucks on one of three diets containing leucaena (LLM), gliricidia (GLM) leaf meals both included at 20% of dry matter or a control diet. Semen volume and spermatozoa concentration values for the control group were higher than the values for the LLM and GLM groups. Seminiferous tubular diameters were significantly wider in the control group than in the other two groups, which were similar. Mild degenerations of seminiferous tubules were observed in some samples from the leucaena and gliricidia groups. Results from the study indicated that the inclusion of leucaena and gliricidia leaf meals at 20% in rations for mature rabbit bucks could cause mild depressive effects on semen production and quality.

Bitto *et al*. (2006) observed similarities between diets for all the parameters evaluated from feeding dietary pawpaw meal suggesting that its inclusion up to 30% level in rabbit diet may support normal body functions and the physiology of reproduction in female rabbits. In a 20-week feeding trial, Odeyinka *et al*. (2008) evaluated the reproductive performance of rabbit does fed *M. oleifera* as a replacement for freshly harvested *Centrosema pubescens* offered to the animals at 2% of their live weights at the ratios of 100:0 (M0), 75:25 (M25), 50:50 (M50), 25:75 (M75) and 0:100 (M100), respectively in addition to the concentrate feed offered to the animals. There were significant differences in the total DM intake of does on the different treatments. However, there was no significant difference in CP intake, the

initial average body weight and gestation length of the does on the different treatments as well as in the litter weight at birth among the treatments. Significant differences were observed in litter size at weaning, average daily weight gain per kid and in milk yield across all the treatments. The authors concluded that *M. oleifera* can be used to replace *C. pubescens* without adverse effect on the reproductive performance of rabbits.

Ogbuewu *et al*. (2009) reported an experiment to ascertain the effects of Neem leaf meal (NLM) (*Azadirachta indica A. Juss*) on semen production and semen quality that rabbit bucks fed graded levels of NLM had reduced sperm concentration for all the groups. Sperm motility was lowest in the treatment groups than the control group. Total sperm per ejaculate was similar between the control and those on 5 and 10 % NLM dietary groups. However, total sperm value for the 15% NLM group was significantly lower than that of the control group. Abnormal sperm percentage of the bucks fed 15% NLM was significantly higher than those bucks on groups 1 to 3. The seminiferous tubule diameters were significantly smaller in the 15% NLM than those on the other 3 dietary groups. All the other variables measured including semen volume, weight of testis and reaction time did not differ among the experimental group. The authors concluded that the inclusion of neem leaf meal up to 15% in the ration of matured rabbit bucks could cause mild depression of spermatogenesis, semen quality and seminiferous tubule diameter.

Cajuday and Poscidio (2010) in a 21-day trial, assessed the effects of *M. oleifera* on the reproduction in male mice at daily doses of 0.5, 5 and 50 mg/30 g BW. Weights of testis (at medium and high doses); epididymis (at all doses); seminal vesicle (at the high dose), seminiferous tubule diameter (at all doses); thickness of epididymal wall (at medium and high doses); higher score for lumen formation (at the high dose) and epididymal maturity (at

all doses) were significantly influenced by *M. oleifera*. No significant effect on the levels of the hormones was observed. The hexane fraction obtained from the leaves of *M. oleifera* did not affect serum FSH and LH levels but enhanced seminiferous tubule, epididymis, testis and seminal vesicle.

Sethi *et al*. (2010) studied the effect of fresh leaves of *Ocimum sanctum* (OS) on male reproductive function (sperm count and reproductive hormones) of buck. Animals in the test group received supplementation of 2 g of fresh leaves of OS per rabbit for 30 days, while the control group was maintained on normal diet for the same duration. A significant decrease was noted in the sperm count in test group rabbits. Serum testosterone levels increased while FSH and LH levels were significantly reduced in rabbits. The authors explained that this pattern of changes in hormone levels could be that tulsi leaves probably contain some androgenic analogue, which increased the circulating testosterone levels sufficiently to inhibit LH but not sufficient to accumulate in the testis at the required concentration for normal spermatogenesis. From their findings they concluded that OS has potential as an effective male contraceptive agent.

Iwuji and Herbert (2012) studied the effect of diets containing T1 (0), T2 (2.5) and T3 (5) % *Garcinia kola* seed meal (GSM) on semen and libido of 36 matured rabbit bucks was investigated in a 3-month experiment. Total sperm count was significantly higher in rabbits fed 2.5 and 5.0 % GSM ($111.33 \pm 1.86 \times 10^6$, $115.67 \pm 33 \times 10^6$) than in those on the control ($104.67 \pm 2.73 \times 10^6$). Also sperm motility had a similar trend with T2 (76.33±0.88 %) and T3 (73.33±0.88 %) having higher values than T1 (64.33±1.86 %). Percent live sperm were significantly higher in T2 than in other treatments. Reaction time recorded a dose-dependent difference and were highly significant among the treatments, T1 = 27.57±1.90s, T2 =

20.75±1.38s and T3 = 11.0±0.38s. The results of the study indicated that *G. kola* seed meal improved semen characteristics and sexual drive (libido) in matured rabbit bucks.

Prabsattroo *et al*. (2012) in search for a novel agent that would be effective, cheap and easy to approach for treating male sexual dysfunction administered moringa extract to rats. The results showed that after single administration, rats subjected to *M.oleifera* extract at a dose of 10mg/kg BW had significantly enhanced libido score. When the treatment was prolonged to 7 days rats subjected to the low dose of extract showed the enhanced intromission number whereas rats subjected to high dose of extract showed the enhanced libido score. No significant change in serum testosterone level was observed. The report concluded that *M. oleifera* could be a potential sexual enhancer particularly for acute and short term application.

Ola *et al*. (2012) compared the sexual receptivity parameters (vulva colour, mating duration and, copulation rate), conception rate, litter performance and productivity of rabbit does fed *Moringa oleifera*, *Tephrosia candida* and *Cajanus cajan* to those fed the conventional *Centrosema pubescens* or sole concentrate diets. Purple was the most exhibited vulva colour in all the does fed the different forages as well as the control group fed only the concentrate diet, while reddish vulva was the least observed. Mating duration was faster for does fed Moringa and concentrate diets. The authors reported that overall productivity was significantly higher for the control group (4.8 born alive/mating), moderate for does fed Moringa or Tephrosia (3.7 and 3.4 born alive/mating, respectively) and lower with the Cajanus or Centrosema diet (2.5 and 2.9 respectively). Thus for dry season, feeding of rabbit under their experimental conditions, Moringa or Tephrosia forages supplemented with concentrate diet seemed to be an interesting option.

Amao *et al*. (2013) observed significant reduction in testes weight, paired testes weight, relative testes weight, testes volume, testes density and daily sperm production from bucks fed both 10% treated and untreated neem rind when compared to those on the control diet. Zade *et al*. (2013) evaluated the effect of the aqueous extract of *Moringa oleifera* seed on reproductive abilities of male albino rats studying their general mating behaviour and libido activity. Oral administration of aqueous extract at doses of 100, 200 and 500 mg/kg significantly increased the mounting frequency, intromission frequency and ejaculation latency with reduction in mounting latency, intromission latency and post ejaculatory interval. It also significantly increased the libido and sperm count in experimental animal. The extract was also observed to be devoid of any adverse effects and acute toxicity. The results of the study demonstrated that aqueous extract of *M. oleifera* seed enhanced sexual behaviour in male rats. It also provided a rationale for the traditional use of *M.oleifera* as acclaimed aphrodisiac and for the management of male sexual disorders.

Cryptorchidism has been known to cause oxidative stress. Afolabi *et al*. (2013) studied the effects of methanolic extract of *M. oleifera* leaves (MEMO) on semen and biochemical parameters in cryptorchid rats. MEMO had no significant effect on testicular weight and malondialdehyde (MDA) concentration while it significantly increased sperm count, germ cell count, testicular parameters and total protein in the cryptorchid rats. The study suggested that MEMO ameliorates cryptorchidism-associated germ cell loss and oxidative stress. Ahemen *et al.* (2013) in an experiment to determine the effect of feeding varying dietary levels of water spinach (*Ipomoea aquatica*) leaf meal on reproductive parameters and organ weights of male rabbits, observed no significant effect of the diet on testicular morphometry and sperm characteristics. This result showed that water spinach meal can be

included up to 15% in rabbit diets without adverse effect on testicular morphometry and sperm quality of male rabbits.

Abu *et al.* (2013) evaluated the effect of *M. oleifera* leaf meal (MOLM) on testicular morphometry and sperm quality. No significant effect of the diets was observed on all the parameters studied. The results showed that MOLM had no adverse effect on the testicular morphometry and epididymal sperm quality of rabbit bucks at inclusion levels of up to 15% and suggested that *M. oleifera* leaf could be used in rabbit diet.

Ebong *et al*. (2014) evaluated the protective effect of *Moringa oleifera* (MO) and *Ocimum gratissimum* (OG) on diabetic rat testes**.** Results from the study showed that only the *Moringa* extract normalized the levels of testosterone, LH and FSH compared with Diabetic Control. Treatment with *Moringa* significantly reversed the levels of the three sex hormones towards normal values. Values obtained were comparable or even better than the reversals by insulin. The OG extract had no effect on the level of the three sex hormones but provided a potentiating effect on the FSH level in the MO + OG group. The results confirmed by histological studies which showed damage on the testes for the DC and OG and reversal of damage to the testes in MO and MO + OG groups. It was concluded that combined extracts more than *Moringa* extract alone, had ameliorative effects on testicular architecture and spermatogenesis in diabetic rats and provided a cheap alternative to treating diabetic associated testicular damage and sexual dysfunction.

Olaniyi and Adeniji (2014) investigated the effect of *Moringa oleifera* leaf meal on reproductive performance (fecundity, hatchability and sperm motility) of African catfish. The fishes were fed with diets containing four different inclusion levels of *M. oleifera* 0, 5, 10 and 15 % for seven weeks. An inverse relationship were observed between the weight

change of the fish and Moringa levels in the diets while number of eggs and fries released had direct relationship with Moringa inclusion level. The fishes were defatted and the weight drastically reduced significantly particularly in groups 2, 3 and 4. The numbers of eggs and fries released by fishes on diets 1, 2, 3 and 4 respectively increased significantly as the level of Moringa in the diets increased. The milts were observed under the microscope for sperm count, % motility and % life. Progressive motility for treatments 1 and 2 were good, forward, directional movement and treatment 3 (Fair, forward, directional movement). The milt for treatment 4 did not show a consistent motility. It was concluded that *M. oleifera* leaf meal above 5% had negative impact on the milt but influenced eggs of the fish positively, higher fecundity and hatchability in female African cat fish.

2.7.4 Effect of leaf meals on organ weight and development in farm animals

The efficiency of feed utilization can be expressed in organ development in terms of weight and functions. A reduction or increase in the size of an organ may be an indication of injury or diseased condition. Understanding the normal structure and function of different tissues is essential for interpreting the changes that occur during disease (William, 2003; Ewuola, 2009). It also shows how to identify a number of tissues and interpret the changes that occur in disease. Histopathology refers to the microscopic examination of tissue in order to study the manifestation of disease or damage in the tissue. Testes and epididymides are expected to still contain certain quantity of sperm cell after an animal has been slaughtered since this organs serve as the production and storage sites of spermatozoa (Holtz and Forte, 1978).

Borin *et al*. (2006) assessed growing indigenous Cambodian chickens, ducks, broiler chickens and White Pekin ducks fed diets containing 0, 7, 14 and 20 % of cassava leaf meal (CLM) to study the effects of CLM level on diet digestibility and gastrointestinal tract (GIT)

and organ development. Weights of small intestine, caeca, gizzard and pancreas (expressed as per kg body weight) increased with increasing CLM in the diet. There was no consistent dietary effect on liver weight. Length of small intestine and caeca (expressed on a mass-specific basis) increased with dietary CLM content. When expressed as per kg body weight small intestine, proventriculus, gizzard, pancreas and liver weights, and small intestine length, were higher in ducks than in chickens and were higher in the indigenous than in the improved breeds except for small intestine weights which were similar. However, chicken had higher weight of caeca and colon in absolute units and per kg body weight.

Ihueukwumere *et al.* (2008) evaluated the performance, nutrient utilization and organ characteristics of broilers fed cassava leaf meal at dietary levels of 0, 5, 10 and 15 % respectively. Feed intake, body weight gain, feed conversion ratio and organ weight of birds on the control (0%) and 5% leaf meals were significantly superior to the birds on 10% and 15% leaf meal. The utilization of dry matter, crude protein, ether extract and ash was significantly poorer at the 10 and 15 % dietary levels. The organ weights (heart, liver, gizzard) were superior at 0 and 5 % groups to the groups on 10 and 15 % inclusion levels of the leaf meal. The result of the study suggested that 5 % inclusion of cassava leaf meal could be used in broiler finisher diets without any deleterious effects on organ weights.

Amata and Bratte (2008) reported that graded level of *Gliricidia sepium* leaf meal had no significant effect on body weight gain, feed intake and feed conversion ratio of rabbits. Heart and lungs weights were not affected by the dietary treatment but kidney and liver weights were significantly higher in 15 and 20 % Gliricidia group compared with the control. The authors concluded from their findings that there was increased detoxification activity of the liver and kidney when rabbits were fed gliricidia beyond 10%. Ogbuewu *et al.*

(2010) assessed the effect of neem leaf meal on organs of rabbit. Non-significant increase in some organ weights were observed in the study as the inclusion levels of NLM increased indicating a higher physiological activity of the liver.

Duruna *et al.* (2011) in a 28-day feeding trial, evaluated the effect of bitter leaf (Vernonia amygdalina) leaf meal as feed ingredient on the performance, feed cost and carcass and organ weights of finisher broilers. The leaf meal was used at 0, 5, 10 and 15 % inclusion levels in the diets. There were significant differences in final body weight, body weight gain, feed conversion ratio and carcass weights between the birds on the 0% inclusion level of the leaf meal and the rest of the groups and between the birds on 5% and the birds on 10 and 15 % inclusion levels. No significant difference was observed in the organ weights. Abdominal fat weight, cost of feed production per kilogram price and profit made decreased significantly as the inclusion levels of the leaf meal increased across the groups. The results of the study suggested that dietary inclusion of *Vernonia amygdalina* leaf meal in broiler finisher diet at a level not exceeding 10% did not have any adverse effect on performance and organ weights.

Isitua and Ibeh (2013) reported the effects of aqueous extracts of the leaves of *Moringa oleifera* and *Caulis bambusae* on the heamatological parameters, selected liver enzymes, insulin level and body weights of rabbits. There were significant increases in CD4 cells, lymphocytes and a decrease in neutrophils. There was an enhancement in the activities of serum enzymes in rabbits exposed to 2.5ml of the extract. There was no significant difference in the histology of major organs, weights and the physical and behavioural pattern of rabbits on extracts and control treatments.

CHAPTER THREE

MATERIALS AND METHODS

STUDY 1: GROWTH PERFORMANCE OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

3.1. Experimental site

This study was carried out in the Rabbitry Unit of Teaching and Research Farm, University of Ibadan, Oyo State. It is situated in south-western agro-ecological zone of Nigeria. Ibadan is about 200m above sea level and the climatic data of Ibadan as summarized in Ministry of Lands and Survey Atlas (2014) of Oyo State is as follows: Mean annual rainfall of 1420mm; temperature range of 21.42 – 26.46^{o}C and mean relative humidity range of 74.55% (Wikipedia, 2014).

3.2 Source and preparation of *Moringa oleifera* leaf meal

Moringa oleifera leaf used for this experiment was harvested from an established orchard within Ibadan metropolis of Oyo State. Leaves were harvested after 90 days of growth and the total fresh weight determined. Dry matter (DM) weight was determined from the fresh weight after oven drying. The *Moringa oleifera* leaf was air-dried under shade for 3- 5 days until it was crispy to touch while retaining their greenish colouration. The leaves was milled and stored in air tight containers until incorporation into the diet. Samples of the air-dried leaves was taken to the laboratory for proximate analysis to determine crude protein, crude fibre, ether extract, nitrogen free extract using standard procedures of the Association of Official Analytical Chemists (AOAC, 1990).

3.3 Sources of concentrate

The concentrate feed ingredients used for the experiment was purchased from a commercial feed supplier in Ibadan, Nigeria.

3.4 Experimental animals and management

Sixty (60) growing New Zealand white × Chinchilla crossbred rabbits of 8-10 weeks of age (5 bucks and 10 does) were used for the experiment. The animals were sourced from the Institute of Agricultural Reasearch and Training, Moor Plantation, Ibadan. The rabbits were subjected to a 2 weeks acclimatization period. Ivomec® injection was administered to treat each animal for endo- and ecto- parasites. The experimental animals were housed in wooden hutches with wire mesh raised from the floor. All experimental animals were subjected to the same housing and management conditions.

3.5 Experimental diet

Four experimental diets were formulated. Diet 1(T1) without *Moringa oleifera* leaf meal served as the control diet (Table 5) and Diets 2 (T2); 3 (T3) and 4 (T4) contained *M. oleifera* leaf meal (MoLM) at 2.5, 5.0 and 7.5 % inclusion levels of the total diet respectively (Table 6) as outlined below:

Treatment 1: 100.0% Control diet + 0% MoLM

Treatment 2: 97.5% Control diet + 2.5% MoLM

Treatment 3: 95.0% Control diet + 5.0% MoLM

Treatment 4: 92.5% Control diet + 7.5% MoLM

Table 5: Gross composition of the control diet for growing rabbits fed varied levels of *Moringa oleifera* leaf meal.

INGREDIENTS	QUANTITY (%)
Maize	42.00
Rice husk	25.00
Soyabean meal	20.00
Wheatbran	10.25
Common Salt	0.25
Premix (growers)	0.25
Dicalcium phosphate	2.00
Methionine	0.13
Lysine	0.12
TOTAL	100.00
Calculated Nutrients:	
Crude Protein (%)	16.34
Crude Fibre (%)	10.51
Digestible Energy (Kcal/kg)	2500.59

Table 6: Calculated nutrients in the experimental diets with the varied levels of *Moringa oleifera* leaf meal.

NUTRIENT	T1 100%CD+0% MoLM	T2 97.5%CD+2.5% MoLM	T3 95.0%CD+5.0% MoLM	T4 92.5%CD+7.5% MoLM
Crude Protein (%)	16.34	16.63	16.92	17.21
Crude Fibre (%)	10.51	10.53	10.55	10.57
Digestible Energy (Kcal/kg)	2500.59	2470.47	2440.36	2410.23

CD – Control Diet, MoLM – *Moringa oleifera* Leaf Meal

3.6 Duration of experiment

After balancing for weight and a two (2) weeks adjustment period, the rabbits were randomly assigned to the four dietary groups (5 bucks and 10 does /group) in a feeding trial that lasted eighteen (18) weeks.

3.7 Experimental design

The experimental design is Completely Randomized Design (CRD).

The Statistical Model :-

$$Y_i = \mu + T_{ij} + e_{ij}$$

Where: Y_i = the effect of the j^{th} observation in the i^{th} treatment

μ = general mean of the population

T_i = the effect of the i^{th} treatment where i = 1,2, …..t

e_{ij} = random error associated with the j^{th} observation in the i^{th} treatment

3.8 Growth performance evaluation

All the rabbits were weighed at the beginning of the experiment before they were allocated to the treatments. The experimental diets were offered to the rabbits twice daily (8am and 2pm). Daily feed consumption was recorded and the feed leftover and/or wastage were weighed daily before supplying fresh feed. Fresh clean water was also provided always throughout the experiment. Records of average daily feed intake and weekly body weight gain were taken. The daily feed intake was calculated by deducting feed leftover and waste from the total daily feed supplied. Also, the daily weight gain was calculated as the weekly weight gain divided by 7. Mortality rate was monitored and Feed Conversion Ratio (FCR) was calculated as the ratio of feed intake to body weight gain.

3.9 Statistical analysis

Data obtained from the study were tested using Analysis of variance (ANOVA) for Completely Randomized Design (CRD) according to statistical analysis system (SAS, 2003) at p = 0.05. Differences between means were separated by the Duncan's Multiple Range Test (DMRT) of the same software.

STUDY 2: HAEMATOLOGICAL, SERUM BIOCHEMICAL AND HORMONAL RESPONSES OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

3.10 Experimental animals

Sixty crossbred growing rabbits (New Zealand white × Chinchilla) were used for the experiment and fed the experimental diet on Table 5 of chapter three for 24 weeks.

3.11 Blood collection

Blood was sampled from the rabbits at 18weeks and at the third trimester of the gestation phase. Bleeding and evaluation was done in the morning between 7.00 am and 8.00 am. Blood was taken from their jugular vein with the hypodermic needle and syringe into a set of sterile plastic bottles containing EDTA for haematological test and another into a set of EDTA-free bottles which was allowed to clot. Haematological parameters assessed include Packed Cell Volume (PCV), Haemoglobin (Hb), erythrocyte, leucocyte and leucocyte differential count. The blood serum was decanted and assessed for serum cholesterol, glucose, protein analysis and hormonal assay. Blood and serum parameters were determined as described by Ewuola and Egbunike (2008) and presented below.

3.11.1 Estimation of Packed Cell Volume (PCV)

The blood sample containing the EDTA was mixed gently and drawn up into a micro-haematocrit capillary tube filled up to 3/4 of its length. The samples were spun for 5 minutes at 10,000 rpm and the PCV was read as a percentage using the haematocrit reader.

3.11.2 Haemoglobin concentration determination

Haemoglobin concentration was determined by a cyanmethaemoglobin method using Drabkin's solution as diluent.

3.11.3 Erythrocyte count

Erythrocyte (Red blood cells) were estimated by taking 0.02 ml of the blood sample from the bottle containing EDTA and mixing with 4 ml of diluting fluid (3 g sodium citrate, 1 ml formaldehyde in 100 ml distilled water) by shaking for about half a minute. About a quarter of the content was expelled before filling the haemocytometer counting chamber and allowed to settle by leaving to stand for about a minute after filling. All the red cells were then counted using the x 40 objective lens and x 8 eyepiece of the microscope, with the aid of a counter. RBC total counts were estimated using the formula below:

RBC Total count = RBC counts x 10 x 5 x dilution factor (200)

= RBC counts x 10,000

3.11.4 Leukocytes and leukocyte differential count

Total leukocyte counts were determined using Neubauer haemocytometer after appropriate dilution, and differential leukocyte counts was performed using the oil - immersion objective examination of blood films stained with the modified Romanovsky's Giemsa stain.

3.11.5 Total serum protein determination

Biuret method of serum total protein determination was employed in this assay as described by Kohn and Allen, (1995).

3.11.6 Albumin and globulin determination

Albumin was determined using Bromocresol Green (BCG) method as described by Peter *et al*. (1982). The globulin concentration was obtained by subtracting albumin values from the total protein.

3.11.7 Alanine and Aspartate amino tranferase determination

Alanine and aspartate aminotransferase (ALT and AST) activities were assayed using Randox commercial enzyme kit as described by Reitman and Frankel (1957) and Schmidt and Schmidt (1963).

3.11.8 Glucose and cholesterol determination

Glucose and cholesterol were determined using spectrophotometric method as described by Braham and Trinder (1972) and Allaine (1974) respectively.

3.11.9 Hormonal assay

Blood sample was taken through the jugular vein from each of the five (5) does per treatment into heparinized tubes then centrifuged at 3000 rpm for 15minutes. Serum was separated and stored at -4^{o}C for analyses. Testosterone concentration was determined by Enzyme Linked Immunoassay (ELISA) using commercial kit (Testosterone-RT ref: RH-1712) while oestrogen and progesterone concentrations were determined by ELISA using commercial kit (Rapid lab, UK) according to the manufacturers instruction.

3.11.10 Statistical analysis

All data obtained were subjected to statistical analysis of variance (ANOVA) using Statistical Analytical System (SAS, 2003). Treatment means were compared using Duncan multiple range test of the same software.

STUDY 3: SEMEN CHARATERISTICS, LIBIDO ASSESSMENT AND FERTILITY RATE OF RABBITS FED DIETS SUPPLEMENTED WITH M*oringa oleifera* LEAF MEAL

3.12 Experimental Animals and management

The rabbit bucks used in the growth study were selected for this reproductive assessment. Bucks on the dietary treatments were used for the semen characteristics and libido assessment per treatment. Four experimental diets were formulated. Diet 1(T1) without *Moringa oleifera* leaf meal served as the control diet and Diets 2 (T2); 3 (T3) and 4 (T4) contained *M. oleifera* leaf meal (MoLM) at 2.5, 5.0 and 7.5 % inclusion levels of the total diet respectively (Table 7 and 8) as outlined below:

Treatment 1: 100.0% Control diet + 0% MoLM

Treatment 2: 97.5% Control diet + 2.5% MoLM

Treatment 3: 95.0% Control diet + 5.0% MoLM

Treatment 4: 92.5% Control diet + 7.5% MoLM

3.13 Reaction time and libido score evaluation

A teaser doe was introduced to the buck every week to monitor their sex drive. In this study, reaction time was considered as an indication of libido. This is the time it takes the rabbit

Table 7: Gross composition of experimental (control) diet for rabbit bucks

INGREDIENTS	QUANTITY (%)
Maize	42.00
Rice husk	25.00
Soyabean meal	20.00
Wheatbran	10.25
Common Salt	0.25
Premix	0.25
Dicalcium phosphate	2.00
Methionine	0.13
Lysine	0.12
TOTAL	100.0
Calculated nutrient:	
Digestible Energy (Kcal/kg)	2540.59
Crude Protein (%)	16.34
Crude Fibre (%)	10.51

Table 8: Gross composition of experimental (control) diet for gestating does

INGREDIENTS	QUANTITY (%)
Maize	43.00
Rice husk	24.00
Soyabean meal	30.00
Common Salt	0.25
Premix	0.25
Dicalcium phosphate	2.25
Methionine	0.13
Lysine	0.12
TOTAL	100.0
Calculated nutrients:	
Digestible Energy (Kcal/kg)	2608.43
Crude Protein (%)	17.86
Crude Fibre (%)	10.01

buck to sniff, groom and mount the female, this time was recorded with a stop watch, and libido was scored as the number of times the buck attempts to mount within a minute.

3.14 Semen collection

A 4-week period was used to train the bucks for semen collection. Semen was finally collected from the buck using the artificial vagina (AV) described by Herbert and Adejumo (1995). Prior to semen collection, the AV was warmed for a few minutes in warm water at a temperature slightly above body temperature and thereafter drained. Semen collection was done between 7.00 and 9.00 am to ensure that optimum quality semen were obtained.

3.15. Semen evaluation

The semen was promptly assessed for semen quality parameters such as semen colour, semen volume, mass activity, sperm motility, sperm concentration and percentage live sperm.

3.15.1 Semen volume and colour

The volume of semen collected was measured using the graduated collection tube. The semen colour was noted and recorded immediately after collection.

3.15.2 Mass activity

A drop of undiluted fresh semen was placed on a sterile glass slide and observed under the microscope with an objective lens at a magnification of x10. Mass activity scoring was done using the intensity of the wave length from absence of wave motion (0, 1) to turbulent motion (+++, 4).

3.15.3 Sperm motility

To determine the motility of the sperm cells, a drop of undiluted semen mixed with a drop of slightly warmed diluents (sodium citrate) was placed on a sterile slide, covered with a cover slip and observed under the microscope at (Mag. ×400) and scored within a rating of 0 -100 %.

3.15.4 Live sperm percentage

Live sperm cells determination was done by placing a drop of semen mixed with one drop of eosin negrosin stain on a slide and observed under the microscope. The unstained cells represented the live cells while the stained cells are the dead ones.

3.15.5 Sperm concentration

Sperm concentration/ejaculate was calculated as: sperm concentration per ml × volume of ejaculate. Sperm concentration per ml of semen was evaluated using a visual count under the microscope using improved Neubauer haemocytometer. A small pipette with a dilution ratio of 1:100 was used. The mixture was uniformly mixed and through capillary action allowed to run under the cover slide placed on the haemocytometer. After few minutes, the sperm cells were counted within five squares diagonally from top to bottom in the ruled areas of the haemocytometer chamber. The total count was obtained by adding the counts from the five squares multiplied by the dilution factor, area of the squares counted from and number of squares counted divided by the depth of the haemocytometer.

3.16 Reproductive Response Evaluation

Does fed the four dietary treatments were used for the mating trial. Natural mating was done. Does were presented to a buck fed similar diet minimum of two times to ensure

successful mating at a ratio of 2 does to 1 buck and the following parameters were monitored.

3.16.1 Conception rate (%)

This is the number of animals that conceived per number of animals that were mated multiplied by 100.

3.16.2 Gestation length

This is the number of days the rabbit doe spent from a successful mating to kindling.

3.16.3 Litter size

This is the number of kits born by a doe at single kindling.

3.16.4 Litter weight at birth (g)

This is the average weight of the litter at birth.

3.17. Statistical analysis

All data obtained were subjected to statistical analysis of variance (ANOVA) using Statistical Analytical System (SAS, 2003). Treatment means were compared using Duncan multiple range test of the same software.

STUDY 4: ORGAN WEIGHTS, ORGAN-HISTOPATHOLOGY AND SPERM STORAGE POTENTIAL OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

3.18 Experimental animals and management

Bucks fed the four dietary treatments with inclusion levels of MoLM at 0, 2.5, 5.0 and 7.5 % were used for this experiment. The bucks were housed individually with feed and water provided adequately.

3.19 Organ assessment

Three animals were randomly selected from each treatment group at the end of the 24 weeks feeding trial, weighed, slaughtered and skinned. The head and paws were removed. The animals were eviscerated, all the internal organs and gastro-intestinal tract removed before the dressed weights were taken using sensitive digital scale.

3.20 Organ weights

The direct weight of organs such as heart, liver, kidney, spleen, lungs, adrenal gland, bile and testes were taken using the analytical weighing balance and recorded to the nearest 0.01gram as absolute weight. Paired organs were weighed separately and recorded, then both were added together to obtain the paired weight for the organs. Percentage relative weights of organs were calculated using the formula below:

$$\text{Relative weight of organ} = \frac{\text{Absolute weight of the organ}}{\text{Live weight of the rabbit}} \times 100$$

3.21 Sperm storage assessment

3.21.1 Testicular Sperm Reserve estimation

The left and right testes were homogenized separately in 2ml of normal saline. The suspension was thoroughly and gently mixed then filtered through doubled layer of sterile gauze and analysed immediately. A dilution ratio 1:20 of homogenate to normal saline was done and sperm cells were counted using the haemocytometer. Testicular sperm reserve was estimated as total number of sperm cells present in the testicular tissues homogenate. All sperm cells were expressed in millions.

3.21.2 Epididymal Sperm Reserve estimation

The epididymis was carefully removed from the left and right testes and each separated into the three different regions of epididymis (caput, corpus, cauda). They were homogenized separately in 1ml of normal saline. The homogenate was thoroughly and gently mixed then filtered through doubled layer of sterile gauze and analysed immediately. A dilution ratio 1:2 of homogenate to normal saline was done and sperm cells were counted using the improved Neubar haemocytometer. Epididymal sperm reserve was estimated as total number of sperm cells present in the homogenized tissues. All sperm cells were expressed in millions.

3.22 Organ histopathology

Liver, kidney and ileum were removed, weighed and fixed in 10% formalin solution and processed for histopathological examination at the Department of Veterinary Pathology, University of Ibadan as described by Ewuola (2009).

3.23 Experimental Design

A Completely Randomised Design (CRD) was adopted.

3.24 Statistical analysis

Data obtained on organ weight and sperm reserves were subjected to statistical analysis of variance (ANOVA) procedure using Statistical Analytical System. Means separated using Duncan multiple Range test (SAS, 2003). Histopathological observations were subjected to descriptive statistics (percentage).

CHAPTER FOUR

RESULTS

4.1 GROWTH PERFORMANCE OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

The results of proximate analysis of *Moringa oleifera* leaf meal is shown in Table 9. The MoLM was rich in crude protein (27.92%), ash (11.50%), crude fiber (11.00%) and ether extract (13.00%). The percentage inclusion level and proximate composition of the experimental diets fed to the growing rabbits are presented on Table 10. The crude protein of diet 1 reduced while that of diets 2, 3 and 4 proportionately increased. It was observed from the analyzed composition of the diets that as the level of MoLM inclusion increased, the values of ether extract, crude protein and ash slightly increased as the inclusion level of *M. oleifera* in the diet increased while the level of crude fibre reduced slightly across the dietary treatments.

The growth indices of the rabbits fed the four experimental diets are presented on Table 11. All the rabbits had similar body weights at the start of the experiment. The final live weight, the cumulative and daily weight gains apparently decreased with increasing levels of MoLM, but not statistically significant. The rabbits on diet 4 (7.5%) had the least cumulative weight gain compared to those fed the control diet and MoLM based-diets at 2.5 and 5 %. It was significantly different from the control. Rabbits on diet 2 had a lower daily feed intake compared to those on the control diet, T3 and T4 although there was no significant difference among the treatments. FCR means were not statistically different among the treatments. Mortality reduced as the inclusions level of the Moringa increased, no mortality

Table 9: Proximate composition (%) of *Moringa oleifera* leaf meal (MoLM)

NUTRIENTS	VALUES
Dry matter	88.00
Crude fibre	11.00
Ether extract	13.00
Crude protein	27.92
Ash	11.50
Nitrogen free extract	24.56

Table 10: Proximate composition (g/100g DM) of experimental diets

NUTRIENTS	T1 100%CD+0% MoLM	T2 97.5%CD+2.5% MoLM	T3 95.0%CD+5.0% MoLM	T4 92.5%CD+7.5% MoLM
Dry matter	90.50	90.44	90.38	90.31
Crude fibre	13.65	13.56	13.52	13.36
Ether extract	5.00	5.23	5.41	5.76
Crude protein	16.80	17.08	17.36	17.63
Ash	10.00	10.04	10.08	10.11
Nitrogen Free Extract	45.05	44.53	44.01	43.45

CD – Control Diet, MoLM – *Moringa oleifera* Leaf Meal

Table 11: Growth performance of rabbits fed varied levels of *Moringa oleifera* Leaf Meal

	DIETARY TREATMENTS			
PARAMETERS	**T1 100%CD+0% MoLM**	**T2 97.5%CD+2.5 % MoLM**	**T3 95.0%CD+5.0 % MoLM**	**T4 92.5%CD+7.5 % MoLM**
Initial live weight (g)	1069.08	1069.83	1085.67	1069.25
Final live weight (g)	2028.57±73.10	1911.54±82.49	1947.00±59.54	1916.00±70.65
Cummulative Weight Gain (g)	959.49±72.68	841.71±62.71	861.33±65.68	846.75±63.52
Daily Weight Gain (g)	6.29±0.79^{a}	5.15±0.40^{ab}	5.17±0.49^{ab}	4.35±0.37^{b}
Dry Matter Intake (g/day)	84.72±4.48	79.17±5.00	83.90±5.17	83.76±5.10
FCR (feed/gain)	13.47±3.34	15.37±4.18	16.23±4.57	19.26±5.79
Mortality (%)	13.33	6.67	6.67	0.00

MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet

a, b: Means in the same row with different superscript are significantly ($P< 0.05$) different

was recorded among rabbits fed 7.5% MoLM while the highest was recorded in rabbits on the control diet.

4.2 HAEMATOLOGICAL, SERUM BIOCHEMICAL AND HORMONAL RESPONSES OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

4.2.1 Haematological parameters of growing rabbits

The results on haematological response of growing rabbits fed varied levels of *Moringa oleifera* leaf meal are shown on Table 12. *M. oleifera* leaf meal significantly ($p<0.05$) influenced the haematological parameters. The packed cell volume of rabbits fed 2.5 and 7.5 % MoLM were not significantly different from each other but the PCV of rabbits fed 5% MoLM diet was significantly ($p<0.05$) lower than that of rabbits on the control. The haemoglobin concentration of rabbit on 5% MoLM were significantly ($p<0.05$) lower than those fed the control diets. The erythrocytes of rabbits on diets 1, 2 and 3 were not significantly different from one another but erythrocyte value of rabbits fed diet 2 was significantly lower than that of rabbits fed diet 3. The leukocytes, eosinophils and monocytes counts were not significantly different among the treatments. The value of neutrophils in rabbits fed 2.5% MoLM was not significantly different from rabbits fed 5% MoLM but significantly ($p<0.05$) different from those fed 0 and 7.5 % MoLM.

4.2.2 Haematological parameters of gestating does.

The haematological parameters of gestating does fed varied levels of *Moringa oleifera* leaf meal are shown in Table 13. The packed cell volume of rabbits fed 2.5% MoLM was not significantly different from that of rabbits fed 0 and 5 % MoLM but significantly ($p<0.05$)

Table 12: Haematological response of growing rabbits fed diets with varied inclusion levels of *Moringa oleifera* Leaf Meal

PARAMETERS	T1 100%CD+0% MoLM	T2 97.5%CD+2.5 % MoLM	T3 95.0%CD+5.0 % MoLM	T4 92.5%CD+7. 5% MoLM
Packed Cell Volume (%)	42.83±1.21^{a}	39.67±0.74^{ab}	37.67±0.97^{b}	39.5±1.81^{ab}
Haemoglobin (g/100ml)	13.71±0.54^{a}	12.69±0.34^{ab}	12.05±0.29^{b}	12.64±0.33^{ab}
Erythrocytes (x 10^{6} /l)	7.00±0.27^{ab}	6.08±0.31^{b}	7.12±0.31^{a}	6.47±0.37^{ab}
Leukocytes (x 10^{3} /l)	5.80±0.48	7.00±1.34	5.78±0.78	6.84±0.45
Thrombocytes (x10^{3} /l)	137.17±5.94^{a}	111.83±6.69^{b}	105.33±7.37^{b}	138.00±5.25^{a}
Neutrophils (%)	26.33±1.56^{b}	36.67±3.14^{a}	30.17±2.27^{ab}	23.33±4.62^{b}
Lymphoctyes (%)	67.67±2.39^{ab}	57.00±3.61^{b}	64.17±1.55^{ab}	70.67±5.30^{a}
Eosinophils (%)	3.67±0.55	5.00±0.86	3.67±1.05	4.50±1.23
Monocytes (%)	2.50±0.42	1.33±0.49	2.00±0.36	2.17±0.48

MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet

a, b: Means in the same row with different superscript are significantly (P< 0.05) different

Table 13: Haematological response of gestating does fed varied levels of *Moringa oleifera* Leaf Meal

PARAMETERS	T1 100%CD+0 % MoLM	T2 97.5%CD+2.5 % MoLM	T3 95.0%CD+5.0 % MoLM	T4 92.5%CD+7.5 % MoLM
Packed Cell Volume (%)	37.38±0.79^{ab}	40.17 ±1.64^{a}	37.00± 1.00^{ab}	33.67 ± 1.61^{b}
Haemoglobin (g/100ml)	12.35± 0.31^{a}	13.00± 0.45^{a}	11.84 ± 0.35^{a}	10.57 ± 0.51^{b}
Erythrocytes (x 10^{6} /l)	6.43± 0.25^{ab}	6.79 ± 0.16^{a}	6.08 ± 0.16^{bc}	5.71 ± 0.27^{c}
Leukocytes (x 10^{3} /l)	4.34 ± 0.41	5.01 ± 0.45	5.36 ± 0.50	4.93 ± 0.71
Thrombocytes (x10^{3} /l)	67.50 ± 0.55	74.52 ± 0.80	89.61 ± 0.11	81.83 ± 0.65
Neutrophils (%)	20.50± 1.69	22.33 ± 3.17	18.80 ± 2.76	20.67 ± 2.50
Lymphoctyes (%)	69.33± 1.61	69.33 ± 3.06	70.60 ± 2.58	70.50 ± 1.43
Eosinophils (%)	6.33 ± 0.80	6.67 ± 1.41	8.60 ± 1.99	6.67 ± 1.48
Monocytes (%)	2.33 ± 0.49	1.67 ± 0.42	2.00 ± 0.44	2.00 ± 0.58

MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet

a b c -Means in the same row with different superscript are significantly different (P< 0.05)

higher than that of rabbits fed 7.5% MoLM. It was observed that the PCV of rabbits fed diet 4 was not significantly ($p>0.05$) different from those rabbits fed diets 1 and 3.The haemoglobin concentrations of rabbits on diets 1, 2 and 3 which were statistically similar but significantly ($p<0.05$) different from that of rabbits on diet 4. The erythrocytes of rabbits fed diet 2 was not significantly different from those on the control but significantly ($p<0.05$) higher than those of rabbits fed diets 3 and 4. The leukocytes, thrombocytes, neutrophils, lymphocytes, eosinophils and monocytes were not significantly different among the treatments.

4.2.3 Serum biochemical and enzyme indices for growing rabbits

The serum biochemical parameters and enzyme activities of rabbits fed varied levels of *Moringa oleifera* leaf meal are as shown on Table 14. Serum glucose decreased with increasing inclusion levels of MoLM in the diets. Serum glucose of rabbits fed diets 1, 2 and 3 were not statistically different from one another but were significantly higher than that of rabbits fed diet 4. The serum cholesterol levels was significantly lower in rabbits fed 7.5% MoLM compared to those on 0 (control), 2.5 and 5.0 % MoLM. Total serum protein, albumin, globulin and urea were not significantly different among the treatments. Values obtained for Alanine and Aspartate amino transferase were not significantly influenced by the levels of the MoLM in the diets.

4.2.4 Serum biochemical and enzyme indices for gestating does

The serum biochemical response and enzyme activities of gestating rabbits fed varied levels of *Moringa oleifera* leaf meal are as shown on Table 15. Among the serum parameters assessed in rabbits fed varied levels of *M. oleifera*, only ALT was significantly ($P< 0.05$) influenced by the dietary treatments. Serum glucose apparently decreased at 2.5% inclusion

level of MLM inclusion in the diet but was not statistically different among the treatments. The serum cholesterol level was lower in rabbits fed diets 2, 3 and 4 compared to diet 1 but all were similar statistically. The total serum protein, albumin, globulin and AST values were not significantly different among the treatments. The ALT values obtained in rabbits fed 2.5, 5.0 and 7.5 % MoLM were significantly ($P< 0.05$) higher than those fed the control diet.

4.2.5 Hormonal indices of growing rabbits

The testosterone levels in bucks and oestrogen levels in does fed dietary MoLM are as presented in Figures 5 and 6 respectively. Results of hormonal assay showed increase in the levels of testosterone and oestrogen with increase in dietary levels of MoLM. The serum testosterone levels in male rabbits were not significantly different among the treatments but rabbits on control diet had lower level compared to those on the MoLM diets. Serum levels of estrogen of rabbits does fed 2.5 and 7.5 % MoLM were identical but significantly higher than those fed 0 and 5.0 % MoLM diets.

4.2.6 Hormonal indices of gestating does

Serum progesterone level in rabbits fed varied levels of MoLM are as shown in Figure 7. The progesterone levels was significantly ($p<0.05$) influenced by dietary levels of MoLM. Progesterone levels of does fed 2.5, 5.0 and 7.5 % MoLM diets were identical but significantly ($p<0.05$) higher than those fed 0% MoLM.

Table 14: Serum biochemical response of growing rabbits fed diets with varied inclusion levels of *Moringa oleifera* Leaf Meal.

PARAMETERS	T1 100%CD+0% MoLM	T2 97.5%CD+2.5 % MoLM	T3 95.0%CD+5.0 % MoLM	T4 92.5%CD+7.5 % MoLM
Glucose (mg/dL)	101.54±15.34[a]	100.40±7.55[a]	99.26±9.62[a]	78.78±5.37[b]
Cholesterol (mg/dL)	84.16±10.22[a]	62.66±6.15[ab]	57.33±5.42[b]	50.80±5.73[b]
Total Protein (g/dL)	9.34±0.47	9.17±0.33	10.52±0.46	9.04±0.69
Albumin (g/dL)	3.41± 0.29	3.91± 0.20	3.57±0.22	3.52± 0.12
Globulin (g/dL)	5.92±0.49	5.26±0.38	6.94± 0.62	5.52± 0.61
Urea (mg/dL)	39.47 ±4.09	44.39 ±5.22	38.76 ±4.40	45.95 ±6.83
ALT (I.U/L)	16.86±1.99	19.53±3.46	19.84 ±3.30	19.18 ±0.69
AST (I.U/L)	22.21± 2.93	25.62 ±4.69	18.72± 2.31	17.92 ±1.90

ALT -Alanine amino Transferase, AST- Aspartate amino Transferase

a b -Means in the same row with different superscripts are significantly different (P< 0.05)

MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet

Table 15: Serum biochemical response of gestating does fed diets with varied inclusion levels of *Moringa oleifera* Leaf Meal.

PARAMETERS	T1 100%CD+0 % MoLM	T2 97.5%CD+2.5 % MoLM	T3 95.0%CD+5.0% MoLM	T4 92.5%CD+7.5 % MoLM
Glucose (mg/dL)	93.11 ± 6.76	81.20 ± 8.11	94.30 ± 6.15	100.72 ± 7.65
Cholesterol (mg/dL)	37.73±13.08	21.90 ± 7.79	35.29 ±10.36	24.64 ± 2.92
Total Protein (g/dL)	5.57 ± 0.20	5.32 ± 0.14	6.05 ± 0.31	5.90 ± 0.19
Albumin (g/dL)	2.12 ± 0.03	2.14 ± 0.04	2.23 ± 0.04	2.15 ± 0.08
Globulin (g/dL)	2.52 ± 0.96	3.18±0.10	3.82±0.28	3.75±0.14
ALT (I.U/L)	19.15±2.00^{b}	38.66 ± 4.15^{a}	39.68 ± 6.57^{a}	48.24 ± 2.70^{a}
AST (I.U/L)	29.76 ± 5.95	33.19 ± 5.23	30.07± 13.71	39.58 ± 14.83

ALT -Alanine amino Transferase, AST- Aspartate amino Transferase

a b -Means in the same row with different superscripts are significantly different (P< 0.05)

MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet

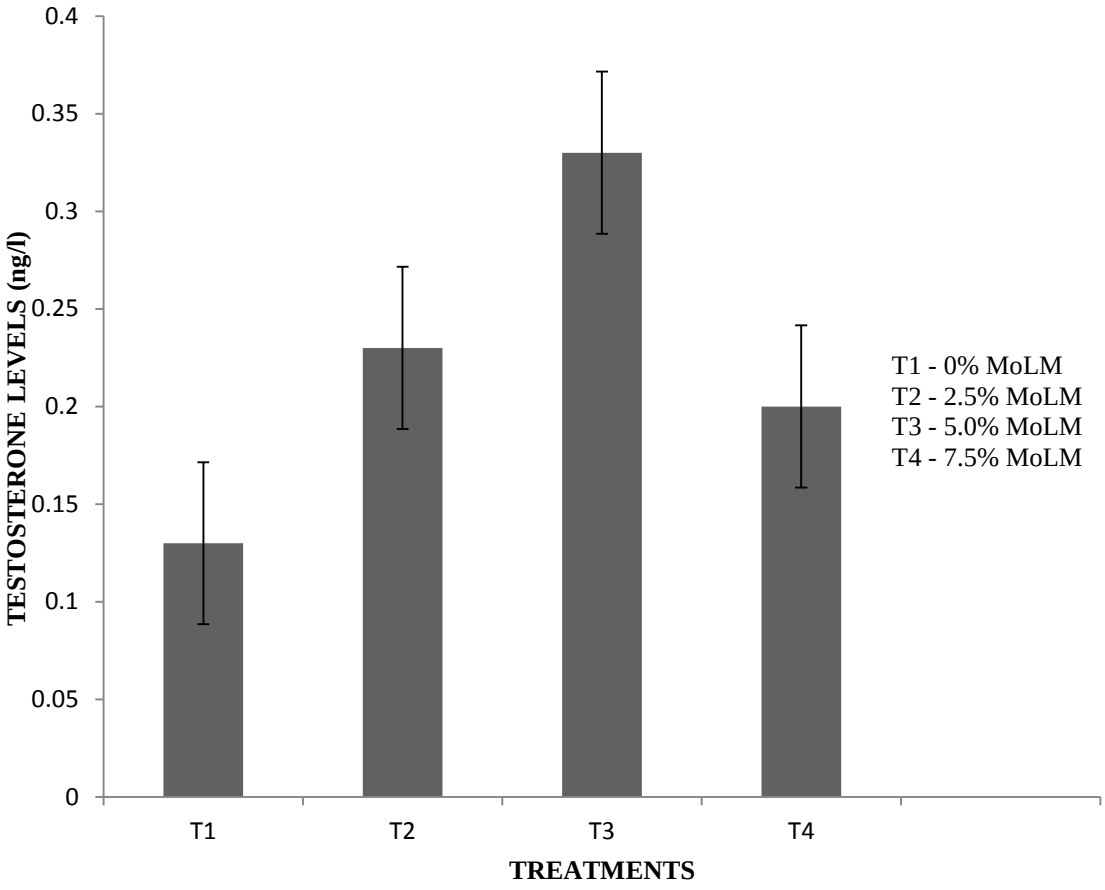


Figure 5: Serum Testosterone levels of bucks fed diets with varied inclusion levels of *Moringa oleifera* Leaf Meal

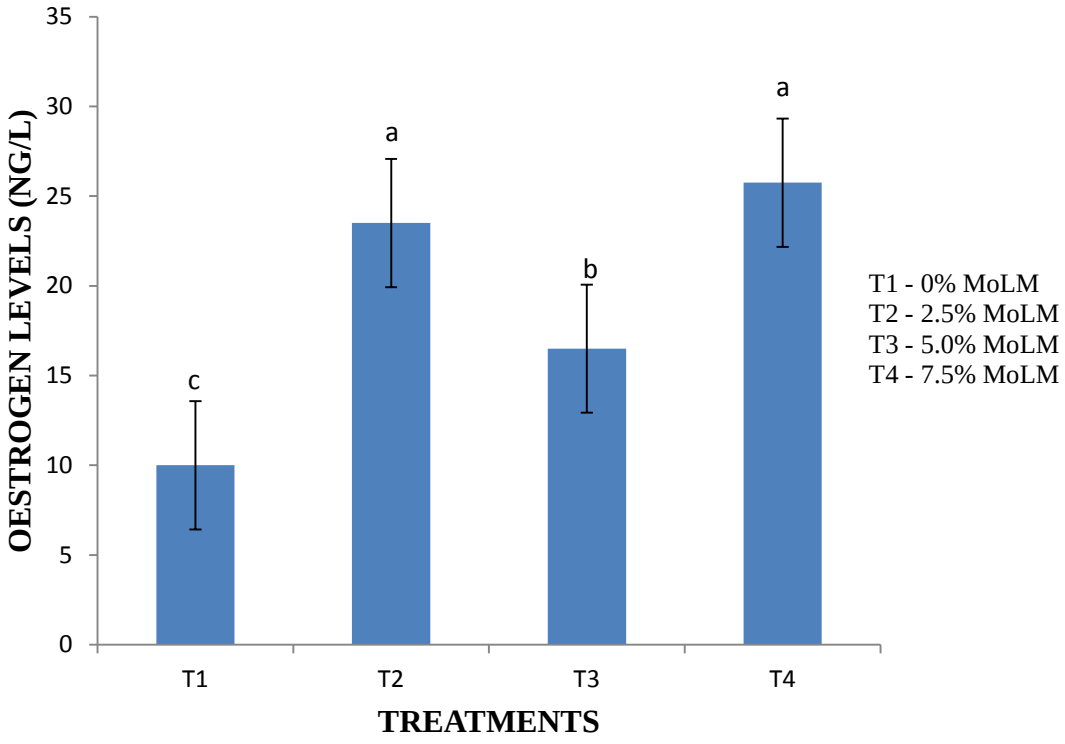


Figure 6: Serum oestrogen levels of does fed varied levels of *Moringa oleifera* Leaf Meal

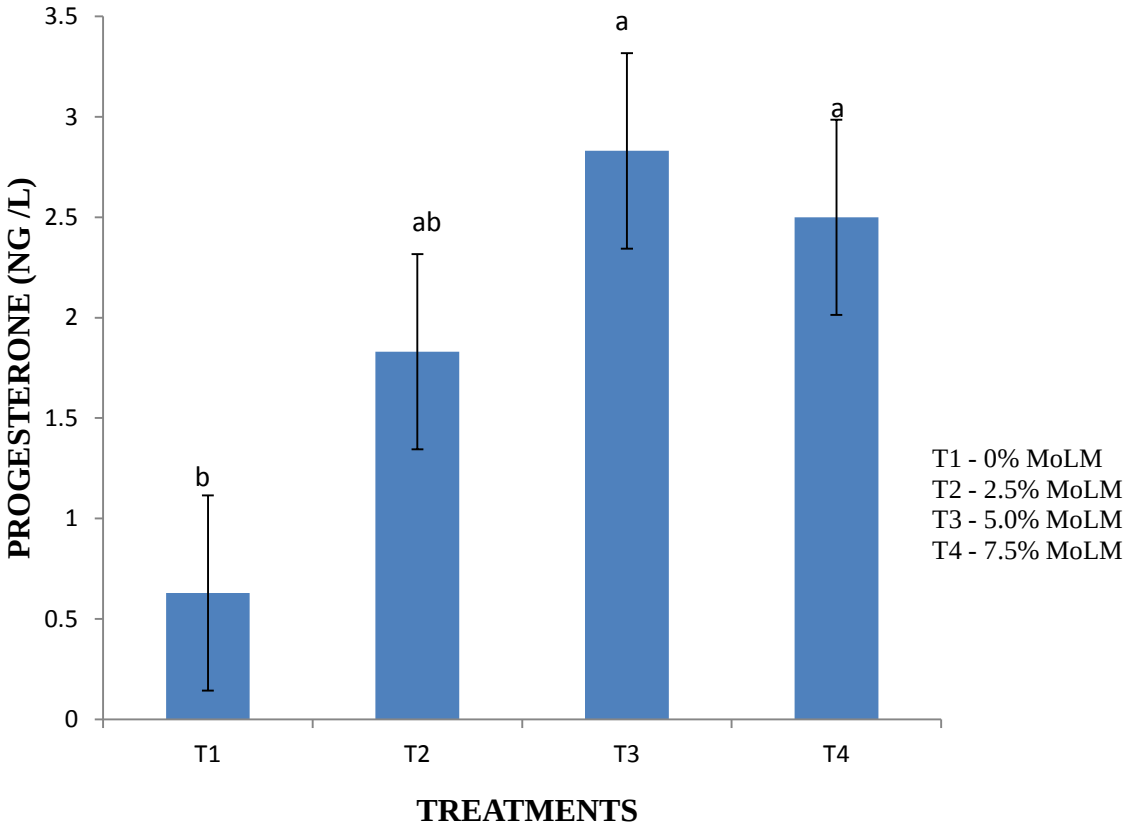


Figure 7: Serum progesterone levels of gestating does fed varied levels of *Moringa oleifera* Leaf Meal

4.3 SEMEN CHARATERISTICS, LIBIDO ASSESSMENT AND FERTILITY RATE OF RABBITS FED DIETS SUPPLEMENTED WITH M*oringa oleifera* LEAF MEAL

4.3.1 Semen Characteristics of rabbit bucks

The results of semen characteristics and libido scores of rabbit bucks fed varied levels of *Moringa oleifera* leaf meal are presented on Table 16. The results showed that semen volume, mass activity, sperm concentration and live sperm cells were significantly ($p<0.05$) different among the dietary treatments. However, semen colour, sperm motility, reaction time and libido scores were not significantly different among the treatments.

Rabbits on diet 4 had the highest semen volume which was significantly ($p<0.05$) different from those on diets 1 and 3 but statistically similar to the rabbits on diet 2. Values obtained for mass activity in rabbits on diets 2 and 4 were significantly higher than those on diets 1 and 3. Sperm concentration was significantly influenced by the inclusion levels of the MoLM and it follows the same trend with the semen volume. The sperm concentrations of rabbits on diets 2 and 4 were not significantly different from each other. However, bucks fed diet 4 had significantly ($p<0.05$) higher sperm concentration than those on diets 1 and 3. Live sperm cells were significantly ($p<0.05$) influenced by MoLM, rabbits on diet 2 had significantly ($p<0.05$) higher values than those on diet 3. Reaction time and libido scores were not significantly different among the treatments but inversely related, the lower the reaction time the higher the libido score.

4.3.2 Fertility rate assessment

The results of the fertility rates of does fed varied levels of MoLM are presented onTable 17. Litter size and litter weight at birth were significantly ($p<0.05$) different among the treatments. The conception rates of the does varied among the treatments. Rabbits on diet 2

Table 16: Semen characteristics and libido scores of rabbit bucks fed varied levels of *Moringa oleifera* leaf meal

PARAMETERS	T1 100%CD+0% MoLM	T2 97.5%CD+2.5% MoLM	T3 95.0%CD+5.0% MoLM	T4 92.5%CD+7.5% MoLM
Semen Volume (ml)	0.62±0.14^{b}	0.81±0.08^{ab}	0.48±0.11^{b}	1.00±0.09^{a}
Semen Colour	Milky	Milky	Milky	Milky
Mass Activity (0-4)	1.75±0.16^{b}	3.00±0.01^{a}	1.75±0.41^{b}	2.50±0.19^{a}
Sperm Motility (%)	82.61±0.27	90.3±0.24	86.62±0.10	89.41±0.28
Sperm Concentration (x 10^{7}/ml)	46.32±1.28^{b}	66.91±1.10^{ab}	50.82±1.15^{b}	94.33±1.31^{a}
Live Sperm Cells (%)	72.58±10.03^{ab}	88.48±3.42^{a}	61.98±11.34^{b}	84.48±7.09^{ab}
Reaction time (seconds)	3.50 ± 1.26	1.75 ± 0.25	2.33 ± 0.33	3.50 ± 1.66
Libido score (mounts/minute)	8.5 ± 1.32	12.5 ± 0.86	11.67 ± 1.20	10.25 ± 1.49

a,b -Means in the same row with different superscripts are significantly different (P< 0.05)

MoLM – *Moringa oleifera* Leaf Meal , CD – Control Diet

Table 17: Fertility rate of rabbit does fed varied levels of *Moringa oleifera* leaf meal

PARAMETERS	T1 100%CD+0% MoLM	T2 97.5%CD+2.5 % MoLM	T3 95.0%CD+5.0 % MoLM	T4 92.5%CD+7.5 % MoLM
Weight of does at mating(g)	2119.09±116.25	2047.20±110.69	1994.00±55.05	2000.60±74.62
WDAK (g)	2272.80±116.57	2149.20±128.64	2053.3 ± 95.34	2147.60±114.48
Gestation length (days)	31.00 ± 0.91	32.17 ± 0.65	32.75 ± 0.63	31.80 ± 0.80
Litter size at birth	6.25 ± 0.85^{a}	5.50±0.62^{ab}	3.75 ± 0.25^{b}	5.60 ± 0.98^{ab}
Litter weight at birth (g)	218.00 ± 32.70	231.67 ± 21.00	179.75 ±10.28	215.80 ± 29.04
AWKB(g)	34.9 ± 2.21^{b}	43.58 ± 3.60^{ab}	48.21 ± 2.21^{a}	39.75 ± 2.25^{ab}

a,b -Means in the same row with different superscripts are significantly different (P< 0.05)

WDAK - Weight of does after kindling, AWKB - Average weight of kit at birth

MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet

had the highest conception rate (100%) with the lowest value (66.7%) in rabbits on diets 1 and 3 (Figure 8).

The average litter size at birth was significantly ($p<0.05$) different among the treatments. Rabbits fed 5% MoLM had the lowest litter size which was significantly ($p<0.05$) different from the control with the highest value. Litter size of rabbits on diets 2 and 4 were not significantly different from the control but significantly ($p<0.05$) higher than that of does on diet 3. The litter weight at birth was not significantly different among the treatment. Rabbits on 2.5% MoLM had the highest litter weight at birth while the lowest was observed does fed 5.0% MoLM.

Regression of sperm motility on MoLM levels in bucks indicated an optimum inclusion level of 2.7% ($R^2=0.56$) as presented in Figure 9 while the regression of conception rate on MoLM levels in does indicated an optimum inclusion level of 2.5% ($R^2=0.61$), this is shown in Figure 10.

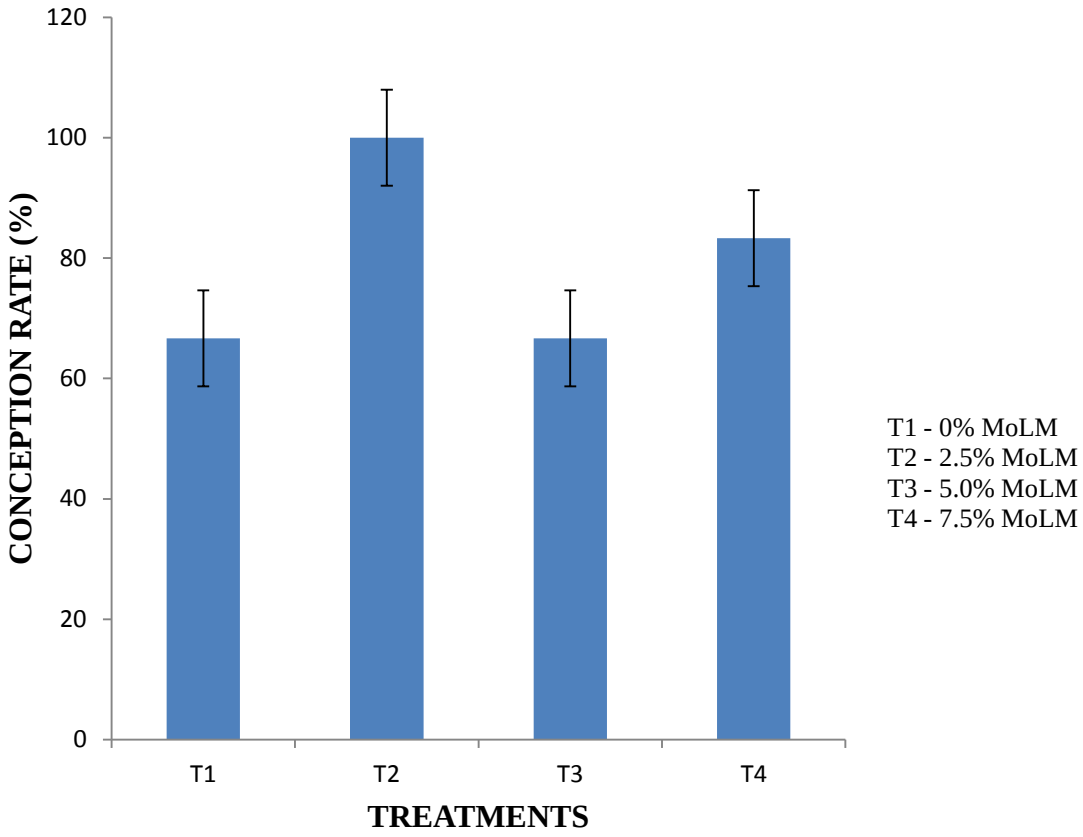


Figure 8: Conception rates of does fed varied levels of *Moringa oleifera* Leaf Meal

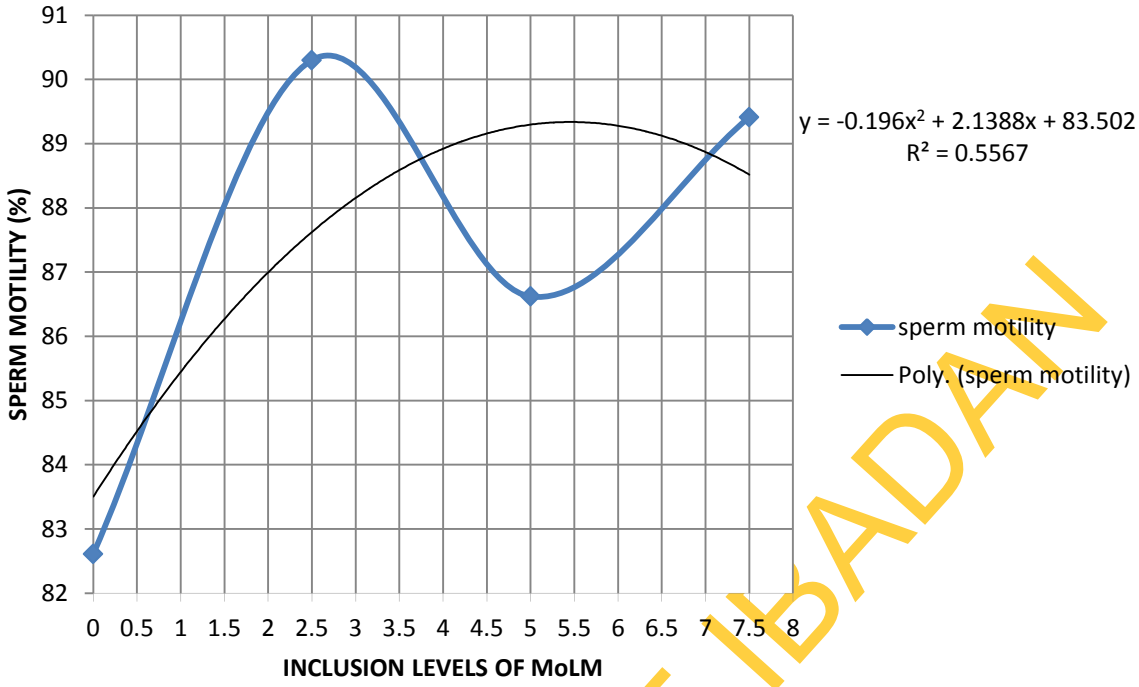


Figure 9: A graph showing the regression of sperm motility on MoLM levels in rabbit bucks.

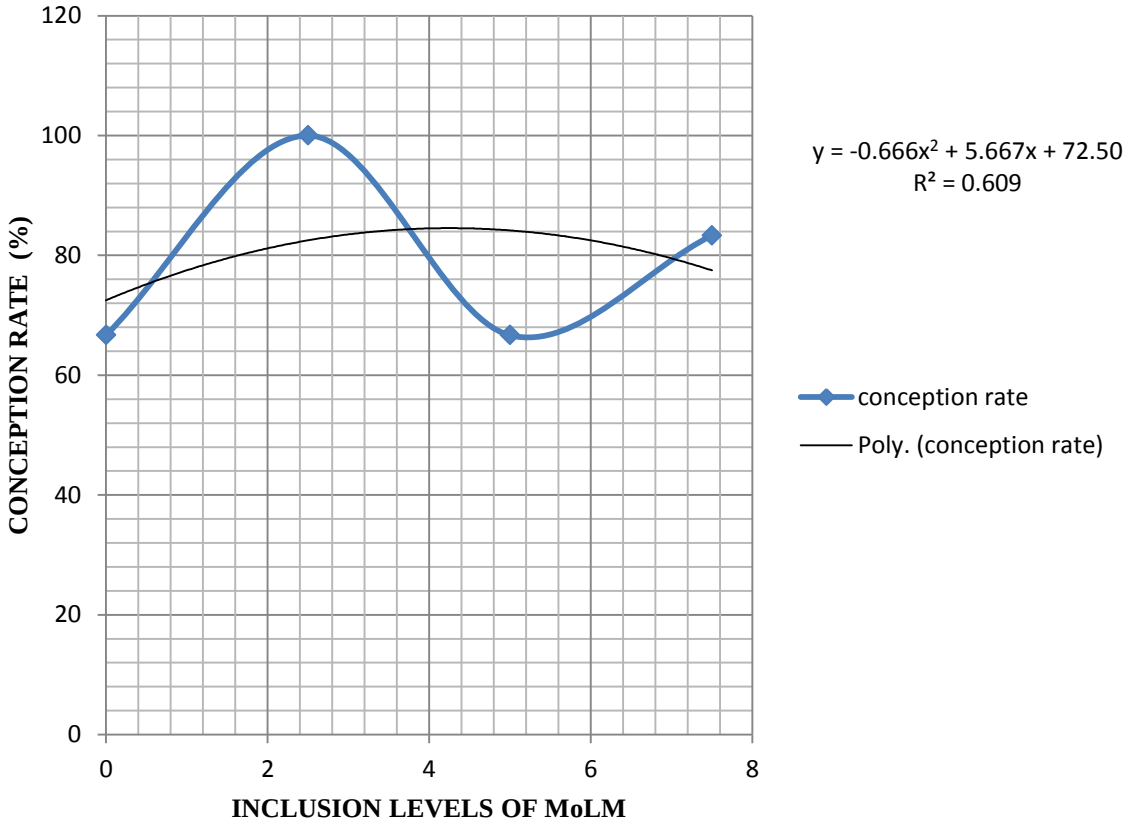


Figure 10: A graph showing the regression of conception rate on MoLM levels in rabbit does.

4.4 ORGAN WEIGHTS, ORGAN-HISTOPATHOLOGY AND SPERM STORAGE POTENTIAL OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

4.4.1 Organ weights

The relative weights of internal organs of rabbits fed varied levels of *Moringa oleifera* leaf meal are presented on Table 18. The relative weights of lungs, adrenal gland and bile were significantly ($p<0.05$) influenced by the dietary treatments. The relative weights of heart, liver, spleen, pancreas and kidney were not significantly influenced by the dietary treatments. The lung weight of rabbits fed 2.5% MoLM were significantly ($p<0.05$) higher than those fed the control, 5.0 and 7.5 % MoLM. The weight of the adrenal gland of rabbits on the control diet was significantly ($p<0.05$) higher than those fed 7.5% MoLM but not significantly different from those fed 2.5 and 5.0 % MoLM. The bile weight of rabbits fed 5.0% MoLM was significantly ($p<0.05$) higher than those on the control and 7.5% MoLM. The weight of the kidney increased as the MoLM in the diets increased but not significantly different among the treatments. Paired testes weight was higher in rabbits on diet 2 but not significantly different among the treatments.

4.4.2 Testicular and Epidydimal Sperm Reserves

The sperm reserves of rabbits fed dietary MoLM are presented on Table 19. The testicular sperm reserve in left, right and both testes are significantly ($p<0.05$) different among the treatments although did not follow any particular trend. Values obtained in rabbits on control diet (52.96×10^6) was significantly ($p<0.05$) higher than those on the MoLM diets ($45.52\text{-}14.40 \times 10^6$). Epididymal sperm reserves were not significantly different among the dietary treatments.

4.4.3 Organ histopathology

The result on histopathology of liver, kidney and ileum are presented on Table 20. The result revealed various levels of damage done to the organs by the dietary treatments. Damage done to the organs by MoLM were rated in percentage as mild, moderate and severe. Mild necrosis was observed in 66.67% of rabbits on 2.5% MoLM while those on 5.0 and 7.5 % MoLM had 100% moderate necrosis of liver, kidney and ileum mucosa.

Table 18: *Relative organ weights (%) of rabbits fed varied levels of *Moringa oleifera* leaf meal

PARAMETERS	T1 100%CD+0% MoLM	T2 97.5%CD+2.5% MoLM	T3 95.0%CD+5.0% MoLM	T4 92.5%CD+7.5% MoLM
Heart	0.21±0.01	0.22±0.02	0.23±0.02	0.22±0.07
Liver	0.56±0.09	0.60±0.04	0.70±0.42	0.51±0.07
Lungs	0.59±0.07^{b}	0.93±0.19^{a}	0.55±0.05^{b}	0.59±0.21^{b}
Spleen	0.04±0.01	0.04±0.01	0.04±0.01	0.03±0.01
Adrenal gland	0.03±0.01^{a}	0.02±0.01^{ab}	0.02±0.00^{ab}	0.01±0.00^{b}
Bile	0.02±0.01^{b}	0.03±0.01^{ab}	0.05±0.01^{a}	0.02±0.01^{b}
Kidney	0.41±0.05	0.46±0.09	0.51±0.12	0.50±0.05
Paired testes	0.27 ±0.06	0.35± 0.08	0.12± 0.07	0.25 ±0.02

a,b -Means in the same row with different superscripts are significantly different (P< 0.05)

MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet, *Relative to live weight

Table 19: Testis weight, testicular and epididymal sperm reserve of bucks fed varied levels of *Moringa oleifera* leaf meal

PARAMETERS	T1 100%CD+0 % MoLM	T2 97.5%CD+2.5% MoLM	T3 95.0%CD+5.0 % MoLM	T4 92.5%CD+7.5 % MoLM
WEIGHT OF TESTIS (%)				
Left testis	0.14 ±0.03	0.18± 0.04	0.12 ± 0.05	0.25± 0.02
Right testis	0.14 ±0.04	0.16± 0.04	0.11± 0.03	0.12± 0.01
Paired testis	0.27 ±0.06	0.35± 0.08	0.23± 0.07	0.37 ±0.02
TESTICULAR SPERM RESERVE (x10^6/ml)				
Left testis	30.88 ± 5.47^a	19.82 ±9.10^{ab}	6.40 ± 1.21^b	7.68 ± 1.02^b
Right testis	22.08 ± 6.34^a	25.60 ± 8.71^a	9.12 ± 2.63^{ab}	6.72 ± 0.78^b
Paired testis	52.96± 11.12^a	45.42± 6.78^{ab}	15.52±3.53^b	14.40±1.53^b
EPIDIDYMAL SPERM RESERVE (x10^6/ml)				
Caput: left	10.99 ± 0.14^a	10.56 ± 0.37^a	5.76 ± 0.25^b	4.00± 0.12^b
right	8.11 ± 0.65^a	6.08 ± 1.90^{ab}	6.51 ± 3.47^{ab}	5.30 ±2.06^b
Corpus left	5.01 ± 1.16^a	1.71 ± 0.49^c	3.31 ± 1.48^{ab}	2.23 ± 0.76^b
right	3.63 ± 0.35	1.81 ± 0.45	3.20 ± 1.35	2.27 ± 1.29
Cauda left	35.41 ± 0.78	55.79 ± 0.37	20.05 ± 0.68	19.45 ± 1.12
right	52.80 ± 1.15^a	32.21 ± 1.31^b	37.73 ± 1.26^b	35.40 ± 2.25^b
Epididymal sperm reserves	118.95± 4.01^a	108.16±3.24^a	76.56±6.18^b	68.65 ± 6.58^b

a, b, c -Means in the same row with different superscript are significantly different (P< 0.05) MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet

Table 20: Organ lesion/necrosis of rabbits fed varied levels of *Moringa oleifera* leaf meal

PARAMETERS	T1 100%CD+0 % MoLM	T2 97.5%CD+2.5 % MoLM	T3 95.0%CD+5.0 % MoLM	T4 92.5%CD+7.5 % MoLM
LIVER				
Necrosis/lesion:				
-Moderate/mild	0(0/0)	100(3/3)	100(3/3)	100(3/3)
-Severe	0(0/0)	0(0/0)	0(0/0)	0(0/0)
KIDNEY				
Necrosis/lesion:				
-Moderate/mild	0(0/0)	100(3/3)	100(3/3)	100(3/3)
-Severe	0(0/0)	0(0/0)	0(0/0)	0(0/0)
ILEUM				
Necrosis/lesion:				
-Moderate/mild	0(0/0)	100(3/3)	33.3(1/3)	33.3 (1/3)
-Severe	0(0/0)	0(0/0)	66.67(2/3)	66.67 (2/3)

MoLM – *Moringa oleifera* Leaf Meal, CD – Control Diet

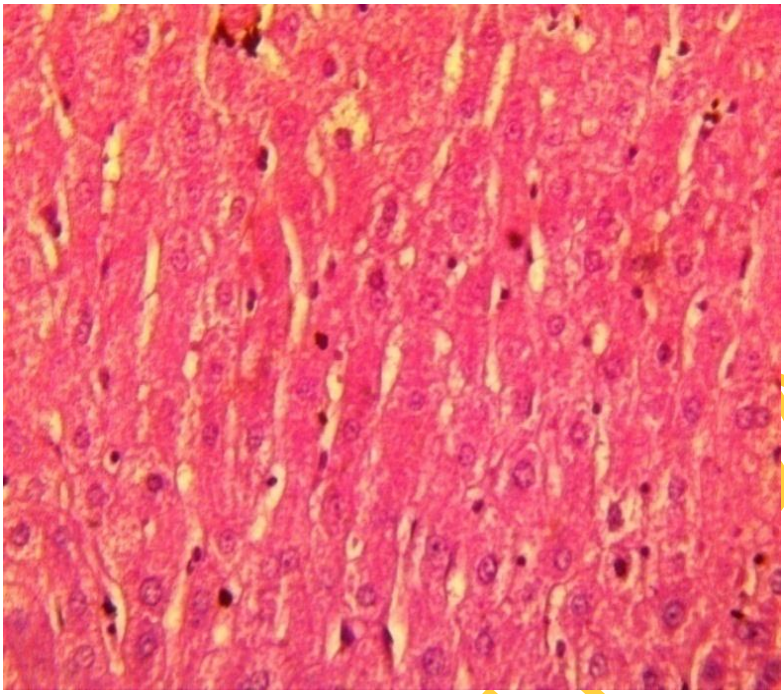


Plate 1. A section of the liver of rabbits fed 0% *Moringa oleifera* leaf meal showing no visible lesions

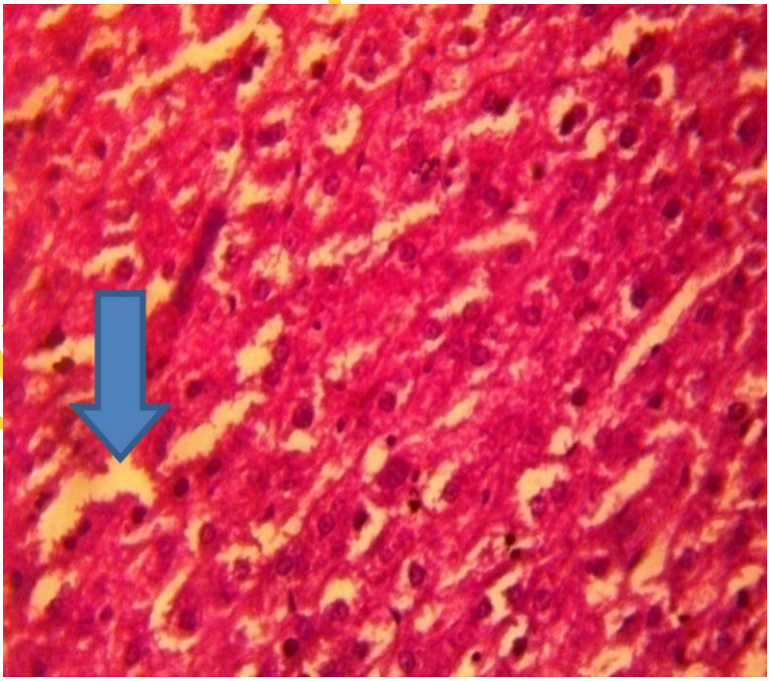


Plate 2. A section of the liver of rabbits fed 2.5% *Moringa oleifera* leaf meal showing mild /moderate changes of the hepatocytes

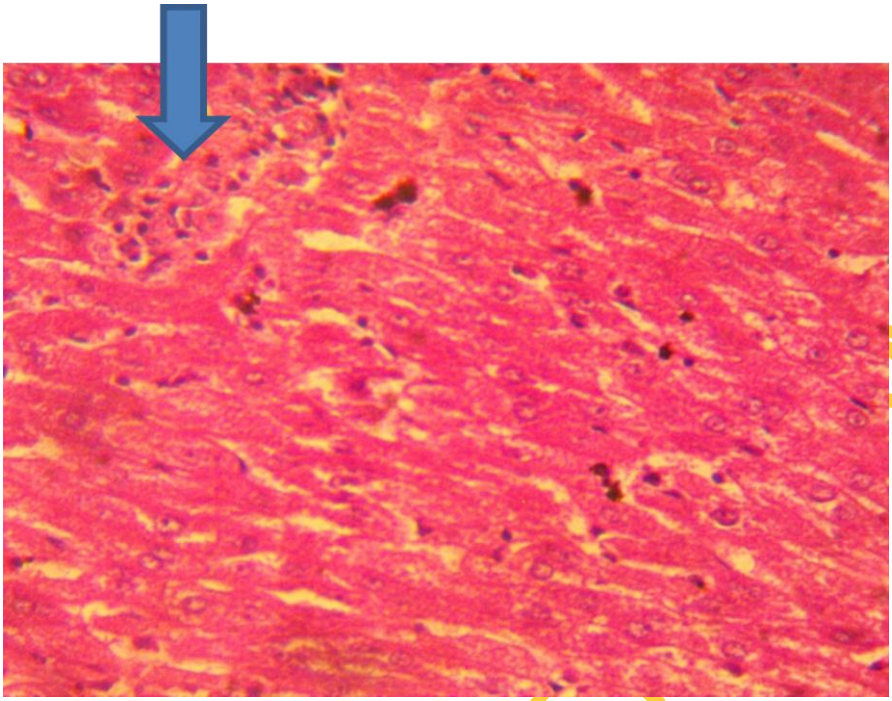


Plate 3. A section of the liver of rabbits fed 5.0% *Moringa oleifera* leaf meal showing mild /moderate changes of the hepatocytes

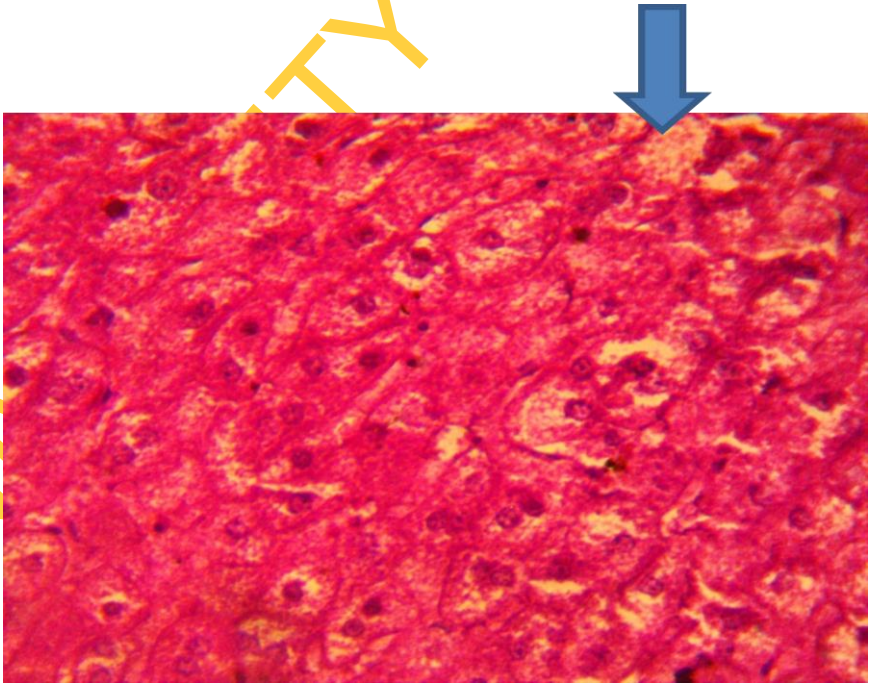


Plate 4. A section of the liver of rabbits fed 7.5% *Moringa oleifera* leaf meal showing mild /moderate changes of the hepatocytes

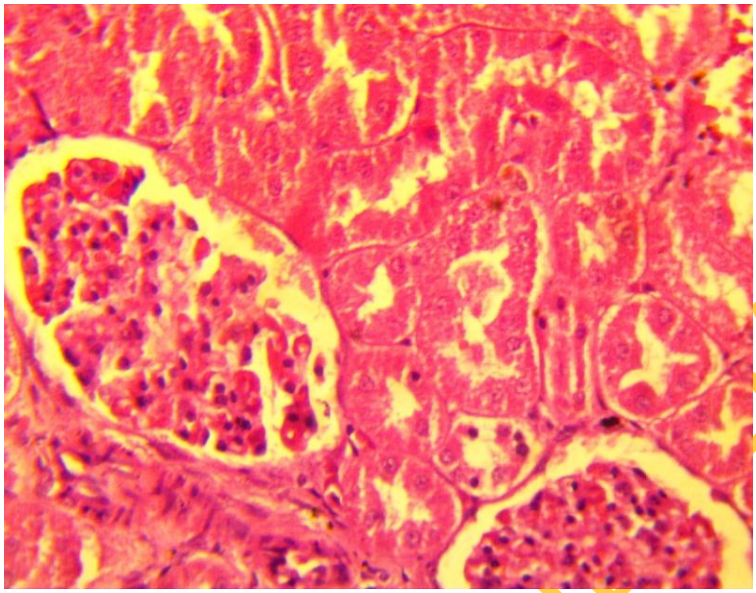


Plate 5: A section of the kidney of rabbits fed 0% *Moringa oleifera* leaf meal showing no visible lesion

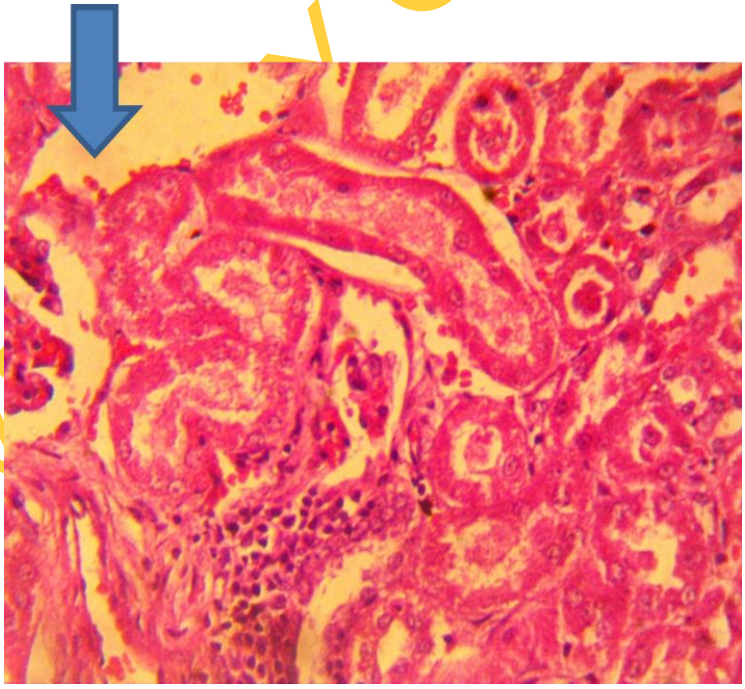


Plate 6: A section of the kidney of rabbits fed 2.5% *Moringa oleifera* leaf meal showing mild sloughing off of the the epithelium of a few tubules of the kidney

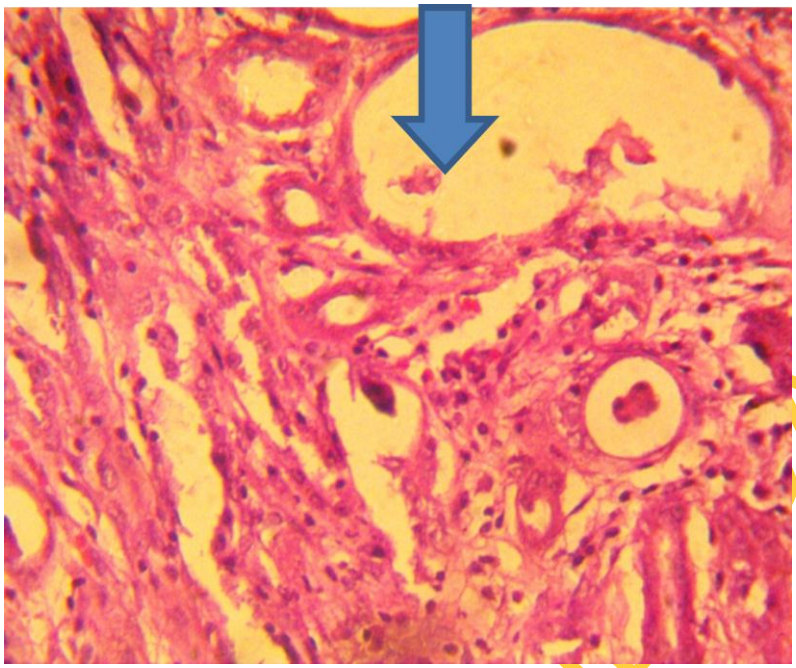


Plate 7: A section of the kidney of rabbits fed 5.0% *Moringa oleifera* leaf meal showing mild sloughing off of the the epithelium of a few tubules of the kidney

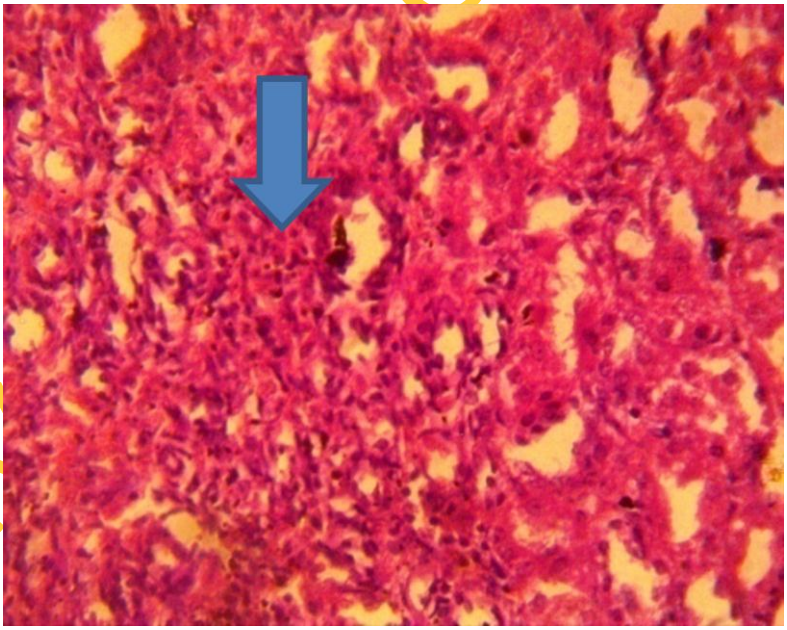


Plate 8: A section of the kidney of rabbits fed 7.5% *Moringa oleifera* leaf meal showing congestion of the renal blood vessels in the kidney

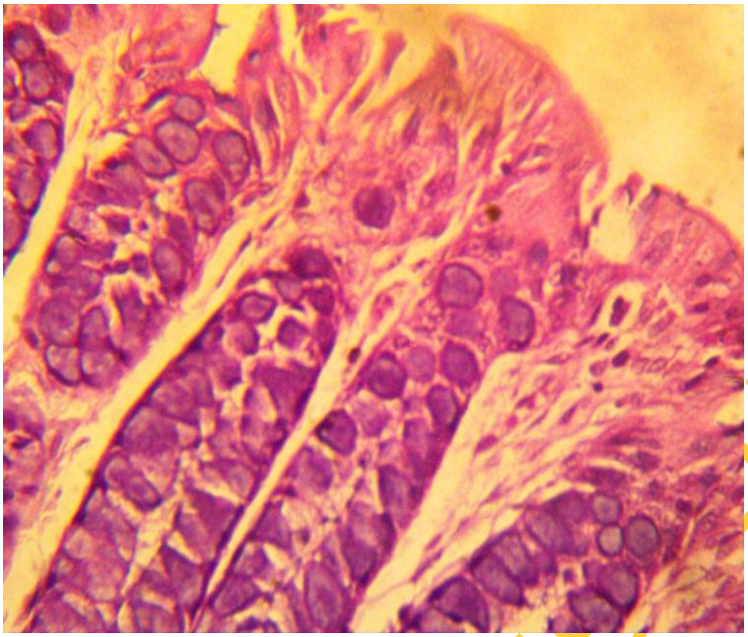


Plate 9: A section of the small intestine of rabbits fed 0% Moringa oleifera leaf meal showing no visible lesion in the ileum with adequate viili height and crypt

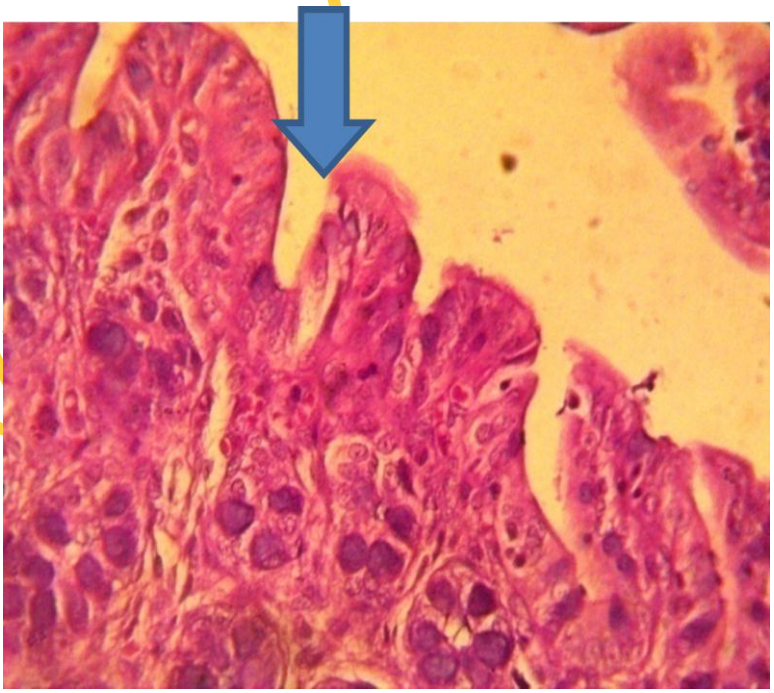


Plate 10: A section of the small intestine of rabbits fed 2.5% *Moringa oleifera* leaf meal showing mild necrosis of the crypts and sloughing off of the enterocytes

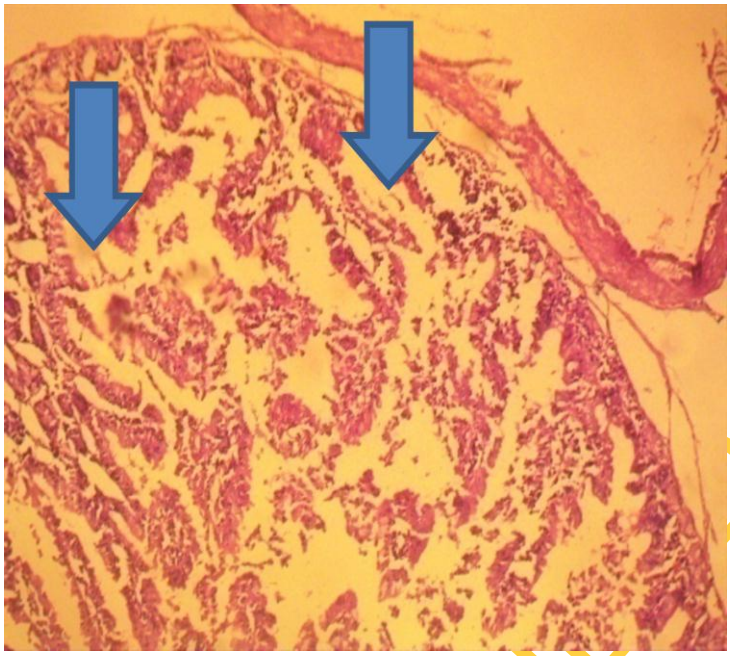
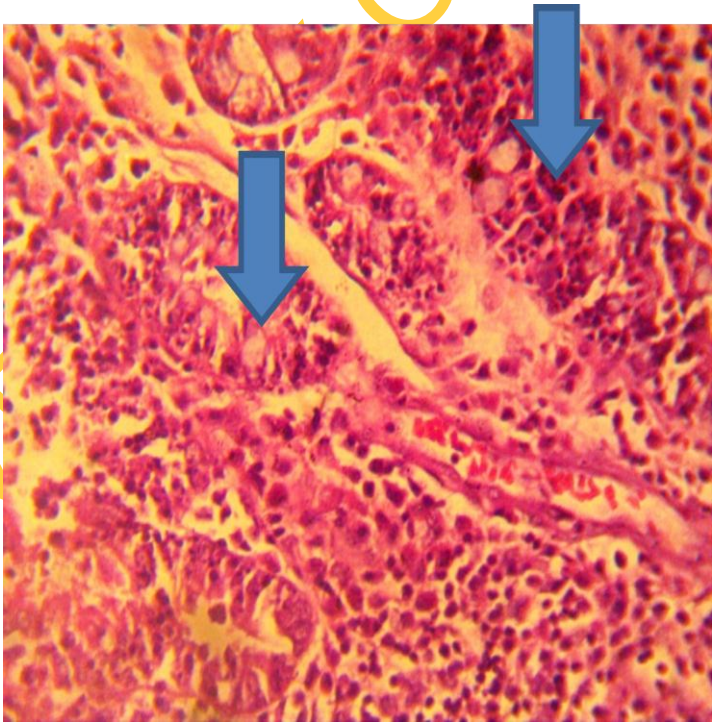


Plate 11: A section of the small intestine of rabbits fed 5.0% *Moringa oleifera* leaf meal showing distortion of intestinal mucosa, severe necrosis of crypts and villi



Slide 12: A section of the small intestine of rabbits fed 7.5% *Moringa oleifera* leaf meal showing short villi with wide spread necrosis

CHAPTER FIVE

DISCUSSION

5.1 GROWTH PERFORMANCE OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

The crude protein (CP) content of the MoLM used was 27.92%. This was similar to the CP values of 27.51, 27.20 and 27.53 % in MoLM reported by Oduro *et al.* (2008), Yamego *et al*, (2011) and Soipe (2011) respectively. However the values were higher than 17.25% reported by Ogbe and Affiku (2012). From various observations CP in MoLM was lower than that of soyabean meal (42%) that is conventionally used as a protein source in rabbit rations but higher than a number of other forages like *Centrocema pubescens*. The values (11 and 13 %) obtained for the crude fibre (CF) and ether extract (EE) respectively for the MoLM in the present study were lower than (19.4% CF) and (17.1% EE) reported by Yamego *et al.* (2011). The variations in the nutrients could be attributed to the age of the plant at harvesting, climatic conditions, agronomic practices as well as methods of processing and analysis (Fuglie, 2001; Fahey, 2005).

The Daily Feed Intake (DFI) did not show any significant difference among the dietary treatments, which implies that the consumption of MoLM-based diets compared favourably with the control diet. Rabbits must eat to meet their energy requirement to sustain rapid growth and development. The results of this study indicated that the presence of *Moringa oleifera* Leaf Meal in the diets at varied levels did not adversely affect the feed intake of the rabbits compared to reports on feed meals from other plants such as neem leaf meal (Ogbuewu *et al.*, 2012). This assertion agrees with the findings of Nuhu (2010) who

reported that MoLM did not significantly influence feed intake. Afuang *et al*. (2003) reported no significant effect of methanol-extracted *Moringa oleifera* leaf on growth and feed utilization by Nile tilapia fishes. The result in this study was at variance with the findings by Nworgu *et al.* (1999) that reported a reduction in feed intake by rabbits with increased forage meal in the diet. The daily feed intake values of 79.17–84.72 g recorded in this study was higher than 60.1- 63.4 g reported by Nuhu (2010).

Weight gain decreased with the increase in the level of MoLM inclusion in the diet. The average daily weight gain was significantly higher for the rabbits on the control diet than those on diet 4 fed 7.5% MoLM. This result contradicts the findings of Nuhu (2010) who reported significantly higher daily weight gain in rabbits fed 5, 10 and 15 % dietary MoLM than those on the control diet. However, the result of this study corroborate the findings of Kakengi *et al*., (2007) and Olugbemi *et al*., (2010) that reported reduction in performance of chicken fed MoLM. Also, Famounyan and Meffega (1986) who reported low body weight gain in rabbits fed sun-dried cassava leaf diets in the tropics. The MoLM is rich in methionine and lysine which are usually the amino acids that are found to be deficient in rabbit rations. The reduction in the average daily weight gain in the rabbits on the MoLM diets suggested the ability of MoLM to induce weight reduction after a prolonged consumption since an increase in dietary crude protein is expected to enhance weight gain. This effect could be attributed to accumulative effect of anti nutritional factors such as tannins and saponin present Moringa leaf which can hinder utilisation of available nutrients such as protein and vitamin A. The rabbits fed diet 4 had 30.84% reduction in weight gain relative to the DWG of rabbits on control diet. This implies that inclusion of Moringa in rabbit may not enhance weight gain.

The feed conversion ratios obtained in this experiment is relatively high. This is possibly linked to poor genetic constitution of the rabbits used since they are mixed breed whose genetic constitution has been altered. The values obtained for feed conversion ratio (13.47-19.26) in this study is higher than the values obtained by Nuhu (2010) (4.22 - 5.13). The generally poor FCRs obtained might probably be due to the relatively low growth rates. Genetic differences might have also contributed to the lower FCRs recorded in this study compared to that of Nuhu (2010). Olugbemi *et al*. (2010) observed that inclusion of leaf meals in broiler diets above 5 – 10 % resulted in depressed performance.

Zanu *et al*. (2012) reported significant decline in final body weight, mean body weight gain, and feed conversion with increase in the dietary inclusion of MoLM in broilers chicken diet. Olugbemi *et al*. (2010) reported a decline in daily and final weight gains in broilers fed varied levels of *Moringa oleifera* leaf meal in cassava based diets. However, Atuahene *et al*. (2008) reported no significant effect of diets containing *Moringa oleifera* leaf meal at 0, 2.5, 5.0, and 7.5 % levels on feed intake of broiler chickens. Du *et al.* (2007) observed no significant depression in growth performance of 3 weeks old broilers (Arbor Acres) that were fed on diets substituted with 0.5, 1.0, 2.0 and 3.0 % levels of *M. oleifera* leaf meal. These various reports corroborate the findings from this study.

It has been reported that MoLM has therapeutic properties (Fahey, 2005) with some of its component such as vitamin C possessing health benefits. In this study, it was observed that mortality reduced as the inclusion level of *Moringa oleifera* Leaf Meal in the diet increased. This reduction in mortality might be due to presence of some phytochemicals such as chlorogenic acids, quercetin, among others in the Moringa leaf that possess medicinal and therapeutic properties. Ologhobo *et al*., (2013) reported that *Moringa oleifera* has antibiotics

properties and may be used to replace conventional antibiotics. These antibiotics properties might have enhanced the ability of the rabbits on the moringa diets to fight against diseases or infections.

Based on the findings on growth performance of the rabbits, inclusion of *Moringa oleifera* leaf meal up to 7.5% in rabbits' diets may not adversely affect feed intake, cumulative and daily weight gain but reduce mortality by boosting their immunity.

5.2 HAEMATOLOGICAL, SERUM BIOCHEMICAL AND HORMONAL RESPONSES OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

Blood is essential for life and productivity. It transports food materials such as glucose, fatty acids, vitamins and electrolytes from the gastrointestinal tract to body tissues where they are used for energy and body building (Robert *et al*., 2003). Since it has been established that feed components affect blood constituents (Harper *et al*., 1979), haematological parameters can be used to assess the effects of test ingredients on health status of animals. In this study, *Moringa oleifera* leaf meal inclusion in rabbits' diet significantly influenced the PCV, Hb and erythrocytes during the growth and gestation phases.

Values obtained for these parameters did not follow the same trend during the growth and gestation phase. During the growth phase, rabbits on the control diet had the highest packed cell volume (42.83%) and haemoglobin concentration (13.71g/100ml) with apparent decrease in the values as the MoLM inclusion levels in the diets increased. However, during the gestation phase, the rabbits on diet 2 had the highest PCV (40.17%) and Hb concentration (13.00g/100ml) which reduced at 5.0 % inclusion levels of MoLM. Haemoglobin and PCV measurements can be used to assess nutritional anaemia. Since the

values obtained for PCV (37.67 – 42.83%), Hb (12.05 – 13.71 g/100ml) and erythrocytes (6.08 – 7.12 x 10^6/l) in this study fall within the reported physiological ranges reported for normal rabbits by Mitruka and Rawnsley, (1981) (Table 1), it can be interpreted that the dietary treatment did not cause any nutritional anaemia and provided adequate nutrients needed by the rabbits. This is an indication that there was efficient erythrocyte production in terms of the volume of red blood cells, its pigmentation and concentration. The result of this study corroborates the findings of Ozovehe (2013) and Aderinola *et al*. (2013) that reported decrease in PCV, erythrocytes and heamoglobin values in fishes and broilers fed diets containing varied levels of Moringa respectively. However, the result of this work is in contrast with the reports of Nuhu (2010) and Ewuola *et al*.(2012) that reported from their findings that *M. oleifera* did not have any significant effect on haematological parameters of growing although higher values were recorded in the rabbits fed moringa based diet. Oyedemi (2011) reported that crude extract of *Moringa oleifera* had no significant effect on the haematological parameters of lactating does. Also, Ologhobo *et al.* (2013) reported higher erythrocyte count when broilers were fed 6g/kg of moringa in their diet.

The higher leukocyte counts for growing rabbits and gestating does in some rabbits on MoLM diets was not significantly different among the treatments. This result may be due to the presence of antioxidants such as flavonoids in Moringa leaves. Flavonoids are reported to exhibit antioxidant activity (Ramanathan *et al*., 1989). Nutritional antioxidants such as vitamins A, C, and E provide additional protection from the stress (Limon-Pacheco and Gonsebatt, 2009) this may have enhanced the ability of the rabbits to maintain good health status during both phases. Similar finding of increase in leucocyte count was recorded by Odetola *et al*. (2012) in growing rabbits fed up to 15% dietary moringa. An increase in leukocyte count above normal is an indication of the presence of exogenous substances and

foreign bodies in the body. Ologhobo *et al*. (1986) reported no record of abnormal rise in values of leukocytes when antibiotics property of *Moringa oleifera* was assessed broilers.

In growing rabbits, the significant decrease in serum glucose and cholesterol values with increasing level of MoLM inclusion in the diets could be attributed to the presence of bioactive compounds such as quercertin glucoside and beta sitosterol present in *Moringa oleifera* leaf. Quercetin glucoside might have inhibited intestinal mucosa uptake of non metabolisable glucose and this might have influenced the level of glucose in the serum. Ndong *et al.* (2007) reported that *Moringa oleifera* significantly decreased the blood glucose of Wistar rats compared to the controls after glucose administration. Phytosterols such as beta sitosterol inhibit the uptake of dietary and biliary cholesterol, decreasing the levels of low density lipoprotein (LDL) and serum total cholesterol. The structure of β-sitosterol is very similar to that of cholesterol, thus taking the place of dietary and biliary cholesterol in micelles produced in the intestinal lumen thereby reducing cholesterol in the body (Moreau *et al*., 2002). Observations in this study agree with the reports of Ghasi *et al*. (1999), Olugbemi *et al.* (2010), and Ewuola *et al.* (2012) that moringa leaves was found to be a potent hypocholesterolemic agent. It may be suggested then that moringa leaf meal based-diets were capable of reducing serum cholesterol, hence assisting in the reduction of low density lipoprotein and deposition of cholesterol in the muscles. The reduction in the cholesterol level of rabbits fed MoLM diets is in agreement with earlier findings by Ogbuewu *et al*. (2012) which indicated that neem leaf meal in the diets of broiler birds and rats resulted in a decrease in the cholesterol and liver lipid levels.

Alanine amino Transferase level is usually elevated when damage is done to tissue cells, especially liver and also in some muscle diseases (Blood and Studdert, 1999). In this study,

ALT values obtained were within the range for normal rabbits, with the control diet having the lowest. This implies that the utilization of protein and by extention the blood protein levels were not negatively affected by feeding rabbits MoLM. The ALT values obtained in gestating rabbits fed MoLM diets were higher (38.66 - 48.24 iu/l) than those fed the control diet (19.15 iu/l) which might be an indication of organ toxicity. Result on ALT in this study corroborate the finding of Oyagbemi *et al.* (2013) who reported increase in ALT in rats from prolong adminstration of methanolic extract of Moringa. Although it has been reported that the levels of tannin and saponnin present in MoLM is insignificant (Makkar and Becker, 1997) prolonged consumption of MoLM may result in liver toxicity. However, results from this study contradicts the findings of Ewuola *et al*. (2011) who observed that serum enzyme activities of gestating and lactating rabbits administered crude moringa extract for eight (8) weeks were not significantly different from the control rabbits. This could be due to the short feeding duration compared to that of this study.

The significant increase in serum progesterone may be attributed to bioactive subtances such as tocopherol, carotene and beta sitosterol in Moringa (Rajanandhl and Kavitha, 2010) which might have influenced hormone synthesis. According to Coppins (2008), quantitative determination of α and β tocopherol in samples of *M.oleifera* leaf showed that the leaf provides a considerable amount of vitamin E. Chew (1999) reported that female rats fed vitamin A deficient diet had reduced ability to secrete progesterone and 20-hydroxy pregn4-en-3one into the ovarian venous blood taken on days 9 and 15 of pregnancy. Thompson *et al*. (1964) reported that feeding retinol restored spermatogenesis in degenerated testes and promoted the normal development of testes that had been infertile and ensured the growth of the seminal vesicle in rats with retinol deficiency. Also, Cajuday and Poscidio (2010) reported that the hexane fraction of *Moringa oleifera* did not affect serum FSH and LH

levels but enhanced development of seminiferous tubule, epididymides, testis and seminal vesicle in rats.

5.3 SEMEN CHARATERISTICS, LIBIDO ASSESSMENT AND FERTILITY RATE OF RABBITS FED DIETS SUPPLEMENTED WITH M*oringa oleifera* LEAF MEAL

Good semen quality with high percentage live sperm cell and high libido of the buck is essential in rabbit reproduction. The assessment of semen quality and quantity is very important and useful especially for diagnosing fertility problems (Holtz and Forte, 1978). In this study, semen quality, quantity and libido score of rabbit bucks fed varied levels of *Moringa oleifera* were influenced by the inclusion levels of the MoLM. The higher percentage of live sperm cells observed some bucks fed MoLM in this study is an indication of the viability of sperm cells with possibly higher fertilizing capacity.

Range of values for sperm concentration in this study (46.32±1.28 to 94.33±1.31 x 10^7/ml) is higher than 126 to 154 (x 10^6/ml) reported by Abu *et al.* (2013) that fed varied dietary levels ofMoringa oleifera leaf meal. This is an indication that the influence of MoLM in increasing testosterone production in rabbit bucks earlier reported in this work might have positively influenced semen quality, quantity and testis size. Testosterone is the major androgen secreted by the male gonad. It plays an essential role in the development of the normal male and maintenance of many male characteristics including muscle mass and strength, bone mass, libido, potency and spermatogenesis (Sanni *et al*., 2012). These are essential attributes that can enhance the ability of a buck to mount successfully and produce viable sperm cells.

Ebong *et al*., (2014) reported that *Moringa oleifera* extract normalized the levels of serum testosterone, LH and FSH in diabetic rats with damaged testicles. Cheah and Yang (2011) in

a review that focused on nutritional elements reported that nutrient components such as Zinc, Selenium, Folate, Vitamins and others which are also present in Moringa are involved in spermatogenesis, sperm maturation and male reproductive system development. Also, antioxidants have been reported to protect sperm cells from further oxidative damage during the entire sperm production (Afolabi *et al*., 2013) and these macronutrients constitute major components of the spermatozoa. Also, these nutritional elements influenced the development of sertoli cells and leydig cells, sperm motility and semen quality, capacity of capacitation and fertilization in rabbits (El-Masry *et al*., 1994). Presence of nutritional elements such as tocopherols, carotenoids and beta-sitosterol in *Moringa oleifera* suggest a basis for increase in testosterone which further enhanced sperm production.

Available reports from other leaf meals were at variance with the result in this study. Ogbuewu *et al*. (2009) reported that all the semen quality parameters except abnormal sperm percentages tend to follow a downward trend as the inclusion rate of neem leaf meal increased in the diet of rabbit bucks and at 5 -15% inclusion level and the sexual drive of the bucks was not improved. Herbert *et al*. (2005) reported that feeding 20% Leucaena and Gliricidia leaf meals to rabbits had a depressive effect on the diameter of the seminiferous tubule, sperm motility, semen volume and sperm concentration. Androgenic and spermatogenic properties of Moringa leaf meal had been reported to enhance the fertilizing ability of the spermatozoa (Sumalatha *et al*., 2010). Increased proportion of live sperm shows that the dietary treatment did not adversely affects spermatogenic cells during the process of spermatogenesis.

Rabbits on diet 2 had the highest conception rate (100%) compared to the control (66.7%), which is higher than 83.33% reported by Oyedemi (2011) when crude extract of MoLM was

administerd to rabbit does. The gestation length of does ranged from 31 to 32 days which is similar to that reported by Oyedemi (2011) which implies that inclusion of moringa oleifera in the diet of gestating does may not alter gestation length generally reported (28- 32 days). The litter size (5.6) observed in this study is higher than 5.2 reported by Odeyinka *et al*. (2008) and 4.8 by Ola *et al*. (2012) but similar to 5.75 reported by Oyedemi (2011). This is an indication that MoLM possess substances that can increase ovulation rate thus resulting in increased conception and litter size. Litter weight at birth ranged from 179 to 231g. The result of improved reproductive performance observed in this study is in agreement with the findings of Odeyinka *et al.* (2008) and Ola *et al*. (2012). In this study, it was observed that does on T2 and T4 with higher estrogen levels had higher conception rate of 100 and 83.33% respectively compared to the control. Also, does with high progesterone levels had low litter size and low litter weight at birth. This is in contrast with the findings of Olayaki *et al*. (2009) who reported increased litter size with increased serum progesterone, but reduced litter and maternal weights when rats were administered aqueous extract of *Cajanus cajan.*

4.4. ORGAN WEIGHTS, ORGAN-HISTOPATHOLOGY AND SPERM STORAGE POTENTIAL OF RABBITS FED VARIED LEVELS OF *Moringa oleifera* LEAF MEAL

Results from this study showed that organ weights were not adversely influenced by MoLM. The increase in liver weights observed in this study could be attributed to the presence of antinutritional factors such as tannin present in the leaf meal capable of distrupting the intergrity of the liver hepatocytes thus resulting in increased Alanine amino Transferase. Ara *et al.* (2008) reported reduced heart weight in rats administered *Moringa oleifera* leaf extract. Findings by Ayssiwede *et al*. (2011) showed that organ characteristics of indigenous

chicken were not adversely affected by dietary levels of Moringa. Isitua and Ibeh (2013) also reported that aqueous extract of *Moringa oleifera* did not have adverse effect on organ histopathology and their weight. However, the organ weights recorded in this study compared favourably with the values reported by Odetola *et al*. (2012). Ogbuewu (2011) reported relative increase in the weight of liver as the Neem Leaf Meal inclusion increases in the diet of rabbits, this he attributed to the inability of rabbits to adequately handle and tolerate anti-nutritional factors at these levels of NLM diets. Durunna *et al*. (2011) reported no significant difference in the organ weights of broilers fed bitter leaf meal.

Ability of testis to store spermatozoa is an indication for selecting bucks for breeding purpose. In this study, testicular sperm reserve decreased with the inclusion of MoLM in the diet of bucks. The testicular sperm reserve in the left, right and both testis were influenced by dietary treatments although did not follow any particular trend. Despite high testicular weight observed in rabbits fed 2.5% MoLM their testicular sperm reserve (45.42 ± 16.78 x 10^6) was lower than that of rabbits on control (52.96 ± 11.12 x 10^6). Rabbits fed 7.5% MoLM with highest paired testes weights (0.37%) had the least testicular sperm reserve (14.40 ± 1.53 x 10^6). This is in contrast with the findings of Ekeocha 2002 who reported that within specie, sperm production is a function of testicular size. It has been reported that prolonged comsumption of MoLM or its extract predisposes organs to damage (Oyagbemi *et al*., 2013) thus resulting in impaired function. Reduced testicular sperm reserve observed in this study may be due to the toxic effect of some anti-nutritional component of the leaf meal such as tannin among others (Makkar and Becker, 1997). Ewuola (2007) reported reduced sperm reserve and daily sperm production due to toxic effect of fumonisin in rabbit diet.This implies that increase in serum testosterone previously reported in this study did not influence the storage capacity of the bucks fed MoLM. Epididymal sperm reserves (ESP) were not

significantly different among the dietary treatments but variations were observed in the sperm reserve were observed in the three parts of the epididymis (caput, corpus and cauda). The ESP was lower in the bucks on MoLM diets compared with the control. The result from this work is in variance with the findings of Abu *et al*., 2013 who reported that Moringa leaf meal did not have adverse effect on epidydimal sperm reserve in rabbit when they were fed up to 15% level. However, Ogbuewu *et al*. (2009); Amao *et al*. (2013) reported reduced sperm reserve in rabbit bucks fed neem leaf meal.

Histopathology of the liver, kidney and ileum of the rabbits revealed varied level of necrotic lesions. Changes observed in the liver hepatocyte varied from mild to moderate. Sloughing off of the epithelium and congestion of the renal blood vessels were observed with the inclusion of MoLM in the diets. The necrosis of the intestinal crypts were observed ranging from mild in rabbits on 2.5% MoLM to severe in rabbits on 5.0 and 7.5 % MoLM, sloughing off of the enterocytes was seen in the ileum. This could be an indication why reduction in weight gain of the rabbits was recorded because of reduced surface for nutrient absorption despite adequate feed intake. Oyagbemi *et al*. (2013) reported that rats that received Methanolic extract of *Moringa Oleifera* at 200 and 400 mg/kg b.w. showed a significantly increased serum ALT, AST, blood urea nitrogen and creatinine which pointed to hepatic and kidney damage. The author concluded that chronic administration of *M. oleifera* leaves might predispose the animal to hepatic and kidney damage. Liver and kidney damages were observed in mice administered ethanolic extract off *Moringa oleifera* (Ugwu *et al*., 2013).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY OF FINDINGS

Feeding MoLM to rabbits up to 7.5% inclusion level does not adversely affect feed intake, it reduced mortality but decrease daily weight gain. Erythrocytes and Leukocytes production were not adversely affected by MoLM. Serum cholesterol and glucose reduced as the level of MoLM in the diets increased. The MoLM in rabbit diet improved the levels of reproductive hormones such as oestrogen and progesterone, but its effect on the levels of testosterone was not significant. Inclusion of MoLM at 2.5% resulted in increased sperm motility, mass activity, live sperm cells, low reaction time and higher libido in the bucks. Highest conception rate of does was recorded at 2.5% MoLM level.

Organ weights were not adversely affected by MoLM. Gonadal and extragonadal sperm reserves were adversely affected by MoLM. Prolonged consumption of *Moringa oleifera* leaf meal above 2.5% as a supplement can depress sperm storage potential of rabbit bucks. Result on histopathology showed mild to severely impaired organs in rabbits fed varied levels of MoLM above 2.5%. Regression of sperm motility on MoLM levels in bucks indicated an optimum inclusion level of 2.7% (R^2=0.56). Regression of conception rate on MoLM levels in does indicated an optimum inclusion level of 2.5% (R^2=0.61).

6.2 CONCLUSION

Results from this study showed that *Moringa oleifera* leaf meal can enhance reproductive efficiency in rabbits thus improving rabbit production at 2.5%. Feeding rabbits 2.5% inclusion level of *Moringa oleifera* will help in maintaining good health status, improve semen quality and fertility rate without excessive damage to the internal organs.

6.3 RECOMMENDATION

Further investigations should be carried out on feeding rabbits bucks 2.7% and does 2.5% inclusion level of MoLM over several gestation phases to assess consistency in reproductive performance at these levels.

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