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RESPONSE OF RABBITS TO MULTI-ENZYME TREATED *MORINGA OLEIFERA* LEAF MEAL

BY

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DECLARATION

This is to affirm that this thesis has been authored by me and has neither been submitted for a degree nor any aspect published by another person elsewhere. All cited literature in the text has been well referenced and any assistance received in writing the thesis has been duly acknowledged.

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ABSTRACT

Two separate experiments were conducted to investigate (1) the effects of harvesting regimes (70, 80, 90 and 100 days) on *M. oleifera* biomass yield, phytochemicals and chemical composition and (2) the effect of "Kemzyme plus P Dry" treated *M. oleifera* leaf meal on the growth performance, hematological parameters, nutrient digestibility of weaned rabbits. *M. oleifera* seeds were sown, in a completely randomised block design with four blocks, in Botanga irrigation site in the Northern Region of Ghana. Agronomic data, biomass yield, chemical composition and nutrient digestibility were collected and analysed. Twenty New Zealand White (NZW) breeds of rabbits with an average initial weight of 908.75±135.3 g were divided into four groups and allocated four test diets for a period of 49 days after one week adaptation period. The experimental diets comprised of T0 (formulated concentrate without *M. oleifera* leaf and Kemzyme plus P Dry), T1 (formulated concentrate with *M. oleifera* leaf and without Kemzyme plus P Dry), T2 (formulated concentrate with *M. oleifera* leaf + 0.03g/day of Kemzyme plus P Dry) and T3 (formulated concentrate+0.03g/day of Kemzyme plus P Dry). There was a significant effect of harvest regimes ($P<0.05$) on the biomass yield, leaf number and stem girth. The harvest at100 days had the highest biomass yield of 785.08 kg DM ha^{-1}. Plant height was positively correlated ($P>0.001$) with biomass yield. The highest ($P<0.05$) DM (204.3 gkg^{-1}) and CP (218.4 gkg^{-1} DM) was obtained at 80 days and 70 days respectively. Statistically, 100 day harvest had the highest ($P>0.05$) NDF (348 gkg^{-1} DM) and ADF (192.3 gkg^{-1} DM). There was a significant difference ($P<0.05$) in IVOMD (37.46-40.62%) and ME (13.19-16.16 MJ/kg DM). The results showed *M. oleifera* plant contains saponins, flavonoid, tannin, phytosterols, reducing sugars and triterpenes. The average daily dry matter intake was significantly higher in rabbits fed

T2 (formulated concentrate with *M. oleifera* leaf +0.03g/day of Kemzyme plus P Dry). Average daily weight gain was found to be higher in rabbits fed with T2. The nutrient digestibility differed among test diet with rabbits fed T2 having the highest (CP: 85.74%, NDF: 79.31%) digestibility. White blood cell (WBC) differentials (Monocytes and Basophil), haemoglobin (Hb) and packed cell volume (PCV) significantly differed ($P<0.05$) with rabbits fed with the T2 having a better performance. In conclusion, for intensive biomass production of *M. oleifera* was highest in the 100 day harvest with the highest crude protein and IVOMD obtained in the 70 days. Rabbits fed with formulated concentrate with *M. oleifera* leaf + 0.03g/day of Kemzyme plus P Dry showed superor performance in feed intake and growth.

Keywords: Digestibility, Multi-enzyme, *Moringa oleifera*, Phytochemicals, Haematology.

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DEDICATION

This work is dedicated to the almighty God the giver of Wisdom, Understanding, Knowledge, and life. I thank him for seeing me through the period of this work. I would also love to show my profound gratitude to beloved family and Miss Elizabeth Buzongmuu for their financial support and prayers.

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LIST OF ABBREVIATION

ADF	Acid Detergent Fibre
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemist
CP	Crude Protein
DM	Dry Matter
DOM	Digestible Organic Matter
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
EFSA	European Food Safety Authority
IVOMD	*In vitro* Dry Matter Digestibility
LSR	Leaf to Stem Ratio
ME	Metabolisable Energy
N	Nitrogen
NDF	Neutral Detergent fibre

OM	Organic Matter
SCFA	Short Chain Fatty Acid
FCR	Feed Conversion Ratio
EFSA	Europen Food Safety Authority

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The daily dietary intake of animal protein (3.24 g) in most developing countries falls grossly short of the suggested 27 g animal protein per caput/day (Ajayi et al., 2007). Appropriate feed containing high protein is needed to meet the daily uptake of protein by animals. Salter (2017) reported that there has been an increase in supply for meat products due to an increase in the human population and consumer tastes for meat and milk.

Tropical grasses are usually deficient in crude protein (Gebregiorgis *et al*., 2011) and test ingredients from agro-industrial products and M. oleifera leaf meal have been established (Adam, 2013; Ansah *et al*., 2014; Alagbe and Oluwafemi, 2019) as alternative feed sources. *M. oleifera* is a fast growing plant that is extensively accessible in developing countries and is economically essential for the agriculture production (Richter *et al*., 2003). *M. oleifera* is capable of regrowth efficiently after pruning and produces excellent quality biomass per unit area (Foidl *et al*., 2001; Nouman *et al*., 2014). Moringa leaves are frequently considered a healthy source of protein (15% to 30% DM), depending on the point of maturity and parts (stem, leaflets and petioles) considered (Price, 2007; Reyes Sanchez, 2004). The fiber content is moderate, with an acid detergent fibre (ADF) value 8% to 30%DM. The content of lignin was observed as 2% to more than 10% DM with an elevated concentrations of minerals (10% DM) and vitamins (Price, 2007; Reyes Sanchez, 2004). It is considered an inexpensive, year-round, high quality feed and a rich source of phytochemicals (Foidl *et al.*, 2001; Siddhuraju *et al*., 2003; Becker and Siddhuraju, 2003; Yang *et al*., 2006).

The formulation of feed from various local raw materials is the priority of all nutritionists to satisfy the daily protein requirement of animals. In recent years, attention of the merits of rabbit meat production in middle-and-low income has been improving as an alternative way to alleviate global food shortages (Cheeke and Lukfahr, 1991). In addition, rabbit occupies a vital midway between ruminants and monogastrics and can effectively use feed rich in cellulose (Hasanat *et al*., 2006). This general understanding is attributed to the high production and early maturity rate of rabbit, rapid growth rate and high potential for genetic selection, efficient use of feed and land use, limited competition with humans for similar foods and high quality nutritious meat (Cheeke and Lukfahr, 1991).

1.2 Problem statement and Justification

Acamovic and Brooker (2005), reported that feeds from *M. oleifera* produce bitter taste as an animal forage. Challenges have been noted in achieving and sustaining specific forage species improvements (*M. oleifera*) (Parsons *et al.,* 2011). Phytochemicals (alkaloids, saponins and tannins) trigger foul taste, not only reducing digestibility but also causing health risks to animals (Herms and Mattson, 1992; Crozier *et al.*, 2001; Engel, 2002; Taiz and Zeiger, 2002).

Mahama *et al.* (2012) reported on the use of biotechnology in animal production to improve animal productivity through better nutrition, better breeding and better health situations. Enzyme additive is a potential solution to standard feed degradation (Chiong *et al*., 2014) and efficient digestibility (Salem *et al*., 2013). Many ingredients in the forage are not completely digested by animals, which makes it difficult to achieve the best promising nutritional value from the forage. Kemzyne Dry P plus feed ingredient is designed to improve the digestibility of raw materials and increase nutrient concentrations of animals (Saleh *et al*., 2010; Raach-moujahed *et al*., 2017). There is a need to search for an alternative or extra source of protein and feed source that can be

easily developed and generate a comparatively short-term economically result using rabbits. A production system that will enable fast growing plants to be integrated, that can generate feed and fast growing animals that would use the feed in the same production system is a possible solution to the challenges in the animal production.

1.3 Objectives

1. To determine the effect of harvesting regimes on *M. oleifera* biomass
2. To assess the nutrient composition, phytochemicals and digestibility of *M. oleifera* leaf
3. To determine the effect of Kemzyne plus P Dry on the growth, hematological and nutrient digestibility of weaned rabbits.

CHAPTER TWO

2.0 Literature Review

2.1 Feed Sources for Animal Production

Crop residues are agriculture by-product that remains after food crops are harvested and processed. The main crops found in the Sahelian zone are millet, sorghum, cowpea and some tubers (Sanon, 2007). In Northern Ghana groundnut haulm is a common feed for animal especially ruminants.

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Millet is the predominant grain crops in the dry subtropical region, contributing 65% of West African countries ' total production, with residue yields projected at a range of 1000 - 2000 kg DM/ha (Williams *et al.*, 1997). As a larger percentage of the land has been used to produce food to satisfy the increasing population, crop residues has become an essential source of forage for animals (Harris, 2002).

Furthermore, their quality depends on the quantity of land under production and the yield of the essential plant parts. They could account for 40 to 60% of the total livestock intake of Dry matter in the Sahelian area, notably in the dry season (De Leeuw, 1997). Sanon (2007) noted that, based on the nature of the remains, the management of crop residues for livestock feed differs.

Nevertheless, certain cereal straws are in situ grazed while legume remains are extracted for sale or stall feeding as a haulm (Tarawali and Hiernaux, 2002). Cowpea haulms, when intercropped with sorghum, the estimated yield range from 400 - 1200 kg/ha (in Mali) and 200-500 kg/ha in

(Burkina Faso) (De Leuw, 1997). As recorded by Savadogo (1999), the nutritional value for cowpea and groundnut haulms was 126 -156 g/kg DM of crude protein (CP), 570-610 g/kg Digestible Organic Matter (DOM), and 9.0 and 9.7 Metabolizable Energy (ME) respectively.

The growing demand and subsequent rising price of standard feed has revitalized interest in the use of agro-industrial by-products and some new legumes as animal feed (Anya *et al.*, 2018). Traditional feed components, primarily the energy sources used in feed preparing (maize, millet, sorghum, etc.) are very expensive owing to their elevated demand for the increasing population of humans. Using agro-industrial by-products correctly formulated with some integrated rich grass protein forage could assist save costs for feeding rabbits.

Soybean meal (SBM) is another product of agro-industrial products and is important as far as the animal feed industry is concerned. The SBM is one of the finest and most widely used protein supplements in animal feeding but it is extremely priced in some nations, and among the oil-seed supplements, may be cheaper than groundnut cake. Soybean meal includes all essential amino acids, but cystine and methionine quantities are suboptimal and a number of toxic elements and inhibitory substances, including allergic, goitrogenic and anticoagulant factors, can also be discovered (McDonald *et al.*, 1992). It is a poor source of B vitamins that must therefore be provided when consistently using soybean meal as a major protein supplement for monogastric animals. Soya meal is rich in crude protein (45%), fat (2%) and cellulose (3.5%) however, monogastric animals partially digest the cellulose content of this cake. The SBM is a source of potassium (K), magnesium and provides a reasonable quantity of elements (Ralph, 1987).

Cereal by-product is poor in protein and lysine content, with an average of only 62% and 65% respectively, with the clear exception of lysine-rich quinoa vegetables (Lebas, 2013). Cereal proteins, on the contrary, are somewhat rich in amino acids from sulfur (about 116%). They

could supply a good amount of phosphorus demands, excluding some by-products of cereals (corn and sorghum bran and oats hulls), but cereal grains have low calcium for all of them. Cereal by-products are a remarkable source of fiber (123% of NDF) but have a slight low lignin content for rabbits (84% of ADL requirements) (Lebas, 2013).

In a selection of agro-ecological settings, maize is a cereal crop cultivated globally.

In developing nations in particular, more maize is generated annually than any other grain to promote family upkeep and the animal sector. More than 90% of all maize generated is mainly used for animal feed, food and alcohol production (Semenčenko, 2013). Cereals are regarded to be one of the prevalent forage plants in animal nutrition due to the unlimited rate of dry matter content and the elevated percentage of grain (Semenčenko, 2014).

Global recognition is given to the significance of browse trees and shrubs and much research is being done under multiple disciplines (Le Houerou, 1980a). Browse species are always an efficient help to the use of agricultural land where, due to climatic, topographic and edaphic restrictions, natural crop production is not feasible. Studies on the development of browse crops will therefore be necessary in order to draw from them the best potential for the improvement of man and his livestock.

It was reported by Shayo (1997) that there were lesser concentrations of CP in older leaves (140 g/kg DM) than younger leaves (186 g/kg DM) of *Morus alba* in in a study conducted in different locations (semi-arid areas of Mpwapwa and Dodoma). The herbage cultivated is a valuable animal feed and is a major source of nitrogen for the animal. A comparative of the yield and nutrient quantity of Leucaena leucocephala, Acioabarter ii, Alchornea cor difolia and Gliricidia sepium cultivated in Nigeria's alley farming regime indicates that the yield of biomass varied from 3.0 to 7.4 t DM/ha/yr while the yield of nutrients were reported as; 40.5- 246.5 Kg N/ha/yr.,

3.6-19.9 kg. P/ha/yr., 20.4-184.0 kg K/ha/yr, 14.7- 104.3 kg Ca/ha/yr and 5.4-17.6 kgMg/ha/yr (Kang and Reynolds 1986). Substituting low-quality diets to at least 7% protein levels will improve feed intake and animal manufacturing (Minson and Milford, 1967). It is valuable for supplementation since browse maintains an elevated nutrient value during the year (Reynolds and Adeoye, 1986).

2.2 Origin and Geographical Distribution of *M. oleifera*

M. oleifera is an adaptable vegetable plant with a diversity of potential. Moringa leaf has been reported to have different species (13) in the genus Moringa and the family Moringaceae (Kristin, 2000). Moringa plants are now extensively cultivated and has become adapted in some areas in the tropics (Fahey *et al.*, 2001). It was further stated that they are native to India, the Red Sea area (Africa). *M. oleifera* is native to Northern India and Pakistan (Bosch, 2004) and was introduced middle-and-low income countries.

More recently, *M. oleifera* has been promoted to reduce human undernutrition after it was identified as a multipurpose plant. However, little evidence is known about the *M. oleifera* plant used as animal feed in Ghana apart from the plant that has been able to give fresh potential for small-scale farmers in Africa to generate family food or as some raw materials for industrial food products.

2.2.1 Cultivation and Production

Palada *et al.* (1996) emphasised that *M. oleifera* development may be achieved through seed sowing or harvest, however in some part of Africa *M. oleifera* seeds are most preferred while vegetative propagation is common in some developed countries. Seed selections is usually considered before sowing, when seedlings are in nursery beds, they could be transplanted (Leone *et al.*, 2015) this practice could help to reduce the use of infected seedlings in sowing. It has been

revealed that by nature *M. oleifera* plant has high biomass yield per unit area and a habit of fast re-growth after pruning has been done (Foidl *et al.*, 2001). The consistent pruning and regrowth leads to increase in *M. oleifera* fresh shoot biomass per hectare within a year (Amaglo *et al.*, 2010).

M. oleifera seeds per kilogram range from 3000 to 9000 in terms of viability, with a high germination rate (80% – 90%) at storage conditions (Leone *et al*., 2015). Moringa tree also has the benefit of a rapidly increasing and deep rooting scheme, it can grow under different climatic circumstances such as subtropical and dry tropical with low water supply and mild salinity, while different soil conditions also promote *M. oleifera* development (Sarwar *et al*., 2017).

2.2.2 Fertilizer application

Isaiah (2013) suggested that *M. oleifera* can be adjusted to a widespread variety of soil types, however it grows well in clay loam soil and is resistant to drought and does not survive prolonged water logging. *M. oleifera* plant does very well in neutral to slightly acidic soil reaction. Specific nutrients regulate the plants quality and health, and any changes in such supplement may affect their characteristics. The application of organic manures, inorganic fertilizer or combinations significantly influenced the agronomic parameters of trees and shrubs. The rate of soil nutrients accumulation depends mainly on the amount and quality of organic matter input.

Also, fertilizer requirements of plant species vary, as such, efforts must be made to ascertain the correct fertilizer to improve plants plant growth and development. Oni (2001) reported that the characteristic of species and genotypes within species play a significant role in limiting effectiveness of plants uptake and use mineral elements. According to Williams *et al.* (2017) the different rates of manure applied significantly affected some morphological traits of

M. oleifera. These researchers suggested that the variation could be attributed to the differences in the content of NPK required for optimum plant growth and development.

Previously, Aluko and Aduayi (1983), noted that using NPK increases seedlings height of *Terminalia ivorensis*. The girth/stem diameter and leaf production of the plants were similarly improved by the combination of organic matter and inorganic fertilizers (Adebagbo, 1981; Aluko, 1989). The application of inorganic fertilizer also affected major morphological characteristics of *M. oleifera*, however, higher rates had lower effect on the seedlings compared to the lower rates. Adegun and Ayodele (2015) reported that soil amended with poultry droppings and cowdung are (valuable sources of nutrients) supported vegetative growth of Moringa plants in their study. It was also concluded that plant cultivated in soil amend with poultry manure were better than those in soil amended with cow dung considering plant height, stem girth and fresh fodder yield.

2.2.3 Moringa oleifera and its Importance

M. oleifera leaves ranks in terms of nutrients as the best of all tropical vegetables and is extremely appreciated by individuals in the tropics and subtropics for its many medicinal properties. Nearly every portion of *M. oleifera* has been noted to be a depot of essential nutrients and antinutrients (Gopalakrishnan *et al*., 2016).

Moringa oleifera have been used to control malnutrition, particularly among infants and nursing mothers (Mensah, 2011). Both Gopalakrishnan researchers and coworkers, and Mensah, have revealed only significant benefits derived from Moringa oleifera in human diet. Little is known about the potential of formulating feed using M. oleifera plant to influence the quality of livestock products

2.2.4 Nutritional value of *M. oleifera*

Moringa leaves are frequently considered a healthy source of protein, ranging from 15% - 30% DM, depending on the point of maturity and the proportions considered (Reyes-Sanchez, 2004; Price, 2007). Similarly, in a separate research, the ADF content of Moringa leaves was reported in a range of 8 - 30% DM. The lignin content was reported to be 2% more than 10% DM, the minerals concentration was about 10% DM, particularly calcium and iron (Reyes-Sanchez, 2004; Price, 2007). It also contains flavonoids, more efficient antioxidants than ascorbic acid (Siddhuraju *et al.,* 2003; Yang *et al*., 2006). It is therefore possible to use *M. oleifera* leaves as an antioxidant in feed (Makkar *et al*., 2007). They have a remarkably high lipid concentrations (5-6%, up to 10% DM) with a significant fatty acid ratio (33-45%) (Moyo *et al*., 2011; Olaofe *et al*., 2013). It has been anticipated that the crude protein content may be smaller (4%) than they require for rumen microbe function if ruminants feed on low quality fibers like stovers crop remains and dead pasture. It has been observed that the nutrient content of Moringa oleifera crops varied at tight and wide ranging plant spacing with distinct harvesting intervals (Basra *et al*., 2015).

Table 2.1: Chemical composition (% DM) of *M. oleifera* leaf

	Fresh	Dry
Parameter	Mean±SD	Mean±SD
Dry matter	26.2 ±8.6	91.2±2.3
Crude protein	24.3 ±4.3	26.8±3.6
Crude fibre	13.6±4.5	12.2±4.7
NDF	28.3±9.4	22.7±7.1

ADF	19.3±7.4	15.3±7.7
Lignin	7±3.3	3.4±2.6
Ether Extrast	5.4±1.2	6.4±1.6
Ash	10.3±1.8	10.8±2.3
Total sugar	11	-
Gross Energy	18.6±0.8	-

Source: (*Aregheore, 2002; Babiker, 2012; Bakhashwain et al., 2010; Busson, 1963; Garcia et al., 2008; Kambashi et al., 2014; Ly et al., 2001; Makkar et al., 1996; Malik et al., 1967; Melesse et al., 2012; Mendieta-Araica et al., 2009; Mendieta-Araica et al., 2011; Nuhu, 2010; Odetola et al., 2012; Reyes Sanchez et al., 2006; Safwat et al., 2014; Sen, 1938; Soliva et al., 2005; Sultana etal., 2014).*

Moringa oleifera leaves' nutrient composition has been revealed to be significantly diverse with harvesting schemes for both harvests determined by tiny and larger spacing of plants and the maximum N leaf content recorded for both harvests at a broader spacing of 30 d cutting interval followed by tiny spacing at a cutting interval of 15d (Basra *et al.*, 2015).

According to Gebregiorgis *et al.* (2011), the correct formulation of *Moringa stenopetala* leaves with Rhodes grass enhanced DMI, body weight, and N retaining capacity in animals. Various flavonoids have been found in Moringa oleifera and *M. stenopetala*, but little is known of other moringa species (Bennett *et al.*, 2003; Siddhuraju and Becker, 2003; Lako *et al*., 2007; Manguro and Lemmen, 2007).

2.2.5 Phytochemicals in Tropical Plants and their uses

Under certain conditions, plants generate non-nutritious chemicals that are used as protective characteristics or preventive characteristics for disease.

In a research, Mohammed (2008) proposed that phytochemicals are widely distributed in crops and are not directly engaged in any metabolic process. It has also been stated that some

phytochemicals play a function as defense chemicals, protecting the plant from damaging herbivor assaults, pathogenic fungi, and parasitic weeds (Mohammed, 2008).

Although plant uses phytochemicals as a defensive mechanism, study has lately shown that they can also be used to treat illnesses. Similarly, phytochemicals recognized in grains are indicated to avoid grain loss owing to premature germination and mold harm (Harris and Burns, 1970), those found in grass or plant act as a protection against insects and livestock. Phytochemicals are grouped as flavonoids, phenolic acids and condensed polymeric phenols (tannins) (Dreyer *et al.*, 1981).

Moringa leaves were discovered to be free of trypsin inhibitors but had a comparatively high saponin content (up to 8%) (Makkar *et al.*, 1997). Moringa plant had a limited amount of cyanogenic glucosides (Makkar *et al.*, 1997). The latter study did not notice glucosinolates in the leaves and only trace quantities in the plants (leaves, stems), but subsequent studies involving various analytical methods showed important quantities of glucosinolates (Bennett *et al.*, 2003; Amaglo *et al.*, 2007; Bellostas *et al.*, 2010).

There is currently a high demand for natural materials containing active substances such as phytochemicals for different purposes (Godinez-Oviedo *et al.*, 2016) as food, feed or medicine.

M. oleifera's varied components are a useful source of different phytochemicals (Anwar *et al.*, 2007; Verma *et al.*, 2009; Mbikay, 2012; Umesha *et al.*, 2013; Hussain *et al.*, 2014; Valdez-Solana *et al.*, 2015).

The phytochemicals provide *M. oleifera* with healing characteristics (Gopalakrishnan *et al.*, 2016). *M. oleifera* is reported to have many beneficial impacts, such as antioxidant (Vongsak et al., 2013), anti-tumor (Khalafalla *et al.*, 2010), anti-inflammatory (Rajanandhet *et al.*, 2012),

antidiabetic (Jaiswal *et al.*, 2009), anti-bacterial (Moyo *et al.*, 2012b), hypolipidemic (Sangkitikomol et al., 2014), immunomodulatory (Sudha *et al.*, 2010), hepato-protective (Al-Said *et al.*, 2012). Consideration of the bioactive compounds present in *M. olerfera* could be a major source of antibiotics for animals fed on the forage.

Some study groups have suggested that condensed tannins maybe absent (Makkar *et al.*, 1997) or present in minor quantities (Bakhashwain *et al.*, 2010; Moyo *et al*., 2011) in *M. oleifera*. The study by Afuang *et al*. (2003) reported that antinutritional factors were not observed in *M. oleifera* leaf meal when ethanol was used for the extraction in the laboratory. Tannins are one of the harmful phytochemicals to animals. Tannins are well defined as water soluble phytochemicals that naturally occur differing in molecular weight from most other natural phytochemicals (Spencer *et al.*, 1988).

Makkar (1993) reported that tannin content from Indian tropical crops (in 62 species). Tannins are not restricted to tropical feeds alone, some species have been reported to condensed tannins (Waghorn *et al.*, 1990). It is very obvious that the incident of tannins is common to most class of plants. Bhat *et al*. (2013) indicated that these crops are probable to be consumed in all farming systems where biomass from trees and shrubs is used as animal feed in industry.

There are even more general hydrolysable tannins in components of plants (leaves, fruits, flowers and galls) and condensed tannins (Kumar and D'Mello, 1995).

Previous study by Bhat *et al.* (2013) proposed that tannin in crops may differ from plant species, genotype, and maturity phase and may differ with plant components (leaves, stems, seeds), growing season, and other particular environmental variables. However, their susceptibility to form chemical complex not only with proteins but also with many other compounds, such as

polysaccharides, nucleic acids, steroids, alkaloids and saponins, is vital (Kumar and Vaithiyanathan, 1990; Mueller-Harvey and McAllan, 1992).

2.3 Biomass Yield Production of *M. oleifera*

2.3.1 Effect of Organic Manure on Biomass Production of *M. oleifera*

The modification of organic manure to soil, improving physical properties, increases nutrient availability, organic carbon, soil cation exchange capacity and increases crop yields (Beaulah, 2001). It has been pointed out that poultry manure is a very inexpensive and efficient source of nutrients, particularly nitrogen (N), but ready accessibility remains a significant problem as large proportions have to be applied to provide optimum output (Aluko, 2017). The shooting height of crops adjusted with poultry manure was the lowest in duration relative to those adjusted with cowdung tests and controls (Imoro *et al.*, 2012).

The quantifiable traits and growth parameters such as plant height, number of branches, number of pods, pod weight, total dry matter production contribute for the economic yield of Moringa plant. The improved growth rate till maturity might be attributed to absorbed nitrogen combining with carbohydrate synthesis, which might lead to the formation of complex nitrogenous substances such as protein, amides to build up new tissues (Childers, 1966).

According to Beaulah (2001) organic manure used for soil amendment resulted to high plant height and. In contrast to the above, treatments comprising combinations of organic and inorganic fertilizers had increased the plant height remarkably. The highest plant height of 3.54 and 4.31 m was recorded during harvesting stage in main and ratoon crop respectively, when poultry manure + Neem cake + Panchakavya + Increased dose of inorganic fertilizers were applied. The highest enhancement in stem thickness under the best combination of organics and inorganics was only up to 36.83 and 57.70 cm in main and ratoon crops. From the above

findings, it is that the organics or inorganics or their combinations have little influence on stem thickness in Moringa.

2.3.2 Effect of Plant Density on Biomass Production

Nadir et al. (2005) noted that competition between crops did not actually affect output of biomass in the first trial, but reduced production of biomass in the second trial, probably partly due to higher competition between crops. It has also been proposed that the higher the plant density, the higher *M. oleifera's* total above ground dry biomass yield over harvest times with respect to places, although the impact on plant height may not be acknowledged at each harvest (Mabapa et al., 2017). The yields of crops may be contributed favorably or negatively by climate and environmental variables. It was emphasized that low temperature and dry conditions adversely influenced the biomass output of Moringa oleifera plants in two distinct locations before the biomass was harvested (Mabapa et al., 2017). It was found that low rainfall conditions attributed to stunted regrowth and reduced biomass yield, and vice versa during the rainy season. (Sanchez *et al.,* 2006; Fadiyimu *et al.,* 2011; Mendieta-Araica *et al.,* 2013).

M. oleifera, planted at greater densities, has been recommended to yield better than reduced densities when irrigated and fertilized (Foild *et al.,* 1999; Foild *et al*., 2001). For different plant species, literature has recorded a strong correlation between plant spacing and yield (Ella *et al*., 1989; Blair *et al.,* 1990; Stür *et al.,* 1994). The plants compete for sunlight, nutrients and water absorption at tight spacing, which can lead to decreased yield per plant while this decrease can be stable on complete yield per unit region (Ella *et al*., 1989; Norman, 1992).

A positive correlation between increased planting density and complete yield of *M. oleifera* was noted by Foidl *et al.,* 2001. Studies on the response of spacing and harvesting regimes to *M. oleifera* growth and foliage yield have been reported to improve the output of biomass per

hectare due to density but not due to interval cutting (Amaglo *et al.*, 2006). According to Latt *et al*. (2000), the frequent harvesting of plants reduces the nutrient assimilation that affects the growth of plants by affecting the leaf development observed for 15 days of cutting intervals.

The use of tropical forage plants and shrubs has been disclosed as an effective means of advancing the supply and quality of forage in smallholder animal systems during the dry season (Robinson *et al*., 1985; Gutteridge and Shelton, 1994).

It is therefore vital to find the ideal time to cut the plant in order to achieve quality forage biomass (Maass *et al*., 1996). Longer cutting frequency has been reported to boost the output of biomass (Guevara *et al.*, 1978; Ella *et al*., 1989; Assefa, 1998; Barnes, 1999; Latt *et al.*, 2000; Tuwei et al., 2003). As a result, frequent cutting will reduce the output of biomass (Ezenwa and Atta-Krah, 1992; Romero *et al.*, 1993; Nygren and Cruz, 1998).

2.3.3 Effect of Moisture on Biomass Production of *M. oleifera*

According to Mabapa *et al.* (2017) biomass continued to increase under favourable weather conditions (temperature and rainfall) at different harvest regimes. This is an evidence that moringa can survive and produce satisfactory yields even under conditions of water limitation. It was reported by Huda (2016) that regardless of the treatment, the biomass production (BM) and dry matter (DM) yield per hectare of *M. oleifera* during first and second cutting was 2604.82, 465.01kg and 1387.85, 239.70 kg, respectively. In spite of minor production in second harvest regime; the biomass production and DM yield during whole autumn season was 3.99 ton/ha and 0.7 ton/ha. Also, it was noted that the average temperature and humidity during first and second harvest regimes were 27 °C and 81 % and 24 °C and 60 %, respectively. However, the weather conditions before the first harvest has improved the biomass yield as compared to the second

harvest. Also, the reduced temperature, humidity and zero precipitation in second harvest regime may be the cause of lesser production of *M. oleifera* compare to first harvest.

Mabapa *et al.* (2017) also, observed that during the early growth stage, a higher plant density sustained higher gravimetric soil moisture at two study areas. However, with time, the gravimetric soil moisture content was decreased as plant competites for light, space, and dwindling moisture concentration among the plant densities. This finding concurs with those of Amaglo *et al*. 2006 and Gadzirayi *et al.* 2013 who showed that relatively close plant spacing resulted in taller plants because of competition for growth factors, especially light and space. These observations indicate that proper field management of soil moisture and fertilizer is necessary in order to supply plants with sufficient nutrients and moisture to reduced competition among the plants.

2.3.4 Effect of Harvest Regimes of *M. oleifera* on Phytochemicals

Studies on *M. oleifera* phytochemicals (leaves) has been made and different phytochemicals were identified (Karim *et al.*, 2015). However, the maturity phase of the *M. oleifera* (leaves) used for the research was not reported by these researchers. It was later revealed that tannins were discovered to be negligible in *M. oleifera* (leaves). Adejuwon and Tsygankova (2019) reported that *M. oleifera* leaves contained phytochemicals in varied concentration and most tested leaves contained high of saponin, alkaloids and flavonoids but considerable quantity of tannins and carotenoids. Different researchers Gupta *et al.* (1989) concluded that unextracted leaves (*M. oleifera*) recorded 2.7 % Total phenol, negligible amounts of Total Tannin (1.4 %) and Condensed tannin was not noticeable. Previous studies by üstündağ and özdoğan (2016) proposed that leaf meal from Moringa oleifera might partly replace costly sources of protein.

However, care must be taken to use *M. oleifera* leaf to feed livestock as it includes high proteins of bioavailability, otherwise it may be hazardous for livestock (Gaia, 2005). Moringa's palatability is considered average when properly linked with separate species of shrubs and tree, Moringa leaves were eaten by livestock only fairly (Garcia *et al.*, 2008c; Garcia *et al*., 2008d; Toral-Perez *et al*., 2008).

While the DMI of Moringa oleifera leaves was similar or greater than that of leucaena when used as a single supplement in a concentrate in feed for cattle (Leucaena leucocephala) or gliricidia (Ndemanisho *et al.,* 2007; Asaolu *et al.,* 2012). Its protein and organic matter are freely digestible in the rumen and in the intestines (Makkar *et al.,* 1997; Makkar *et al.,* 1996; Kakengi *et al.,* 2005; Ndemanisho *et al.,* 2007; Gutierrez *et al*., 2012).

However, the information available are extremely variable, with digestibility ranging from 40 to 80% in vitro and in vivo organic matter, probably due to the high variation in fiber content. The leaves and stems of *M. oleifera* contain small quantities of tannins and condensed tannins. (Makkar *et al.,* 1996; Sarwatt *et al.,* 2002; Aregheore, 2002; Murro *et al.*, 2003; Bakhashwain *et al.,* 2010).

The glucosinolate concentrations observed in moringa leaves have not impaired ruminant nutrition (Bennett *et al.,* 2003; Amaglo *et al.,* 2010). However, the leaves contain saponins, which may impair palatability. The fermentable nitrogen and energy in Moringa leaves was reported to encourage microbial protein synthesis (Soliva *et al*., 2005). Rumen in sacco DM leaf degradability varying from 82 to 95.6% (Garcia *et al*., 2008d; Ndemanisho *et al*., 2007; Sarwatt *et al.,* 2004). However, in distinct animal species and distinct pore sizes of nylon bags, reduced values (less than 70%) were observed (Gutierrez *et al*., 2012; Garcia *et al*., 2008a).

In Nigeria, the inclusion of *M. oleifera* leaf meal in animal diets declined their metabolizable energy content, OM digestibility and short chain fatty acid production (Tona *et al.,* 2013). Animal production sustainability comes with sufficient supply of excellent protein and fiber content sources. Most of these sources in smallholder farmers are extremely suggested for monogastrics to assist save feeding and manufacturing costs.

2.3.5 Effect of Harvest regimes on Chemical Composition *M. oleifera*

According to Sanchez *et al*. (2006) both the first and second year, harvest of *M. oleifera* did not influenced the crude protein (CP) content and IVDMD. Similarly, Ventura and Pulgar (1997) observed that the crude protein (CP) content was not significantly different between plant densities while total nitrogen and IVDMD declined progressively (Assefa, 1998; Nygren and Cruz 1998). Sanchez and co-workers suggested that at longer harvest regimes, the CP and IVDMD remained high. This was earlier explained that N content in the leaves and young stems usually do not lessening with maturity (Miquilena *et al*., 1995).

Sanchez *et al*. (2006) reported that CP content and IVDMD of *M. oleifera* was within the range of 193–264 CP gkg^{-1}DM and 648–790 gkg^{-1}DM, respectively, reported for Moringa by other workers (Makkar and Becker 1996; Foidl *et al.* 1999; Aregheore 2002; Al-Masri 2003; Manh *et al.* 2003).

However, neutral detergent fibre (NDF) concentrations in two (2) years were affected significantly by harvest regimes, while the acid detergent fibre (ADF) concentration was affected in the second year of the study. Young stems are generally of high quality, on the other hand, the quality falls faster than in leaves, since epidermis and fibrous cells change into secondary cellular wall and lignin content increases with increased age of the plant. Different researchers have reported different values for NDF and ADF contents, however, all the values were within

the range of 151–564 g kg^{-1}DM and 92–515 gkg^{-1}DM respectively, at different harvest regimes (Makkar and Becker, 1996; Foidl *et al.*, 1999; Aregheore, 2002; Al-Masri, 2003; Sanchez *et al.*, 2006). The differences in agro-climatic conditions, soil type and fertilisation, age of trees, stage of maturity of the leaves, different parts of the plant sampled may have resulted to some differences in the observed values (Sanchez *et al.*, 2006).

The ash content reported under different harvest regimes for *M. oleifera* was within the range of 88–134 g kg^{-1} DM (Makkar and Becker, 1996; Al-Masri, 2003). Different factors may influence the concentration of mineral elements in plants, e.g. minerals in the soil and their accessibility to the plant, soil type and soil pH and stage of growth (Lukhele and Van Ryssen, 2003).

2.4 *Moringa oleifera as Forage*

Some plant leaves have been used as feedstuffs for rabbits and other animals as a partial substitute for the conventional grains and forages. *Leucaena leucocephala* has been successfully used in rabbit diets when incorporated in low levels.

A twenty (20) week feeding trial was conducted by Odeyinka *et al.* (2008) to investigate the reproductive performance of rabbits fed *M. oleifera* as a replacement for *Centrosema pubescens*. Freshly harvested *C.* pubescens and *M. oleifera* leaves were offered to the animals at 20% of their live weight at the ratios of 100:0 (MO), 75:25 (M25), 50:50 (M50), 25:75 (M75), and 0:100 (M100), in addition to the concentrate feed offered to the animals. There were

significant differences in the total DM intake, litter size at weaning, average daily weight gain per kid and milk yield of does, on the different treatments ($P<0.05$). However, there was no significant difference in crude protein intake, initial average body weight, and gestation length as well as litter weight at birth. Odeyinka *et al.* (2008) concluded that *M. oleifera* can be used to replace Centrosema pubescens without adverse effects on the reproductive performance of rabbits. Little is known on the use of the tender stem and petiole of Moringa plants in feeds for livestock's especially monogastrics.

Another experiment was investigated to determine the nutritional potential of two leafy vegetables (*M. oleifera* and *Ipomoea batatas*), Oduro *et al*. (2008) reported that *M. oleifera* leaves contained crude protein 27.51%, crude fibre 19.25%, crude fat 2.23%, ash 7.13%, moisture 76.53%, carbohydrates 43.88%, and caloric value 1296.00 kJ/g (305.62 cal/g). Calcium and Iron content in mg/100 g (DM) were 20.09 and 28.29, respectively. It was noted from their findings that either the combination of the two or one could help improved the growth performance of rabbits considering the available nutrients in the plants.They concluded that *M. oleifera* leaves could contribute to the nutrient requirements of humans and should be strongly recommended in Ghana.

2.5 Nutrient Digestibility of *M. oleifera* in farm Animals

Some plant leaves have been used as feedstuffs for rabbits and other animals as a partial substitute for the conventional grains and forages. *Leucaena leucocephala* has been successfully used in rabbit diets when incorporated in low levels. *Moringa oleifera* appears to have the potential as a forage for animals to improve and maintain high nutritional quality (Table 2.2 and Table 2.3). In literature, the dietary value and structure of distinct trees and shrubs have been

heavily acknowledged to reveal their significance to the animal sector (D'Mello and Devendra, 1995).

Table 2.2 Comparative analysis (%) of forage quality of stem and leaves of M*oringa oleifera* as tree and fodder crop

Moringa parts/fractions	DM	CP	EE	CF	Ash	NDF	ADF
Moringa fodder leaves	7.8	21.87	6.5	4.5	12	8	6
Moringa fodder stem	8.0	8.75	2	20	12.5	28	21
Moringa fodder leaves and stem	7.6	15.31	3	14.5	12	16	12
Moringa tree leaves	8.6	23.51	3	7.5	13.5	11	6
Moringa tree stem	8.6	10.93	1	26.5	10.5	36	26
Moringa tree leaves and stem	9.2	16.41	2.5	17.5	11	21	15

DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Source: Nouman *et al.* (2014).

The leaves of *M. oleifera* were discovered to be very small in nitrogen free extract but had considerable amounts of crude protein relative to other forages used (Owoleke *et al.*, 2016). The 7.0–8.0 g/100 g DM range was proposed as a critical limit below which ruminant and microbial consumption of forage would be adversely impacted in the rumen (Van Soest, 1994).

However, in comparison with some other browse grass, some tropical plant such as Moringa oleifera has been noted as much preferable as forage. It was reported earlier that diverse morphological parts of *M. oleifera* contain various levels of CP (71.2-267.9 gkg-1DM), crude

fiber (210.0-490.0 $gkg^{-1}DM$), NDF (48-842 g $kg^{-1}DM$), ADF (39-805 $gkg^{-1}DM$) and ADL (11-452 $gkg^{-1}DM$) (Makkar and Becker, 1997).

According to Blais *et al.* (1999), the content of nutritional lignin is negatively associated with digestibility of energy and digesta accumulation in caecum. The researchers also clarified that extreme and inadequate concentrations of nutritional fibers lead to a weakening of the efficiency of the rabbit.

It was later concluded that there are recommendations for optimum fiber concentrations and the least quantity of large particles, possibly given for breeding, rabbit fattening and mixed diets (Blais *et al.*, 1999).

The nutritional value of Moringa pods, fresh (raw) leaves and dried leafy powder are presented in Table 2.3. It could be observed that the nutritional value varied among the various parts of Moringa pants as reported (Booth and Wickens, 1988; Nouman *et al.*, 2014).

Table 2.3: Analysis of nutritional value of Moringa pods, fresh (raw) leaves and dried leafy powder per 100 g of edible portion.

Components	Pods	Leaves	Leaf powder
Moisture (%)	86.9	75.0	7.5
Calories	26	92	205
Protein (g)	2.5	6.7	27.1
Fats (g)	0.1	1.7	2.3
Carbohydrates (g)	3.7	13.4	38.2
Fibre (g)	4.8	0.9	19.2
Minerals (g)	2	2.3	-
Ca Calcium (mg)	30	440	2003
Cu Copper (mg)	3.1	1.1	0.57
Fe Iron (mg)	5.3	7.0	28.2

K Potassium (mg)	259	259	1324
Mg Magnesium (mg)	24	24	368
P Phosphorus (mg)	110	70	204
S Sulphur (mg)	137	137	870
Oxalic acid (mg)	10	101	1600
Vitamin A (mg)	0.11	6.8	18.9
Vitamin B (mg)	423	423	-
Vitamin B1(mg)	0.05	0.21	2.64
Vitamin B2 (mg)	0.07	0.05	20.5
Vitamin B3 (mg)	0.2	0.8	8.2
Vitamin C (mg)	120	220	17.3
		AMINO ACIDS	
Arginine (mg)	90	402	1325
Histidine (mg)	27.5	141	613
Isoleucine (mg)	110	422	825
Leucine (mg)	163	623	1950
Lysine (mg)	37.5	288	1325
Methionine (mg)	35	134	350
Phenylanaline (mg)	108	429	1388
Threonine (mg)	98	328	1188
Tryptophan (mg)	20	127	425
Valine (mg)	135	476	1063

Source: Booth and Wickens (1988)

2.6 Rabbit Production

Recent global knowledge of the merits of the production of rabbit meat exists (Omole et al., 2008; Ansah *et al.*, 2014; Sánchez-Laiñ *et al.*, 2018). This issue has made a significant contribution to the sector's growth since the first domestication of the European rabbit.

2.6.1 Domestication and Benefits of rabbit farming

The European rabbit (*Oryctolagus cuniculus*) advanced in the Iberian Peninsula and southern region of France (Callou et *al.*, 1996). Domestication occurred in the 16th century during the Middle Ages, rabbits were raised in hutches in distant monasteries (Sandford, 1992). The production of hutch raised rabbits in the backyard was commonly used by some families throughout Western Europe and Northern Africa (Lebas *et al.*, 1997). The domestication of

rabbit was mainly used for the provision of meat (Payne and Wilson, 1999) and household income (FAO, 2001). Rabbit meat is high-quality protein and low fat relative to other meat animals (Holmes *et al.*, 1984). Furthermore, rabbit meat has low concentrations of sodium and cholesterol (Owen, 1976; Lebas *et al.*, 1997), making it safe for fat and other associated illnesses. Rabbit production is also suitable for smallscale farmers and could minimize dietary imbalance in most emerging world populations (Vorster and Hautvast, 2002).

Rabbit slaughter rates differ by race, age, and dietary level (Mississippi Agricultural and Forestry Experiment Station, 2010). It was discovered that Dutch rabbit had a greater dressing proportion (63.3%) when related to New Zealand White (NZW) (59.9%). Regarding age, when it started to decrease, dressing proportion of rabbits risen to 13 weeks of age. It was earlier revealed that higher-nutritional level rabbits (NDF and high CP) yielded more than 60% of carcass yields compared to lower-nutritional yields. Thus, there are several reasons that make rabbit suitable meat sources; proverbial prolificacy, early maturity, rapid growth rate, high genetic selection potential, high feed conversion efficiency, and economic space utilization (Lukefahr and Cheek, 1991; Hassan *et al.*, 2012).

Establishing a rabbit business needs low inputs and has been a vehicle for empowering females living in poverty in certain developing countries such as Cameroon (Lukefahr *et al.*, 2000). Rabbit production poses minimal economic risks compared to other animal businesses such as dairy, enabling resource poor families to either downscale or upscale the company without incurring heavy losses.

2.6.2 Challenges facing Rabbit farming

The difficulties facing small-scale rabbit farmers in Africa are regular rabbit feed shortages, absence of regular sources of quality genetic reserves (Oseni *et al.*, 2008), bad housing (McNitt *et al.*, 2000), high rabbit disease levels (Schiere, 2004).

Insufficient market access connected with low consumer supply due to absence of knowledge of the advantages of the rabbit (Nyete, 2000; Schiere, 2004). The primary marketing limitation is also the low acceptance of rabbit meat (Schiere, 2004). For example, Luzobe (1997) pointed out that only 35.5% of Ugandans had eaten rabbit meat and, as Lukefahr (1998) suggested, this could be due to the fact that the rabbit looks morphologically like rats that are not traditionally consumed. Perhaps this challenge is quite distinct from other nations like Ghana. Although little is known about rabbit requirements and consumption, some researchers point out that there are plenty of opportunities for rabbit manufacturing in Northern Ghana (Ansah *et al*., 2014).

2.6.3 Rabbit Breeds

Rabbit breeds are phenotypically characterized (for example shape, body size, and color of the coat) (Lebas *et. al.*, 1997). Using this classification basis, the American Rabbit Breeders Association (ARBA), (2010, 2012), has recognized 47 separate rabbit breeds, only a few of which are maintained in some African nations (MOLD, 2010).

Table 2.4 Rabbit Breeds, live weight, purpose, origin and description

Breed name	Weight	Purpose	Origin	Color description
New Zealand White	Buck 4.5kg Doe 5kg	Meat	America	All-white rabbit with red eyes

The Californian	Both buck and doe 4.1Kg	Meat	America	All white except for ears, nose, feet And tail, which are a dark gray or black.
The Dutch	1.6-2.5kg	Exhibition purposes	Holland	Grey, brown/ black body with white belt around the neck/shoulders
The Rex	Buck 3.6kg does, 4kg.	Meat/fur	America	Grey body with or without white spots
Checkered giant	5 kg	Meat	Germany	Either black or blue-spotted rabbit
Chinchilla	2.3–3.4kg	Fur	France	Under-color slate blue at the base, the middle portion pearl grey, merging into white and tipped with black much like the chinchilla
Flemish giant	5-7 kg	Meat	America	Vary from silver grey to black color
French lop	5-6 kg	Meat	France	White, grey black body with drooping ears hence the names lop.
Angora	2.5-4Kg	Fur	America	Vary from white, grey and brownish long fur covering the head, neck and body

Source: American Rabbit Breeders Association (2007)

Rabbits are classified as: small rabbits (at maturity about 1.4 – 2 kg), mediumsized races (4 – 5.4 kg), and large races (6.4 – 7.3 kg) (USDA, 1972). White New Zealand and white California are races of medium size. Because of their excellent growing features and also a quality meat: bone ratio (Oseni *et al.*, 2008; Mailafia *et al.*, 2010), these breeds are widely used in meat production.

The NZW is well recognized as a breed of dams based on its exceptional maternal genetic benefits for litter size, milking and nurturing qualities (Lebas *et al*., 1997; McNitt *et al.*, 2000). The excellent characteristics of both races are due to their accurate choice for reproductive results of better quality (King, 1978; Owen, 1981).

It have reported that rabbit breeding experiments in the U.S.A. have acknowledged the NZW as commonly lesser to crosses for post weaning fryer growth, feed use and carcass lean yield traits (Ozimba and Lukefahr, 1991; Lukefahr *et al*., 1992). Additional common meat breeds are

Flemish giant, French lop and Checkered giant mainly due to their large size. Smaller breeds, are mostly kept as pets and include Chinchilla, Dutch and the Angora (Moreki, 2007).

In a study comparing growth performance and mortality rates amongst the common meat rabbit breeds, purebred New Zealand white and Californian litters performed in the same way but the crossbreds of the two were superior for the above traits compared to the purebreds (Ozimba and Lukefahr, 1991). However, in most developing countries, the practice of unselective crossing with supposed better exotic breeds has often led to the dilution or loss of the adapted breeds (Boulet, 1999). In Africa, a major constraint to the growth of small-scale rabbit production was reported to be the absence of consistent sources for quality genetic stocks of rabbits (Oseni *et al.*, 2008).

2.6.4 Growth potential of rabbits

Growth rate in rabbit production is a significant parameter considered (Chen *et al.*, 1978). Growth rate depends primarily on breed type, birth weight and maternal (nutrition and milk production) effects (Vakulenko, 1985). Previous study showed that the doe properties are significant variables that influence body weight at birth and up to weaning (Lebas et al., 1997). The weight of most young rabbits risen to an average of 119-308 over the first three (3) weeks of nursing (Zerrouki *et. al.*, 2007).

2.6.5 Rabbit Housing

Housing is a major factor in the production of rabbits (Mailafia *et al.*, 2006). Housing is designed to safeguard rabbits from negative weather, predators, ectoparasites and endoparasites (Hoy, 2006). Poor housing is recognized as prevalent to some developed countries, affecting rabbit development (McNitt *et al.*, 2000).

Mortality, morbidity, physiological parameters, rabbit behavior and production efficiency are indicators for assessing the suitability of rabbit accommodation (Hoy, 2008). House floors, walls, wire mesh and other materials or machinery should not cause pain, suffering or injury to the rabbit.

In addition, the housed rabbits should be supplied with appropriate feed and water to meet their nutritional needs. Clean and well ventilation should be ensured in order to prevent the accumulation of toxic gasses such as ammonia (Hoy, 2008). As far as individual cage housing is concerned, rabbit behavioral patterns change and this is suggested as a result of the decreased room that hampers free motion and decreases the likelihood of social interaction (Lehmann, 1987; Szendro and McNitt, 2012). It is essential that rabbits have sufficient room to decrease conflict between people and prevent abnormal conduct regardless of the type of housing (Lehmann, 1987).

2.6.6 Diseases of rabbits

The immunomodulating impact of the *M. oleifera* leaf and its extracts has been recorded (Rajeshwari *et al*., 2008). According to Isitua *et al.* (2013) aqueous extracts of *M. oleifera* leaves fed to male rabbits (5 ml/d/head) for 30 days increased CD4 cells, blood insulin concentration and increased enzymatic activity. The largest cause of rabbit production and mortality decrease is the incidence of disease. In combination with feeding, rabbit health determines body condition score and breeding performance (Sanchez *et al*., 2012).

Rabbits are primarily prone to nutritional deficiencies and contaminated feed ingestion (mycotoxins) circumstances (Szilagyi *et al.*, 1994). Aflatoxins are generally discovered in low-cost rabbit feed (like maizemilling waste) and when eaten, these toxins cause oxidizing proteincausing immune suppression to the body tissues of animals (Kumar *et al.*, 2008).

Coccidiosis is also a prevalent viral cause of digestive disorders in commercial production, caused by a protozoan Eimeria genus parasite that is supposedly always present in rabbit farms and is nearly impossible to eliminate (Vancraeynest *et al.*, 2008).

Earlier, it has been revealed that eating is a widespread disease that affects rabbits and is a skin parasite condition influenced by Sarcoptes scabiei, leading to scab formation and alopecia as a consequence of pruritis (Merck, 2010).

This results in loss of appetite, body condition, and growth rate stunts (Merck, 2010). Mange is also prevalent in Africa's rabbits (Adu *et. al.*, 2008). Infections with pinworms and ear mites also occur in rabbits (Rai, 1988).

2.6.7 Rabbit Meat Consumption

Rabbit meat production is a quickly increasing sector, which is forecast to boost by 38.3% (1990 and 2009) over the decade (FAOSTAT, 2010). Most of the world's rabbit production methods in middle-and-low income countries are focused on home consumption and as a good source of revenue similarly significant (FAO, 2001). China is regarded as the world's largest producer and consumer of rabbit meat with other major manufacturers: France, Spain, Italy and Egypt (EFSA, 2005). Consumer demand for rabbit meat was low compared to other meats (Hoffmann *et al.*, 1992) and supply for rabbit meat has also increased in South Africa (Hoffman *et al.*, 2004). However, a study showed that rabbit meat markets exist in Nigeria but are not structured and this is attributed to consumer-oriented production processes (Adu *et al.*, 2008).

2.7 Alleviation of Detrimental phytochemicals in feeds

To overcome the impacts of harmful phytochemicals in forages, a collection of medicines and feeding techniques have been created, however, there is limited documentation on *Moringa oleifera* forages to promote the plants nutritional significance as a forage. The approaches to

minimizing harmful phytochemicals have been revealed to be based on the knowledge that some phytochemicals are water soluble polymers (form complexes), basically protein-based (Mueller-Harvey, 2006).

These were further clarified that under high acidity (pH 3.5) and high alkalinity (pH 7.5) the compound created is broken. The prevalent techniques reported were physical, chemical and biological techniques, most of which were established at the laboratory stage and tested on some forage products in animal experiments. The approaches directed at inactivating or removing harmful phytochemicals from tanniniferous feed, saponins, alkaloids and energy from forage.

2.8 Feed preferences and feeding systems of rabbits

The appropriate use of various byproduct feed resources by the rabbit has been created (Abu and Ekpenyong, 1993; Onifade and Tewe, 1993). However, various studies on the relative effects of feeding diets based on agroindustrial products and maize have been acknowledged as a predictable feed component on quantitative nutrient consumption, nutrient digestibility estimate and the resulting growth efficiency (Onifade *et al.*, 1998; Dougnon *et al.*, 2012; Ansah *et al.*, 2014).

Feed is generally a crucial factor that plays a vital role in the development of animals inshowing their genetic potentials. The composition, timing and feeding managements all affect the growth quality of individual animals.

In particular, rabbits are really selective in feeding behaviour, and particular plant components are selected in the wild. In general, rabbits choose new leaves preferred to stems and young plant materials are preferred to dry ingredients rather than old and green, leading in a diet that is greater in protein and digestible energy and lower in fiber than the complete offered plant material (McNitt *et al*., 2000).

Rabbits are susceptible to modifications in feed than other animals, but they may refuse to accept a fresh diet and starve to death instead of accepting the fresh feed for several days. (McNitt *et al.,* 2000). Ideal growth performance can be achieved by feeding forages or leaf meals focused in rabbit diets. (Safwat *et al.,* 2014).

Earlier study by Lukefahr and Cheeke (1997) indicated that in comparison with cattle and sheep, rabbits can use herbal biomass more effectively. This is one of the reasons why rabbits can be grown effectively on diets composed entirely of forages (Cheeke, 1986). This is a significant feature in African subsistence farming systems where concentrate diets are very costly and often include components that are directly used as human food (Akinmutimi, 2007). If palatable greens are fed adlibitum concentrate can be decreased by 50% without ill effects on rabbit performance (Cheeke *et al.,* 1987).

Tropical crops contain significant amounts of protein, fat, minerals and carbohydrates that can promote development and production, many of which have antinutritional factors on animals' ddigestibility (Safwat *et al.,* 2014). It was mentioned earlier that it is not appropriate to mix approximately unprocessed feedstock because of the large ability of the rabbits to select some part of a mixture, eat only the palatable and then destroy the formulated nutritional balance (Lebas, 2013).

In addition, if a rabbit finds the most appealing part, it scratches inside the feeder and could waste up to 40-50% of the dispersed blend (Lebas, 2013). Common practice is to allocate dry concentrates (cereals or cakes) separately, in limited amounts in the morning. This makes it easy to control the actual intake visually. The quantity to be dispersed must be fully consumed within 6-7 hours. Under many circumstances, concentrated raw materials could be economically replaced by proper rabbit feeds, if possible pelleted. In this condition, it is appropriate that the

pellets do not represent more than 40-50% of the daily ration of dry matter, the other part being made up of different forages. It should be noted that in no condition can a balanced diet be constituted by rabbit pellets + cereals, particularly for reproduction.

Some new forages may be distributed throughout the evening, in boxes but not on the ware house floor, in such an amount that only very few or none of the amount distributed remains in the rack the next morning. It is necessary to throw away the remaining part. Dry forages, if used at the same time as greens, could be separated ad libitum but in a separate portion of the tray. Forestry, the primary source of fiber for rabbits, is not necessarily available in green form throughout the year with an ideal nutritional value (Lebas, 2013). Subsequently, dried forage production and storage is allowed for small-scale farmers. Fresh leaves can be collected in small quantities, day after day, in rainy season or at the start of the dry season, and dried in the vicinity of the farmer's house. Various trees and shrubs (such as agriculture by-products, water plants etc.) can be used for feeding animals in tropical areas (Chiv-Phiny, 2007). Water spinach, Mulberry and Cassava leaves, and seeds of sweetpotato, for instance, are rich in protein and good energy sources.

Various researchers have also recommended that *M. oleifera* leaves be a useful source of ruminants and even monogastric livestock (Dougnon *et al.,* 2012). It has been noted previously that forage crops often have low nutritional quality and high fiber content that can adversely affect feed consumption and thus decrease nutrient supply for monogastric animals (Cheeke *et al.,* 1980). Lukefahr (1992) reported on appropriate sources of feed and fundamental main dietary requirements and proposed that feed can be acquired from available sources. The examples of these sources are; wild, native seedlings, cultivated forage plots, farm crop residues,

farm surplus food, agricultural by-products, kitchen waste and market sources (Lukefahr, 1992; Ansah *et al.*, 2014).

Pound *et al.* (1984) proposed that an accurate strategy for small-scale farmers would be to develop trees, shrubs and water plants that generate much greater unit area protein yields in the form of leaf biomass rather than traditional protein crops like sunflowers, soybeans and groundnuts as parts of their farming schemes. Ansah *et al.* (2014) also recorded products such as corn mill residue, milled mango seed kernel, spent grain brewers and maize bran on various agro-industrial products.

It was subsequently suggested that 70% of maize bran could be used in the rabbit diet without any negative impacts (Ansah *et al.*, 2014). Approaches for the efficient use of these substitute feeds are more likely to succeed if the production system matches the available funds (Preston and Leng, 1987). Aside, good management practices and balance of diets is essential for rabbits (Aduku and Olukosi, 1990; Safwat *et al.*, 2014). Although rabbit production in Africa is generally considered lowcost feeding owing to locally accessible forages (Mailafia *et al.*, 2010), rabbits can also be enhanced with concentrate (bought or produced locally) which will improve productivity and breeding. Water limitation to 24 hours has been recorded to decrease growth rates (19-11%) of ad libitum access (Boisot *et al.*, 2004). Limiting water access was observed to adjust the consumption of freshly sewed rabbits resulting in lower mortality rates (Adjare, 2003; Verdelhan *et al.*, 2004; Bovera *et al.*, 2013).

However, Rayana *et al.* (2008) noted that increasing rabbits' feed consumption and weight gain decreased with the degree of limitation and that there were no variations in increasing rabbit's mortality rates. *M. oleifera* (Fresh leaves), with mild fiber content, tends to be rich in protein. Smallholder rabbit farmers in Benin and Ghana use the plant effectively as a traditional nutrient

forage and may be beneficial in the dry season in the absences of some forages (Adoukonou, 2014).

Nigerian report showed that Moringa leaves are used as a normal forage fed (with concentrate) in rabbit research (Ola *et al.,* 2013). Moringa leaves raises breeding rabbit's body weight by 2% with a concentrate relative to fresh *Centrosema pubescens* and *Tephrosia candida* (Odeyinka *et al.,* 2008; Ola *et al.,* 2012). Because rabbits eat new leaves and tender stems (Osei et al., 2012), they could be used successfully for the routine pruning proposed for increasing *M. oleifera* forage production (Palada *et al.,* 2003). A slight but remarkable reduction in serum glucose and serum cholesterol concentration was correlated with *M. oleifera* leaf meal (Rajeshwari *et al.,* 2008; Ewuola *et al.,* 2012b).

M. oleifera leaf meal was effectively included in some feeding studies at 30% (Dahoudaet *et al.,* 2013) and 40% of the test diet (Safwat *et al.,* 2014) the conclusion from these researchers confirmed that the Moringa leaf meal had great impact on the growth parameters.The primary limitation on using *M. oleifera* leaf meal for feeding increasing rabbits, however, is the dietary dietary balance needed rather than the product itself. A number of researchers revealed that *M. oleifera* leaf meal was served to males at an amount of 15% in rabbit weaning (Abu *et al.,* 2013), and increasing at a rate of 7.2% in substitution for soya bean meal (Ayodele *et al.,* 2014).

When *M. oleifera* leaf meal replaced 20% of soybean meal protein, rabbit's growing features were much higher than those allowed for dietary control (Ayodele *et al.,* 2014). The fecal protein digestibility was discovered to be high (70-71%) in studies with proteinrich Moringa oleifera leaf meal (Adeniji *et al.,* 2012) and progressively improved in *M. oleifera* as 65% to 80% (Ewuola *et al.,* 2012; Nuhu, 2010). This beneficial impact on CP and DM digestibility of

Moringa oleifera leaf meal was also observed at reasonable add-on levels as low as 3% (Djakalia *et al.,* 2011).

However, various surveys have shown that *M. oleifera* leaf meal contained a lesser amount of protein (20-23%) and more fiber (NDF 31%, ADF 26%, Safwat *et al.*, and 2014), only about 50 % of the calculated CP digestibility was recorded (Lebas, 2013). It has been noted that extreme and inadequate concentrations of nutritional fibers can influence the growth efficiency of chickens (de Blas *et al.,* 1999). Furthermore, the researchers clarified that the greater dietary fiber concentration generally results in low energy efficiency and net energy intake. Optionally, low fiber diets are associated with elevated complete mean retention time and then decreased intake capacity and increased risk of digestive illnesses (de Blas *et al.*, 1999).

Low fiber levels, however, have been suggested to indicate elevated levels of nutritional starch connected with elevated levels of diarrhoea, especially in early weaned rabbits (Blas *et al.*, 1994; Lebas and Maitre, 1989).

Fiber reaction on passage frequency, gut flora control, caecal fermentation, and rabbit performance has been assessed for a long time (de Blais *et al.*, 1999). Studies have recorded a strong impact on the physical and chemical features of fiber in animal diet. Increases in the quantity of fine particles will increase the digestibility of NDF, the acidity and weight of the caccal content, the fermentation time and the microbial CP recycled through caecotrophy, and will decrease the rate of passage and intake (de Blais *et al.*, 1999).

A small quantity of large particles is also important in order to allow an adequate turnover rate of caecal matter and to maximize microbial productivity. A range of 10.6 to 13.8 MJ/kg DM for low-protein and highprotein meals was recommended for the digestible energy content found in

Moringa oleifera leaf meal (Lebas, 2013). It is therefore proposed that the different composition and dietary value of crops such as *M. oleifera* leaf (fresh or dry) meal should be evaluated first of all on a case-bycase basis before being used in balanced animals such as diets for experimental rabbit testing.

2.9 Enzymatic Removal of Deleterious Phytochemicals in Forage

In animal forages, anti-nutrition variables can decrease the consumption of feed and some dietary digestibility. Forestry supplementation as phenolic sources is a problem to be resolved properly to minimize harmful effects on ruminants (Yusiati *et al.,* 2018). Different trials are underway to enhance tannin-rich feed and forage considering microbial strains by biodegrading their tannins. *Aspergillus niger* van Tieghem (hungus) with complex tannin – protein – degrading activity was separated from faeces of animals (hill cows) (Bhat *et al.,* 1997). This has earlier been recognized as a prolific tannin degrader with elevated tannase activity on feed (Sharma *et al.,* 1999) and has been tested on various tannin-rich tree forages such as *Robinia pseudoacacia* (Rakesh *et al.,* 2003).

Recently, *Enterobacter ludwigii* bacterial strain has been isolated from goat rumen, the enzyme has been subjected to tannin-rich forage to decrease tannin in livestock forage (Singh *et al.,* 2012). The enzyme tannase, alone or a Kemzyme plus P dry (different hydrolytic enzymes) has been tried for the improvement of different forage values (Duen˜as *et al.,* 2006; Duen˜as *et al.,* 2007; Urbano *et al*., 2007; Schons *et al*., 2011).

The enzyme tannase, alone or a Kemzyme plus P dry (combined with other hydrolytic acids) has been tested for the enhancement of various forage values (Duen leich *et al*., 2006; Duen ass et al., 2007; Schons *et al.,* 2011; Urbano *et al.,* 2007). It has been noted that the use of tannin-

tolerant microbes in an animal's rumen by inoculating bacterial crops has merits in increasing the productivity of animals consuming high tannin forages in the tropics (Bhat *et al.*, 2013)

There is little and irregular proof of the use of microbial cultures for the detannification and consumption of tannin-rich feed. Miller *et al.* (1995) found that ruminal microbes from animals adjusted to high tannin diets may be convenient to non-adjusted ruminants in order to promote the digestion of tannin-containing forage by crude protein. Bhat *et al*. (2013) clarified this later that the prospect of this strategy appeared positive on some forage, using a single bacterial species. Jia *et al.* (2014) proposed that there are distinct conditions where single enzyme was unable to finish responses, but it was recommended that various enzymes work together in a cascade. This limitation has been fixed by using Kemzyme plus P dry complexes (MECs) to encourage general catalytic effectiveness, which has encouraged scientists to synthesize artificial MECs to control the output of required compounds in *in vitro* multistep response cascades (Jia *et al.*, 2014) as well as reducing deleterious phytochemicals in agriculture by-products, to facilitate the use of certain nutrient forages. These arguments call for further studies on the use of bacterial culture or Kemzyme plus P dry in tropical forage to demonstrate the above claims to enhance the use of such forage.

The Kemzyme plus P dry has been intended to advance the digestibility of crop residues and boost the nutrient concentrations of forage/feed according to EFSA (2016). It was described that the Kemzyme plus P dry consists of both exogenous and endogenous enzymes, intended to provide extensive and convenient alternative to break down complicated substrates (some phytochemicals) requiring more than one digestive enzyme activity (EFSA, 2016). Improving the digestion of complicated substrates of non-starch polysaccharides increases the amount of the

animal's accessible energy and amino acids. Kemzyme plus P dry was used in the poultry industry to enhance the poultry product (EFSA FEEDAP, 2015).

Studies of tolerance recommend that Kemzyme plus dry is secure for chickens and turkeys for fattening and laying hens at an extreme dose of 1000 mg additive / kg feed and at a peak dose of 500 mg additive / kg feed in weaned piglets (EFSA FEEDAP, 2015). However, there is also a growing concern of Kemzyme plus P dry to feed rabbit with agro-industrial products and Moringa oleifera. Little is documented on the use of Kemzyme plus P dry in tropical forage (grasses and shrubs) to improve plant utilization and digestibility, since the additive is safe for consumers.

Free-ranging animals can choose their diets to prevent the worst tannin impacts. In Moringa oleifera seeds fed to some animals in a trial, a deadly impact was noted (Makkar and Becker, 1999). There are some recommendations in the cutting and carrying feeding systems of small-scale farmers that mixtures of varied forage are used on occasions, perhaps in part as a strategy to prevent necessary toxicity owing to excessive amounts of some species of fodder. Lowry (1990) indicated that smallholder farmers generally decrease damaging complications of phytochemicals by mixing leaves which reduces toxic impacts. Different study has verified how appropriate blend formulation (tannin forestry and tannin-free forage) has generated less harmful impacts than tannin forestry as a single feed (Melaku *et al.*, 2005; Pamo *et al.*, 2006; Patra *et al*., 2003, Sharma *et al.*, 2008).

Forages from tannin plants tend to have lower soluble nitrogen content, which improves the nutritional value (Albrecht and Muck, 1991; Rioux *et al.*, 1995; Salawu *et al.*, 1999). The impacts observed from microbial degradation in livestock are reduced consumption of feed, reduced use of nutrients and toxicity at greater intake concentrations (Van Soest, 1994),

however, the amount of microbes used has not been reported. Previously, tannins were regarded to be toxic to microorganisms as a result of inhibition of the enzyme, substrate deficiency, action and metal ion deprivation (Reed, 1995).

Studies show that condensed tannins are more impervious to microbial decomposition, whereas some microorganisms can degrade hydrolysable tannins more easily (Aguilar and Gutierrez-Sanchez, 2001).

Microbial tannase, tannin acyl hydrolase (E.C.3.1.1.20), is one of the most important engineering enzymes in the food, forage, leather and pharmaceutical sectors (Lekha and Lonsane, 1997). Tannase is a major enzyme catalyzing the conversion of hydrolysable tannins (glucose and gallic acid gallotannins) (Van de Lagemaat and Pyle, 2005).

2.10 Characterization of Kemzyme plus P dry

The scientific community has pursued studies on cellulases, hemicellulases, pectinases and others in many sectors, including meat, beer and wine, animal feed, textiles and laundry, pulp and paper, agriculture and a few fields of research and development (Bhat and Bhat, 1997).

2.10.1 Characterization of the Active Substances

The additive Kemzyme Plus Dry contains five main activities: endo-1, 3(4)-beta-glucanase (EC 3.2.1.6, glucanase) produced by Aspergillus aculeatinus (CBS 589.94, formerly classified as A. aculeatus), endo-1, 4-beta-glucanase (EC 3.2.1.4, cellulase) produced by Trichoderma reesei (CBS 592.94, formerly classified as *T. longibrachiatum*), alpha-amylase (EC 3.2.1.1, amylase) produced by Bacillus amyloliquefaciens (DSM 9553), bacillolysin (EC 3.4.24.28, protease) produced by Bacillus amyloliquefaciens (DSM 9554) and endo-1,4-beta-xylanase (EC 3.2.1.8, xylanase) produced by *Trichoderma reesei* (NIBH FERM BP 4842, formerly classified

as *T. viride*) on distinct fermentation procedures, each of the primary enzymes is generated. In the final formulation (e.g. alphagalactosidase, pectinase, galactomannase), which originates from the various fermentation procedures, several side operations can be discovered.

Various groups of microorganisms are proficiently engaged in hemicellulose degradation. More than 100 years ago, microorganisms that degrade hemicelluloses, especially xylan, a significant component of hemicelluloses, were disclosed (Hoppe-Seyler, 1889). The article outlined a method of gas production related to the suspension of wood xylan and microbes of river mud. It was further clarified that microbial enzymes behave in support of converting xylan into simple sugars that constitute it. Wong *et al.* (1988) reported that the β-1, 4-endoxylanases, which cleave internal glycosidic bonds within the xylan backbone; arabinofuranosidase which hydrolyzes arabinose side-chains; α-glucuronidase which removes glucuronic acid side-chains from the xylosyl units; xylan esterase which releases acetate group; and finally β-xylosidase, which hydrolyzes xylobiose to xylose.

2.10.2 Conditions of use

According to EFSA (2007), EFSA FEEDAP (2012, 14, 15, 16) and EFSA BIOHAZ Panel (2013) the additive is designed for use in poultry and decorative bird species between 250 and 1000 mg/kg feed (offering 588–2350 glucanase U, 4 500–18 cellulase U, 100–400 amylase U, 425–1 700 protease U and 8 750–35 000 xylanase U per kilogram feed). For piglets, the suggested dose is 500 mg / kg food (glucanase 1, 175 U/kg, cellulase 9000 U/kg, amylase 200 U/kg protease 850 U / kg, xylanase 17 500 U/kg). Three basal diets (starter, grower, and finisher) based on meals of wheat, maize, and soybean were supplemented with Kemzyme ® Plus Dry (or

focus) at 0, 500 (0.5 adjusted dose) or 100 000 (100) mg / kg feed. Enzyme activities were confirmed by analysis.

Feed provided 41 days of ad libitum in mash form. On a pen based basis, birds were weighed on days 1, 14 (before splitting), 28 and 41. During each period, feed consumption per pen was registered. Mortality was low (5, 2 and 6 birds in the control, 0.5× and 100× groups, respectively) and was not affected by dietary treatments. Feed to gain ratio was significantly improved by the addition of the enzyme at 100 compared with the control (1.74, 1.71 and 1.70 in the control, 1× and 100× groups respectively. Feeding the birds 100 times the suggested dose did not affect performance parameters negatively. Therefore, the FEEDAP Panel proposed that at the extreme recommended dose, the product is secure for chickens for fattening

2.10.3 Safety for the consumer

The toxicological experiments with the individual enzymes were conducted. The results of the genotoxicity studies and the sub chronic oral toxicity study do not indicate any reason for consumer safety concerns arising from the use of Kemzyme plus P Dry.

2.11 Proximate Composition of Experimental Diets

Animals such as goat were also considered in a rapidly increasing sector under the animal industry. Overall ruminant development such as cows requires 16 % crude protein (CP) (Luginbuhl and Poore, 1998), but the CP value of Moringa oleifera is above the CP requirement of goats, making the plant appropriate for use as a complement to the CP. Moringa oleifera leaves' CP was revealed to be 30.3 % (Moyo et al., 2011) and this was lower than the 35.88 % CP of sunflower seed cake frequently used as protein concentrate (Mapiye et al., 2010). It was also concluded that Moringa may produce a potential source of additional protein in animal diets

(Moyo et al., 2011). Various researchers observed different crude protein (16, 22.42, 23.27, 27.4 and 40 %) (Gidamis *et al.*, 2003; Sarwatt *et al.*, 2004; Nouala *et al.*, 2006; Reyes-Sanchez *et al.*, 2006; Oduro *et al.*, 2008; Sanchez-Machado *et al.*, 2009).

The variety in CP has a specific dietary advantage since it can satisfy animal dietary and energy needs and increase the immune system to reduce illnesses (Kyriazakis and Houdijk, 2006; Brisibe *et al.*, 2009).

Fonolla *et al.* (1985) fed brewers dry grains (BDG) with 28.85% Crude Protein mixed with 50% dried bean pods or 3% mineral and vitamin premix and 30% sugar beet molasses. The results from the aforementioned study indicated that the digestibility of nutrients in grains other than CP and ether extract was increased by the addition of bean pods. Jimoh and Atteh (2015) also recorded significant improvements in the digestibility of crude fiber and wheat fiber fractions when using xylanase and multi-enzyme cocktails comparison to other single enzyme diets. The amount of fiber fractions such as NDF and ADF is sometimes negatively correlated with the digestibility of dry matter, particularly in monogastric animals (Van Soest *et al.*, 1991). It was stated that low digestive ingredients (cellulose, lignin) could decrease digestive tract disorder and that the fiber demand in the fattening diet of rabbit (Gidenne *et al.*, 1998) and lignified cellulose in the diet could decrease the digestibility.

The crude protein content of dried brewers' grains was 37.9% (Lima *et al.*, 2003) and this was greater compared to the Gilaverte *et al.* (2011) report with 26.1% of rabbit CP brewer grains. However, Lima *et al.* (2003) found that the quantity of neutral fiber detergent (51.7%) was smaller than the value (62.9%) reported by Siddaramanna *et al.* (2009) and Gilaverte *et al.* (2011; 62.1%). The differences in feed ingredients inclusion used to prepare the diets maybe a factor for the differences observed.

Lima *et al.* (2003), Gilaverte *et al.* (2011) and Siddaramanna *et al.* (2009) concluded in their studies that dried brewers grains may be incorporated in the rabbit growing diet (about 28%) without affecting growth performance and meat quality of rabbits. Using the suggested feed could assist decrease the addition of alfalfa hay and soybean meal in diets for rabbit fattening while improving financial indexes and reducing feed costs. The bread waste digestibility of dry matter and observed Moringa leaf ranged from 65.0 to 78.4 % (Federick, 2010). It was further clarified that the digestibility of crude protein was 65.1-87.8% greater than previously recorded values (26.3-62.5%) (Iyeghe-Erakpotobor *et al.,* 2006). According to Madubuike and Obidimma (2009), weaner pigs altered their feed consumption based on the availability of energy and raw fiber in the feed supplied. Metabolizable dietary energy for growing rabbits has been reported to range from 2400 to 2800 kcal/kg (Pond *et al.,* 1995).

Another study disclosed that the crude bread feed protein fell below the recommended rabbit value, while the metabolizable energy met the maintenance and manufacturing requirements (Aduku and Olukosi, 1990). Studies have shown that bread waste and *M. oleifera* may have alternative energy and protein sources for weaner rabbits, especially in the dry season. The suggested CP content of diets for optimal growth in animal was above 160 gkg^{-1} (NRC, 1977 and Obinne and Okorie, 2008). The study conducted earlier by Djakalia *et al.* (2011) suggested that *M. oleifera* leaf meal maybe included at a reduced rate (about 3%) since the the DM and CP met the required range for animals. Chen *et al.* (1995) noted that DM, OM, CP, and NDF digestibility were observed for lactating cows when fungal derived enzyme complexes were added with amylolytic and proteolytic activity, but did not influence milk output or DMI. The understanding of the deficiency of effect for single or multi-enzymes are not well defined.

Numerous variables may interfere with the effectiveness of the catalytic action of exogenous enzymes, such as variances in enzyme activity, frequency and composition of implementation, mode and moment of enzyme interaction, in vitro ruminal activity and environmental enzyme stabilization, and enzyme-feed specificity (Beauchemin *et al.*, 2004; Adesogan, 2014). Animal feed digestion relies on several variables; the enzymes current, the physiological environment in which they operate, the feed's characteristics and the complete digestive tract's processing ability.

The three groups of enzymes that the digestive tract secretes to catalyze the different step-by-step hydrolysis in the digestive tract are carbohydrase, protease and lipase (Ranjhan, 1999). Feed properties that may affect its digestion include its vulnerability to enzymatic hydrolysis and the effects of inhibitory substances (glycosides, tannin etc) (Ranjhan, 2001).

The animal's digestive tract's processing ability leads to chemical degradation, solution emulsification, and colloidal suspension and synthesization. This is due to chemical reactions between feed components, enzyme action and other chemicals synthesized from the body of the animal or by microorganism and physical action (Ranjhan, 2001). According to Mongeau and Brassard (1979), nutritional fiber containing feed is known as plant polysaccharides and lignin that withstand digestive enzymes to hydrolysis. It also, suggested that the fiber is not chemically uniform and the parts vary from plant to plant (Mongeau and Brassard, 1979).

Subsequently, in monogastric livestock this imposes anti-nutritional impacts and affects their products. This study gap has been recognized by the scientific community and various approaches are under study to solve the challenge in monogastric animals. However, the effects was suggested to be attributed to the difference in activity of enzymes. Increasing the digestibility of crude fiber may be feasible in the phytase present. This is because phytase

breakdown will cause the nutrients to be released and made accessible to the corresponding enzymes (Ravindran *et al.,* 2000).

Changes in nutrient digestibility between nutritional components and comparable feed ingredients have been disclosed (Classen *et al.,* 1995; Rose *et al.,* 1996; Hessing *et al.,* 1995; National Research Council, 1994). Some researchers later proposed that such findings include variations in fiber content, antinutritional factors, harm to production, and less digestible types of the same nutrient (e.g. amylose vs. amylopectin (Anderson-Hafermann *et al.*, 1992; Miller *et al.,* 1994; Zhang and Parsons, 1994). However other papers explained that experimental diets or individual enzyme components can help to increase general dietary digestibility and decrease ingredient inconsistency by disrupting cell walls to enable better access to compressed nutrients by digestive enzymes, destroying antinutritional factors and adding digestive enzymes to livestock in circumstances where they are overwhelmed (Campbell and Bedford, 1992; Jeroch and Danicke, 1995). It has been reported that fibre constituents can only be digested in rabbits through microbial fermentation (de blais *et al.*, 1999) and the use of enzymes increased the digestion of the fibre (Freiria *et al.*, 2018). However, suitable amounts of multienzymes in feed have the ability to promote enhanced in animal fiber digestion (Freiria *et al.*, 2018).

The use of distinct operations of carbohydrase may be more beneficial than a single enzyme acting (Juanpere *et al.*, 2005). However, because of the existence of protease that can digest carbohydrase, this may have a restriction on the hydrolytic activity of carbohydrase. A study by Ravindran *et al.* (2000) stated that the apparent metabolizable power value of wheat (5.34%) improved by adding phytase (400 units / kg diet).

Rutherford *et al.* (2002) noted that, in relation to considerably higher phytate phosphorus loss including rice bran in the diet, microbial phytase (Ronozyme P) enhanced digestibility of amino

acids. Parkkonnen *et al.* (1997) considered that xylanase improves the permeability of the wheat aleurone layer, the phytic acid storage site, thereby improving phytase accessibility to the phytate.

Jimoh (2018) found that phytase enhanced crude protein and ether extract digestibility relative to other non-phytase feeds. Different researchers have stated that the use of exogenous phytase can considerably enhance the digestion of phosphorous by destroying plant-derived phytates (Thiel and Weigand, 1992; Jimoh, 2018).

Phytic acid is generally regarded to be hydrolysis resistant in avian gut due to absence of any endogenous phytases, although this reality is not unquestioned (Bedford, 1996). Whether there are endogenous phytases or not, both phosphorous (Kornegay, 1995) and mineral phytases have been shown to be considerably improved (Thiel and Weigand, 1992). Phytate's capacity to generate complicated proteins and digestive proteases has also been suggested to be liable for its adverse impact on protein digestion (Anderson, 1985). Hence its breakdown in the intestinal lumen may lead to a faster pace of proteolysis and a better digestion of proteins. However, feeds containing any significant quantities of antinutrients were revealed to be detrimental to monogastric efficiency and digestibility of nutrients (especially protein) (Schulzl *et al.,* 1992; Schulzl *et al.,* 1995; Jansman et al., 1994). The potential for enhanced animal growth and development by enzymatic degradation of remaining lectins and trypsin inhibitors in soybean meal is clear and confirmed in raw soybean (Hessing *et al.,* 1995).

Raach-Moujahed *et al.* (2017) revealed that Rovabio and Kemzyme Plus Dry diets (without phytase) resulted in the largest weight gain ($P<0.05$, 2010.56 g average) compared to control and Cibenza DP100 diets (1917.97 g average). However, there were no variations in total dry weight gain (54.62g/d), total feed intake (91.67 g/d), feed conversion ratio (1.67), and mortality rate

(3.54%) between the 4 diets tested. It was found that adding Kemzyme Plus Dry and Rovabio Excel in a diet based on corn and soybean meal seemed to be more effective in improving broiler WG at 35 days than Cibenza DP 100.

Previously enhanced feed consumption was recorded in various research on supplemented diets for livestock supplied with yeast culture (McLeod *et al.,* 1990;). Most feed enzymes are well designed to increase the ration of digestible CP and energy to improve the growth rate in animals. It was earlier proposed that the ratio of digestible CP and digestive energy is appropriate at 10.8 in the diet (De Blas and Mateos, 1998).

Wang *et al.* (2009) had earlier reported that dietary supplementation with Bacillus probiotics in grower pig increased feed intake. Weichselbaum (2009) further discussed the reports from these researchers, who proposed that the possible reason could be that probiotics containing diverse strains of microorganisms with distinct abilities and some strain could provide some advantage to the host as others do not.

2.12 Growth potential of rabbits

McDonald *et al.* (1998) clarified that feed is a material capable of being digested, absorbed and used after ingestion by animals, while growth is increased in weight and size, associated with shape modifications and leading to animal maturity. Gain in body weight, generally expressed as mean weight gain per day (g/d), is the prevalent significant measurement of growth in animals (Nsoh, 2018). Similarly, animal development is weight gain as a proportion of original weight, eliminating the impacts of initial weight as larger animals tend to gain more weight than smaller animals of the same period (Mavromichalis, 2006). It was also reported by Jabeen *et al.* (2008) that the efficiency of animal feed concentrates could be enriched by supplementation with *M. oleifera* leaves meal. Inaddition, Sultana *et al.* (2014) noted that soybean meal added with M.

oleifera leaf meal significantly influenced growth performance of birds body weight, body weight gain, better health status and feed conversion ratio (FCR).

The feed consumption obtained by *M. oleifera* leaf inclusion diet had a range of 60.10-63.40 g/day for rabbits (Federick, 2010). Similar experiment conducted by Mufwa *et al.* (2011) reported a lower values for feed consumption ranging from 44.73 to 57.90 g/day. The two studies has been showed weight gain improvement but the formal results was much better than the latter. In addition, Odeyinka *et al.* (2008) reported high weight gain above 6.78-8.64g/day when rabbits were fed with feed components with moringa leaf meal exclusive.

The feed conversion ratio (FCR) observed when rabbits were fed with experimental diet had a range of 2.63-3.00 at the end of the experimental period (Okorie, 2003). However, similar trial was made by Fedderick (2010) who reported the feed conversion ratio having a greater mean value than values when compared to the study by Okorie. The differences in the feed conversion ratio noted in the two studies could possibly be attributed to the differences in the feed ingredients used to prepare the experimental diet and the age difference of the rabbits used for the experiment.

The consumption of feed sometimes influences different variables from the environment and feed. There has been an improvement in the pace of development of feed additives compared to feed without additives, which demonstrates a better use of nutrients with additives (Rexen, 1981; Hollister *et al.*, 1990; Schutte *et al.*, 1993; Singh *et al.*, 1995).

In India, it has been noted that rabbits grown for meat have a feed conversion ratio of 2.5 to 3.0 for high-grain diet and 3.5 to 4.0 for natural feed diet without animal feed (TNAU, 2016).

Feed additives/enzymes are designed to enhance feed consumption in animal production and this may influenced feed conversion ratio. The feed conversion ratio observed with yeast (6.40) and effective microorganism (EM) (6.43) were also lower than the values from the control diet (8.20) (Shanmuganathan *et al.*, 2003). This was further explained that the development in growth performance was higher than the increased rate of feed intake indicating better nutrient utilisation with additives. Singh *et al.* (1995), reported that feed prerequisite per unit gain was declined by 3% in New Zealand White (NZW) rabbits given Saccharomyces cerevisiae (strain N0. 2094, ITCCF). Regarding the same experiment about 5% development in body weight gain had been observed. The implication of Singh and co-workers findings was that the feed required for the body gain maybe reduced depending on the additives and this may not have much influence on the body gain.

Hollister *et al.* (1990) also observed an enhanced percentage of feed for rabbits fed on Lacto-Sacc diets. Li and Umemura (1995) and Inciong (1996) recorded greater feeding performance in poultry diets supplemented with effective microorganism. Probiotics containing feed was revealed to be efficient in some monogastric animal species (Abe *et al*., 1995).

Improved growth efficiency in rabbits were also noted when supplied with feed components with distinct probiotic supplementation structure (Kritas and Morrison, 2005). It was also reported that an average 5.65 (FCR) in NZW rabbits fed commercial feeds from their 5^{th} week to their 20^{th} week of age. The original body weight of rabbits in their trial experiment was 735 g and the average increase in body weight was 20.2 g/day. Shanmuganathan *et al.* (2003) recorded an initial body weight of 1,986 g on rabbit and an average weight gain of 12.65 g/day owing primarily to its greater era. The difference in age may have led to the broad spectrum of observed (FCR) values.

2.13 Feed intake and blood profile weaner rabbits

Madubuike and Ekenyem (2006) indicated that animal hematological and serum biochemical assays correspond to their nutrients the animal's physiological nature. Therefore, samples of blood from livestock can always be collected and analyzed to determine whether a non-conventional feed ingredient has had any adverse impact on the animal's blood profile or physiology.

Important signs of the physiological status of animals have been recorded as hematological parameters (Adenkola and Durotoye, 2004; Khan and Zafar, 2005; Makinde, 2017). Differences in hematological parameters are of significance in the assessment of animal reactions to multiple physiological and disease circumstances (Schalm *et al.,* 1975; Yadav *et al.,* 2002; Khan and Zafar, 2005). Different trials were performed to evaluate the impact of experimental diets on the blood profile of both ruminants and non-ruminants. Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and Hemoglobin (Hb) were investigated (Ansah *et al.,* 2014). Monogastric animals like the rabbit neutrophil are also referred to as heterophiles and are the second most common white blood cells seen in peripheral smears of blood.

Rabbit neutrophil consists of tiny acidophilic granules and varying numbers of big red granules (Zimmerman *et al.,* 2010). Rabbit erythrocyte is a biconcave disk with a mean diameter of 6.7 to 6.9 mm (Jain, 1986) and an average thickness of 2.15-2.4 mm (Hawkey *et al.,* 1989; Schermer *et al.,* 1967).

However the average lifespan of an erythrocyte is about 45-70 days (Mitruka *et al.,* 1977). It was also noted that lymphocyte is the most prevalent form of white blood cell in peripheral blood smears in both tiny and big forms, the former about the size of a red blood cell and the latter about the size of a neutrophil (Kozma *et al.,* 1974). It has been revealed that rabbit eosinophil has

a horseshoe shaped nucleus (Kozma *et al.,* 1974; Benson *et al.,* 1999) quite larger than a neutrophil (12–16 mm).

It was further outlined that there are plenty of acidophilic granules in the cytoplasm that are about 3-4 times bigger than those in neutrophils that can occupy much of the cytoplasm room (Kozma *et al.,* 1974; Benson *et al.,* 1999).

Rabbits have small to moderate amounts of basophils in the peripheral circulation compared to other species, some of which account for up to 30 % of WBCs in clinically ordinary humans. (Benson *et al.,* 1999).

The rabbit monocyte (about 15–18 mm in diameter) is the biggest of the WBCs. Monocyte has an amiboid nucleus with diffuse, slightly stained nuclear chromatin (lobulated, horseshoe or bean-shaped) (Reagan *et al.,* 2008; Zimmerman *et al.,* 2010). It was commonly predictable to demonstrate excellent efficiency in livestock with ordinary blood composition. Contrary to the Adegoke *et al.* (2018) results, broiler chicken organizations fed on a distinct diet have the highest growth efficiency indicators, but not blood composition. Conclusively, at proven concentrations of incorporation, all nutritional additives can strongly affect organ and system health. Dried cassava pulp was fed to animals according to Otsyina et al. (2007) and noted the blood parameters (PCV, Hb, RBC, WBC) of the animals test diet were all similar with the control diets (P> 0.05). The findings showed that sundried cassava pulp does not affect the animal's blood profile. The PCV (33-50) and Hb (9.4 -17.4) were all recorded for rabbits within the standard range except for reduced maize suggested by Mitruka and Rawnsley (1997) and Ross *et al.* (1979).

The high Hb and effective RBC dietary protein digestion and absorption reported in BSG and MB may be reflected in the experimental diets. According to Togun et al. (2007), hematological values in rabbits have been revealed to decline within the normal range.

This was further explained by various studies that these indicate that such diets have not had any adverse effects on hematological parameters throughout the period, but that when the values fall below the normal range, anaemia is indicated. (Mitruka and Rawnsley, 1977; Radostits, 1994; Ameen *et al.*, 2007).

Low hematological values were reported on the counts of PCV (30 %), Hb (10.30 g/ dl) and RBC (7.10 x 106/ml) (Bawala *et al.*, 2007), it was suggested that this could be due to the damaging impacts of high nutritional levels.

The physiology and nutritional status of the animals may be ascribed to differences observed in PCV and MCV for humans in separate therapy groups (Esonu *et al.*, 2001). Eheba *et al.* (2008), however, recorded a decline in WBC count, revealing a decrease in the production of defensive mechanisms to fight infection. Togun *et al.* (2007) noted that a considerably reduced lymphocyte count indicated a decrease in experimental rabbits' capacity to generate and release antibiotics when infections happen (Campbell and Lasley, 1975).

Table 2.5: Referenced ranges of normal hematological parameters in the New Zealand white rabbit (oryctolaguscuniculus)

	Rabbit Gender		Age 1 to 3 Months
Parameters	**Adult Male**	**Adult Female**	
RBC ($\times 10^6$/mL)	5.46–7.94	5.11–6.51	5.15–6.48
PCV (%)	33–50	31.0–48.6	38.1–44.1
Hgb (g/dL)	10.4–17.4	9.8–15.8	10.7–13.9
MCV (fL)	58.5–66.5	57.8–65.4	66.2–80.3

MCH (pg)	18.7–22.7	17.1–23.5	19.5–22.7
MCHC (%)	33–50	28.7–35.7	24.2–32.6
Platelets ($\times 10^3$/mL)	304–656	270–630	-
WBC (×103/mL)	5.5–12.5	5.2–10.6	4.1–9.79
Neutrophils (%)	38–54	36.4–50.4	18.8–46.4
Lymphocytes (%)	28–50	31.5–52.1	44.6–77.8
Eosinophils (%)	0.5–3.5	0.8–3.2	0–2.4
Basophils (%)	2.5–7.5	2.4–6.2	0.1–4.5
Monocytes (%)	4–12	6.6–13.4	0–13.1

Sources; Mitruka et al. (1977), Jain (1986) and Zimmerman et al. (2010).

CHAPTER THREE

3.0 Materials and Methods

Materials and methods consisted of the cultivation, management and collection of agronomic information from *M. oleifera* in the field, phytochemicals screening and in vitro digestibility of *M. oleifera*, and preparation of test ingredients, experimental diet, experimental rabbits and management, digestibility trials, chemical analysis, blood profile analysis and statistical analysis of the data.

3.1 Experiment I

M. oleifera plant cultivation, plant height, stem, leaf number, phytochemicals and in vitro digestibility data collection.

3.1.1 Study Area

Planting of the *M. oleifera* plants was at the Botanga irrigation field at Kumbungu District in the Northern Region Ghana (9°28'28.75"N latitude and 0°50'53.48"W longitude). The area receives

an annual rainfall averaging 600mm, considered enough for a single farming season. The annual rainfall pattern is erratic, starting in April, intensifying as the season progresses and increasing the average from 600 mm to 1000 mm.

Temperatures are usually high, with an average of 34 °C. The maxi mum temperature could rise as high as 42 ^{o}C and the minimum as low as 16 ^{o}C. Low temperatures can be experienced from December to late February. It has a unimodal pattern of rainfall that begins at the end of April and ends in October. The average annual rainfall is 1043 mm. Temperatures generally fluctuate between 15^{o}C (minimum) and 42^{o}C (maximum) with an average annual temperature of 28.5^{o}C. The average annual relative humidity of the day is 54% (SARI, 2007). The area experiences dry cold harmattan winds from November to February and warm dry conditions from March to mid-April. The dry season is therefore from November to the end of April.

Phytochemical screening was performed at the Center for Plant Medicine Research Laboratory in Mampong Akuapem in the Eastern Region.

3.1.2 Soil Preparation, Propagating and Experimental Design

Experimental plot of *M. oleifera* plants was established on August, 2018 to December, 2018. A total land area of 9.4 x 7.4 m^2 was used, consisting of 12 plots each measuring 1.2 x 1.2 m^2. Hoe and cutlass were used to prepare the land. The design of the experiment was randomized to complete block with each treatment replicated 3 times. Poultry manure of 6 kg was applied before sowing, as described elsewhere (Amaglo *et al.*, 2010). Seeds of *M. oleifera* were sowed at 2 cm depth with 2 to 3 seeds per drill at a spacing of 20 cm to 20 cm. Germination begun on the first week after sowing, giving a population of 450 plants on the entire plot. Management practices (watering, weeding etc.) were ensured during *M. oleifera* plants establishment.

3.1.3 Measurement of Agronomic and Biomass Yield

Five seedlings were randomly selected from each for agronomic measurement. Seedling height was measured from the soil surface to the shoot apex with a thread and a meter rule. The number of leaves was also countered and recorded. The stem girth was measured at the base of each stem with the help of the Vernier caliper. Four harvests were carried out during the experimental period. The first harvest was on 9th October, 2018 followed by the second harvest 19th October, 2018, the third harvest was on 29th October, 2018 and the forth harvest was performed on 8th November, 2018. Plants were cut to stump height of about 20 cm from ground level as described by Amaglo *et al.* (2010). Biomass yield on each plot was taken and weighed using electronic scale. Leaves were separated from stems and air-dried for chemicals analysis.

3.2 Chemical Analysis and Phytochemicals of *M. oleifera*

3.2.1 Dry Matter

This was determined by collecting fresh samples of each variety (whole, leaf and stem). About 0.2 g of each sample was weighed into aluminum pan and placed into forced air oven at 60 ^{o}C for 48 h. The weight after oven drying was recorded and used for computing the dry matter percentage of each treatment (AOAC, 2000).

3.2.2 Ash

Ash was determined according to the procedure of AOAC (2000). Approximately 2 g of dried sample was weighed into a known weight of pre-dried crucibles. The crucibles containing the samples were placed in a muffle furnace and heated to 550 ^{o}C for 4 h. The crucibles were then cooled in a desiccator and its weight taken. The ash content was calculated as:

Ash (g/kg DM) = Weight of ash/Weight of sample×1000

3.2.3 Crude Protein

The crude protein content of the samples was determined according to the method of AOAC, (2000). A gram (1g) of each dried sample was weighed and placed into Kjeldahl digestion tubes and blank determination was done by digesting filter paper in each set of digestion. Approximately15 ml of concentrated sulphuric acid (H_2SO_4) and two Kjeldah tabs were added to the content of each digestion tubes. The Kjeldahl tabs contained potassium sulphate (K_2SO_4) and copper sulphate ($CuSO_4$) which increase the boiling point and acted catalysts respectively. The tubes were mounted on Kjeldahl digestion block with fume exhaust set (J.P. Selecta RAT 2 Spain) and heated gradually to 420 ^oC and maintained for 3 h. The tubes were removed and allowed to cool to room temperature after which, 50 ml of distilled water was added and distilled using an automated Kjeldahl distillation apparatus (J.P. Selecta, s.a, Pro-Nitro II Spain). The apparatus draws 50 ml of previously prepared 35% sodium hydroxide (35 % NaOH) into the digestion tubes and 25 ml of 4% Boric acid (4% H_3BO_3) into a 25 ml erlynmeyer flask to trap the liberated ammonia during the distillation period of 9 min per sample. The distillate was collected and titrated against 0.1N HCl (hydrochloric acid). The average titre values were recorded and the percentage nitrogen (% N) as well as the percentage crude protein (% CP) calculated using the formulae:

% Nitrogen = $(T- B)\times N\times 1.4/$ weight of sample (g)

% Crude protein = %Nitrogen × 6.25

Where:

T – Sample titre value

B – Blank titre value

N – Concentration of HCl

3.2.4 Neutral Detergent Fibre and Acid Detergent Fibre

Neutral detergent fibre and ADF were determined exclusive of residual ash by sodium sulfite and α- amylase following the procedure of Van Soest *et al*. (1991) and was run on the Ankom 200 fiber analyser.

About 0.45-0.55 g of each sample was weighed directly into filter bags (Ankom F57) and labeled. The filter bags were then sealed within 4mm of the top with an electronic heat sealer. One blank filter bag was included in each run to determine blank bag correction. The bags with samples were then placed on the bag suspender and inserted into the Ankom fiber analyser vessel with a bag suspender weight on top to keep it submerged. Neutral Detergent Fibre and ADF solutions were then added respectively. Neutral detergent fibre solution was prepared by dissolving 30.0 g Sodium dodecyl sulfate, USP; 18.61 g Ethylene diaminetetra acetic disodium salt, dehydrate; 6.81g Sodium borate; 4.56 g Sodium phosphate dibasic, anhydrous; and 10.0ml Triethylene glycol, in 1L distilled water; Whilst that of ADF was prepared by dissolving 20.0 g Cetyl trimethylammoni um bromide (CTAB) into 1L of 1.00N H_2SO_4.

For NDF, two liters of NDF solution were added to every 24 sample bags in the fiber analyser vessel. 20 g (0.5 g/50 ml) of sodium sulfite and 4.0 ml of alpha-amylase was added to the solution in the vessel. The fiber analyser was then allowed to run for 75 mins. After 75 mins, the solution in the vessel was exhausted and the content rinsed with 2 L of hot water (70-90 ^{o}C). Rinsing was repeated three times for 5 mins and 4.0 ml of alpha-amylase added to the first and second rinses. After rinsing, the samples were placed in acetone for 3-5 mins after which they were oven dried at 102 ^{o}C for 2 h and weights recorded.

For ADF, the procedure was the same as that of the NDF except that for ADF the fiber analyser was allowed to run for only 60 mins and also sodium sulfite and alpha - amylase was not added.

3.3 *In Vitro* Gas Production

3.3.1 Experimental Design

The design for the *in vitro* gas studies was a factorial design in a randomized complete block. The 4 x 3 factorial design was used with each replicated 2 times in each period. The factors were four varieties and three botanical fractions which are whole, leaf and stem.

3.3.2 *In vitro* Gas Experiment Procedure

The *in vitro* gas production technique of Theodorou *et al*. (1994) was adopted. Where approximately 200 mg of oven dried samples from each treatment was weighed into 50 ml test tubes. The McDougall's buffer was prepared a day before the incubation.

McDougall's salivary buffer solution was prepared from solutions A and B. Solution A was made by dissolving 19.60 g $NaHCO_3$, 9.28 g $Na_2HPO_4 \cdot 2H_2O$, 1.14 g KCl, 0.94 g NaCl and 0.26 g of $MgCl \cdot 6H_2O$ in 2 L of distilled water. Solution B was made by dissolving 2.65 g of $CaCl_2 \cdot 2H_2O$ in 50 ml of distilled water. Complete salivary buffer was prepared by adding 2 ml of solution B to solution A, which was then warmed to 39 ^oC with continuous stirring and flushing with carbon dioxide (CO_2) immediately before starting to incubate samples. About 30 ml of buffered rumen fluid was dispensed into each test tube containing the samples. The buffer and the strained rumen fluid were mixed in a ratio of 4:1(cite here).

Rumen fluid was obtained from 3 different cows at the Tamale Abattoir. The rumen fluid was collected from the rumen after the animals have been slaughtered and rumen taken out (Mutimura *et al.*, 2013). The rumen fluid was collected into a thermos flask that had been pre-warmed to a temperature of 39 ^{o}C. Incubation procedure was as reported by Menke and Steingass (1988). The rumen fluid was squeezed through a four layer of cheesecloth. The test tubes and the samples were placed in a water bath at a temperature of 39 ^{o}C. The gas production was measured using a digital manometer at 3, 6, 12, 24 and 48 h.

The gas readings were then fitted to the exponential curve of Orskov and McDonal d (1979) without an intercept using sigma Plot 10th edition (Systat Software Inc. 2006). The degradation parameters (b and c) were derived from the exponential model.

$Y = b (1 - e^{-ct})$

Where Y = gas volume at time

t (ml) b = asymptotic gas

production (%), t = time (h)

c = fractional rate of gas production (ml/h)

The digestible organic matter (DOM) was calculated using the equation DOM (%) =

16.49 + 0.9042 GP + 0.0492 CP + 0.0387ash by Menke and Steingass (1988) whilst the metabolizable energy was calculated using the equation ME (MJ/ kg DM) +2.20

+0.136 *GP+0.057 *CP according to Menke *et al.* (1979). Where, GP= gas production (ml/200mg DM at 24 h) CP= Crude protein (g/kg DM).

3.4 Determination of phytochemicals in *M. oleifera*

3.4.1 Tannin detection

About 2-3 drops of 5 % Ferric Chloride is added to 3ml of aqueous extract a sudden change in colour to black, bluish-black or dark green shows presence of phytochemicals (Ciulei, 1981).

3.4.2 Reducing compounds

The alcohol extract (0.5-1ml) is diluted with water (1-2 ml) add Fehlings solution (A and B) (0.5– 1 ml) solutions and heat them. A brick red precipitate denotes the presence of reducing compounds (Ciulei, 1981).

3.4.3 Polyuronides

About 2 ml of the aqueous extract are added dropwise in a test tube where 10 ml of alcohol or acetone have already been placed. If a thick precipitate is formed denotes presence of polyuronides (Ciulei, 1981).

3.4.4 Saponins detection

Shake 2 ml of diluted solution (1:1) in a test tube of 1.6 cm diameter for 15 minutes. The occurrence of a foam column of at least 1 cm in height persisting minimum 15 minutes, indicates the presence of saponins (Ciulei, 1981).

3.4.5 Alkaloids

The alcoholic extract is evaporated to dryness and about 5 ml of 10 % HCl is added to the residue containing alkaloids as salts of some organic acids. The alkaloids now become salts of the mineral acid. From the aqueous solution, the alkaloids are precipitated as bases with the help of ammonia solution (pH=8, 9, 10%) and extracted with a non-polar solvent (ether, chloroform). The ether or chloroform solution is evaporated to dryness in an evaporating dish. The residue is dissolved in hydrochloric acid solution (1.5 ml, 2%). The acidic solution in which the alkaloids are under a salt form is divided in three test tubes: one is the reference and in the other two test tubes 2-3 drops of Mayer's reagent are added. The occurrence of an opalescence or yellowish-white precipitate with Mayer's reagent.

To 25 ml alcohol extract, hydrochloric acid solution (10%, 15ml) is added by refluxing and heated up for 30 minutes. During the hydrolysis the solution becomes opalescent due to the precipitating aglycones obtained by the division of the glycosides. After cooling, the solution is 3 times extracted in separating funnel, with ethyl ether (10-12 ml). The ether extracts are placed together (30-36 ml) and dehydrated with anhydrous sodium sulphate, resulting in ether and an aqueous solution. The ether extract will serve to identify the anthracenosides, flavonosides, steroid glycosides and triterpenes by means of the following series of reactions characteristic of each group.

3.4.6 Identification of anthracenosides

The ether extract (4 ml) is concentrated to 2 ml, then ammonia solution (25%, 1-2 ml) is added by shaking. A cherish-red colour of the alkaline solution indicates the presence of aglycones of anthracenosides (Borntrager's reaction) (Ciulei, 1981).

3.4.7 Identification of Phytosterols and Triterpenes

The ether extract (10ml) is evaporated to dryness. The residue is dissolved successively in acetic anhydride (0.5 ml) and and chloroform (0.5 ml). The solutions are transferred to a dry test tube. Concentrated Sulphuric acid (1-2 ml) (Liebermann-Burchard's reaction) is added at the bottom. At the separating level of the two liquids, reddish-brown or violet-brown ring is formed, the superior layer being green for phytosterols and red or violet for triterpenes.

3.4.8 Identification of Flavonosides

The ether (5 ml) is evaporated to dryness. The residue is dissolved in methanol (50%, 1-2 ml) by heating, then metal magnesium and 5-6 drops of concentrated hydrochloric acid are added. The solution becomes red for flavonols and flavonones (Shibata's reaction) (Ciulei, 1981).

3.5 Experiment II

Feed trial, growth parameters, blood profile and digestibility of rabbits

3.5.1 Study Area

The feeding trial was also carried out at the Animal Science Department of the University for Development Studies, Nyankpala campus, Tamale. Nyankpala is located at 9° 25' N, 0° 59' W and at a height of 183 m above sea level and in the dry savannah ecological zone of Ghana (SARI, 2007).

New Zealand White rabbits were purchased from the University of Ghana farms, Accra and Kemzyme plus P dry was purchased from Kemin Industries (Pty) Ltd, Olifantsfontein, South Africa and distributed to MT 19:26B, Amakom Kumasi.

3.5.2 Source of Animals and Experimental Design

Twenty (20) weaned New Zealand White (NZW) rabbits (0.700-1.18 kg) were sourced from the University of Ghana farms, Accra. The Completely Randomized Design (CRD) was used in grouping the rabbits and placed on four different diets and each dietary group had 5 replicates. The feeding trial lasted for 49 days after a week adjustment period.

3.5.3 Management of the experimental rabbits

The rabbits were kept in wire mesh cages each raised 1m above the ground. The dimension of the hutch was 50cm length x 60 cm width x 60 cm high. The weights of the rabbits (initial) were taken before subjecting rabbits to experimental diet. The rabbits were allowed one week period of adaptation to the feed. Feed and water were served in cleaned bowls every morning after the bowls are thoroughly cleaned. Coccidiostat (Embazing forth) was added to water (1g/litre) to avert diarrhoea and coccidiosis. The experimental diets comprised of T0 (formulated concentrate without *M. oleifera* leaf and Kemzyme plus P Dry), T1 (formulated concentrate with *M. oleifera* leaf and without Kemzyme plus P Dry, T2 (formulated concentrate with *M. oleifera* leaf +0.03g/day of Kemzyme plus P Dry) and T3 (formulated concentrate+0.03g/day of Kemzyme plus P Dry). Each animal was given 100 g of feed a day for the 49 days. The records were kept on feed offered and leftover on daily basis, in order to estimate the amount of feed consumed by the rabbit per day. The weaner rabbits were weighed on weekly basis throughout the experimental period. At the end of 49 days, three rabbits from each treatment making a total of twelve (12) rabbits were selected for the Hematological analysis.

3.5.4 Source of Feeds

Maize bran (MB)

Maize bran was obtained from the local porridge producers within Nyankpala and dried thoroughly in the sun to prevent it from getting mouldy. It was then bagged into a jute sack for the feed preparation.

Table 3.1: Inclusion levels of the various ingredients

Ingredients (%)	Treatment			
	T_0	T1	T2	T3
Maize	50	10	10	50
Maize Bran	20	20	20	20
Soy Bean	29	29	29	29
Salt	0.25	0.25	0.25	0.25
Di calcium phosphate	0.25	0.25	0.25	0.25
Vitamin premix	0.5	0.5	0.5	0.5
M. oleifera	-	40	40	-
Kemzyme plus P dry	-	-	0.03g (30mg)	0.03g (30mg)
Total	100	100	100.03	100.03

Premix composition (g/kg): vitamin, 12, 500 IU; vitamin D3, 2500 IU; -itamin,50.00 mg; vitamin K3, 2.50 mg; vitamin B1, 3.00 mg; vitamin B2, 6.00mg;vitamin B6, 6.00 mg; niacin, 400 mg; calcium pantothenate, 10mg;biotin, 0.8 mg; vitamin B12, 0.25 mg; folic acid, 1.00 mg; chlorinechloride,300 mg; manganese, 100 mg; iron, 50 mg; zinc, 45 mg; copper,2.00 mg; iodine, 1.55 mg; cobalt, 0.25 mg; selenium, 0.10 mg; antioxidant, 200 mg. Four treatment groups labeled, T_0 (control), T1, T2 and T3 was used in this experiment.

M. oleifera fresh leaves

Healthy fresh *M. oleifera* regrowth were brought from the trial field. Fresh leaves were harvested from available trees and the leaves were trimmed from its twigs on a plastic container. The trimmed leaves were then weighed and properly mix with the other ingredients as required.

Soybean meal

Soy bean meal was purchased from McDan Company in Tamale and was brought to the study area for the preparation of the feed.

3.6 Performance Characteristics of Rabbit

3.6.1 Weight gain

Each animal was weighed before the introduction of the feed. This gives the initial Weights (w_0) g. After each week of feeding, the weight of the rabbit was taken again (w_1). Weight gain is calculated:

Total weight gain = Final weight - Initial weight

3.6.2 Feed intake

Feed intake was obtained by calculation.

Total Feed Intake (g) = Feed offered (g) - feed left over (g)

3.6.3 Feed conversion ratio

Feed conversion ratio, is referred to as the amount of feed (g) consumed to gain a unit of live weight (g), and was calculated as:

$$\text{Feed conversion ratio, (FCR)} = \frac{\text{Total Feed intake (g)}}{Total\ weight\ gain\ (g)}$$

3.6.4 Average feed intake

$$\text{Average feed intake (g/rabbits)} = \frac{\text{Total Feed Intake}}{Number\ of\ rabbits}$$

Mortality percentage

$$\%\ \text{Mortality} = \frac{\text{Number of dead Rabbits}}{Total\ number\ of\ rabbits\ stock} \times 100$$

3.7 Hematological and Serum Biochemical Studies

3.7.1 Sample collection

Twelve (12) rabbits were selected out of the twenty (20), bled through the anterior vena cava to collect about 5-7 ml of blood into two labeled sterile vacutainer tubes. One contained 1.0mg/ml of ethylene diamine tetra acetic acid (EDTA) and 0.1mg/ml heparin and was used for hematological analysis.

3.7.2 Hematological Assessment

The Sysmex Hematological Auto-Analyser 7 was used for the hematological analysis. The parameters investigated were: Hemoglobin (Hb), Hematocrit (HCT) or Packed Cell Value (PCV), Red (RBC) and White Blood Cells (WBC) counts as well as Mean Cell Hemoglobin Concentration (MCHC) and Platelets (PLTS) number.

3.8 Chemical Analysis and Nutrient Digestibility

Digestibility study was conducted using 12 out of 20 weaners rabbits at 8-10 weeks old kept for 49 days of the trial. Fecal sample were collected daily for five (5) days continuously on the third (3^{rd}) week of the experiment, weighed and packed in zip-bag and stored in freezer at 4 ^{0}C immediately after collection. At the end of each collection period the samples were bulked for each weaner rabbit for proximate analysis using described method (AOAC, 2000) referred to chemical analysis mentioned earlier. Nutrient composition of each experimental diet were done using (AOAC, 2000). The parameter determine were feed and nutrients intake digestibility coefficient of the rabbits. Digestibility of the nutrients was determined by the formula: Digestibility coefficient = Nutrient digested/ Nutrient intake x100 %.

3.9 Data Analysis

The one-way analysis of variance (ANOVA) in randomized complete block design (RCBD) from Genstat 18th edition was used for analyzing the biomass yield, plant height, leaf number and leaf to stem ratio (LSR), growth performance and hematology parameters. The *in vitro* gas digestibility trial of the *M. oleifera whole* plant and chemical composition in experiment two were analysed as two- way ANOVA. Means were separated at 5 %.

CHAPTER FOUR

4.0 RESULTS

4.1. Experiment I

The results of this study are presented in three (3) parts. The first part demonstrates the effects of harvesting regimes on *M. oleifera* biomass (70, 80, 90 and 100 days). The second part shows the nutrient composition, phytochemicals and digestibility of *M. oleifera* whole plants. The third part also demonstrates the effect of multi-enzyme on the growth, hematological parameters, and nutrient digestibility of weaned rabbits.

4.1.1 Biomass yield, plant height, leaf number, plant girth and leaf to stem ratio of M. oleifera

The effects of harvest regimes on the average number of leaves, plant girth, LSR (Leaf to stem ratio at harvest) and height of the *Moringa oleifera* are shown in Table 4.1.

Table 4.1 Mean biomass yield, plant height, leaf number, plant girth and leaf to stem ratio of *Moringa oleifera*

Parameters	Harvest regimes (Days after germinations)				Sed	P value
	70d	80d	90d	100d		
Biomass yield (kg ha^{-1} Dm)	356.55^{a}	489.95^{ab}	669.23^{ab}	785.08^{b}	108.06	0.029
Plant Height (cm)	53.48	67.09	69.69	74.79	6.71	0.082
Number of Leaves	9.40^{a}	11.13^{b}	11.07^{b}	14.60^{c}	0.43	0.001
Plant Girth (cm)	0.92^{a}	1.06^{ab}	1.09^{b}	1.26^{c}	0.0411	0.001
Leaf to stem ratio	1.420	1.523	1.451	1.422	0.1384	0.865

Mean with different superscript are significantly different at P<0.05

The results show that apart from the plant height and LSR that was not significantly (P>0.05) affected, the remaining parameters such as biomass yield production, plant girth, number of leaves at harvest regimes (70, 80, 90 and 100 days) were all significant (P<0.05). The biomass yield production ranged from 356,551.0 - 785,079.0 g/kgDm for 70d and 100d respectively. The leaves number ranged between 9.40 and 14.60 for the 70d and 100d respectively. The highest (P>0.05) plant height was recorded in the 100d. The plant girth had a ranged between 0.92-1.26 with 100d having the highest. The LSR was in the range of 1.420-1.523 with 90d having the highest.

Table 4.2 represents the results of a correlation analysis between biomass yield, plant height, leaf number and stem. A positive correlation (Table 4.2) was reported between plant height, leaf number and biomass yield with plant height having a significant (P = 0.001) influence on biomass yield.

Table 4.2 Correlations between biomass yields, plant height, stem and leaf number of *Moringa oleifera*

Parameters	Biomass Yield	Plant Height	Leaf Number	Stem
Biomass Yield	-	0.0842***		
Plant Height	0.0842***	-	0.6857	0.7714
No. of Leaves	0.2935	0.6857	-	0.9338
Stem	0.3474			-

*** $P > 0.001$

4.1.2 Phytochemical Screening of *Moringa oleifera* plants parts (whole, stem and leaf)

The result of preliminary phytochemical analysis of *Moringa oleifera* plant parts (whole, stem and leaf) tested by basic colored-reactions and is tabulated in Table 4.5. The phytochemical screening revealed the presence of various phytochemicals in the ethanolic extracts of *Moringa oleifera* plants. None of the harvest regimes on *Moringa oleifera* plant parts had all the phytochemicals investigated i.e. alkaloids, saponins, polyuronoids, and anthracenosides tannins, reducing sugars, flavonoid, phytosterols and triterpenes.

Table 4.3: Phytochemical Screening of *Moringa oleifera* plants parts (whole, stem and leaf)

Phytochemical Constituents	Harvest regimes											
	Whole 70d	Stem 70d	Leaf 70d	Whole 80d	Stem 80d	Leaf 80d	Whole 90d	Stem 90d	Leaf 90d	Whole 100d	Stem 100d	Leaf 100d
Saponins	+	+	-	-	-	+	+	+	+	+	+	+
Polyuronoids	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	+	-	+	+	+	+	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Anthracenosides	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenes	+	-	+	-	-	-	-	-	-	-	-	-
Phytosterols	-	+	-	+	+	+	-	+	-	-	-	-
Flavonoids	+	-	+	+	-	+	+	-	+	+	-	+

These results were obtained by color reactions and qualitative comparison. Reacting polyphytochemicals; F, flavonoids; A, alkaloids; T, tannins; S, saponins; P, Polyuronoids; RS, Reducing sugars; An, Anthracenosides; Ph, Triterpenes; Tr, Triterpenes. Negative detection is

indicated by -, positive reaction is indicated by +. All reactions were done by duplicates on at least three plants.

Reducing sugars were found to be present in all the plant parts under the harvest regimes. However, alkaloids, polyuronoids and anthracenosides were not found in all the harvest regimes. Reducing sugars were found in all the plant parts under the harvest regimes.

Similarly, tannins were detected in the plant except 70d (stem). Triterpenes was found to be present in 70d (only whole and leaf) but absent in all the harvest regimes. Flavonoids were found to be present in all the stems of the *M. oleifera* plant parts across all the harvest regimes. Phytosterols were not observed in plant parts under 100d but were commonly distributed in 70d, 80d and 90d. Triterpenes were present in 70d (whole and leaf) but were not detected in the other harvest regimes.

4.1.3 Nutrient composition and digestibility of *M. oleifera* leaf

The results of the chemical composition analyses of *Moringa oleifera* production at harvesting regimes are presented in Table 4.3. There were significant ($P<0.05$) differences in total DM, ADF and CP among harvesting regimes. There was no significant differences ($P>0.05$) among ash Hem and NDF. The whole part of the plant maintained a higher CP concentration in 70d when compared to the remaining treatment.

Table 4.4: Chemical composition (gkg^{-1} DM) of *M. oleifera* whole plant at different harvest regimes

Harvest Regimes	DM	CP	Hem	NDF	ADF	Ash
70d	175.9 [a]	218.4[c]	135.3	256.7	121.3[ab]	7.46
80d	204.3 [b]	151.4[a]	185.3	282.0	96.7[a]	8.45
90d	186.5[c]	167.5[b]	170.7	306.0	135.3[ab]	7.86
100d	195.1 [b]	164.0[a]	155.7	348.0	192.3[b]	7.43
Sed	5.05	11.93	28.3	45.0	26.91	0.718
P-value	0.001	0.002	0.389	0.289	0.039	0.497

Mean with different superscript are significantly different at P<0.05, DM= Dry matter, CP=Crude protein, NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre.

The harvesting regimes revealed different trends in hemicellulose concentration of the *Moringa oleifera*. The highest hemicellulose was obtained from 80d and had a ranged of 135.3-185.3 g/kgDM. There was significant difference (P<0.05) among the four treatment in IVOMD and ME expect the test ingredients under SCFA that was not significant difference (P>0.05) regarding the whole plants (leaf, stem and petiole). It can be observed that the IVOMD from the treatment were higher at 70d and reduces on the subsequent days until it increased again on the 100d (37.46-40.62 ml/g DM).

The highest (P<0.05) metabolisable energy recorded was in 70d and the range was 12.37 - 16.16 ml/g DM. It can be observed that the short chain fatty acids (SCFA) were higher on the 100d and the remaining three harvesting regimes were the same (0.016 - 0.017 ml/g DM).

Table 4.5: Chemical composition and in vitro dry matter digestibility (gkg^{-1} DM) of *M. oleifera* whole plant at different harvest regimes

Harvesting Regimes	IVOMD (%)	SCFA(Mmol/L)	ME(MJ/kg DM)
70d	40.62^{b}	0.016	16.16^{a}
80d	37.46^{a}	0.016	12.37^{a}
90d	37.72^{a}	0.016	13.24^{b}
100d	38.74^{ab}	0.017	13.19^{b}
Sed	0.970	0.0015	0.1458
P-value	0.011	0.722	0.001

IVOMD= in vitro organic matter digestibility, SCFA = short chain fatty acids and ME = metabolisable energy.

4.2 Performance Characteristics of Weaner Rabbits

4.2.1 Health of Weaner Rabbits

All the weaner rabbits were mostly healthy during the study and readily consumed test ingredients. However, the signs of diarrhea was observed on some rabbits during the second and four week, medication was administered using coccidiostat and antibiotics (antibact 3x) considering manufacturers recommendation while ensuring proper housing management. The mortality % recorded was 5% (one out of 20 rabbits) which occurred in the fifth (5th) week. The post mortem done on the rabbit indicates that the rabbit was battling with bacterial infection and antibiotics were given to the rest.

4.2.2 Growth Performance of weaner Rabbits

The performance characteristic of rabbits fed the test ingredient diets is shown in Table 4.4. There was significant difference (P<0.05) among the total feed intake, average of the daily feed intake and final weight gain, except feed conversion ratio, total weight gain, average daily weight gain and initial weight gain of rabbits were not significantly (P>0.05) affected.

Rabbits fed diets T2 and T1 had higher (P<0.05) feed intake when compared to T0 and T3, while statistically the feed conversion ratio was higher (P>0.05) in rabbits fed diet T0 compared to T2, T1 and T3 diets.

Rabbits fed diets T2 and T1 had higher (P<0.05) total weight gain when compared T3 and T0, while the average daily feed intake was substantially higher (P<0.05) in rabbits fed diet T2 compared to T1, T0 and T3 diet.

Table 4.6: Growth performance of rabbits fed different test ingredients

	Test Ingredient					
Parameters	T0	T1	T2	T3	SED	P- value
Initial weight (g)	827.0	956.0	958.0	894.0	135.3	0.740
Final weight (g)	1363[a]	1738[ab]	1980[b]	1548[ab]	181.7	0.022
Total weight gain (g)	536	780	1024	654	169.3	0.059
Average daily weight gain g	10.9	15.9	20.9	13.3	3.46	0.059
Average daily feed Intake (g)	51.32[a]	72.72[ab]	81.66[b]	50.94[a]	7.58	0.001
Total feed intake (g)	2966[a]	4353[b]	4660[b]	2817[a]	440.9	<.001
Feed conversion ratio	5.193	4.592	4.774	3.948	1.167	0.759

[abc] *means with different superscript along rows differ significantly (p<0. 05).*

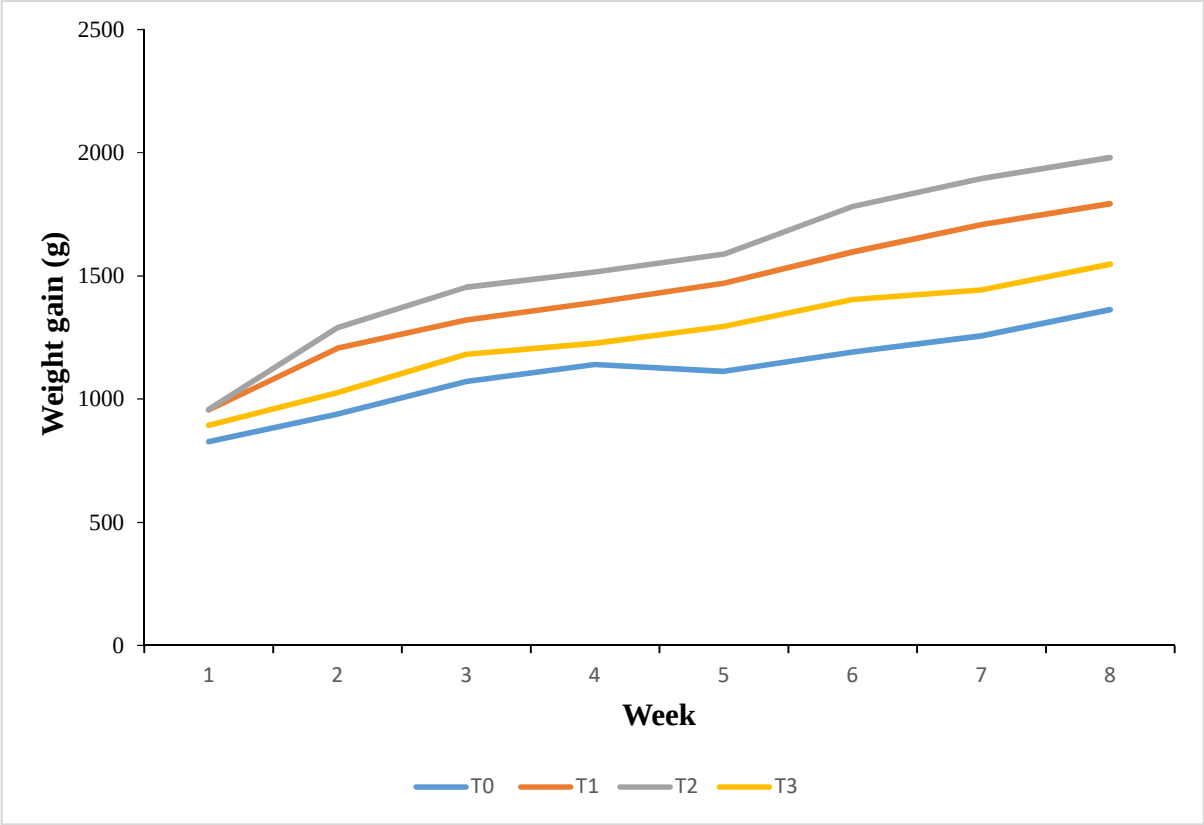

Figure 4.1: Mean weekly weight gain of rabbits fed test ingredients for 49 days

The graph shows the rate at which weaned rabbits responded to the test ingredients considering their weekly body weight gain (Figure 4.1). All the figures are presented as mean weight (g), rabbits fed diet T2 increased dramatically over the period when compared to diet T1, T3 and T0 in an increasing order.

4.3 Proximate Composition of Test ingredients and fecal samples

4.3.1 Proximate Composition of Test ingredients

The various tables (Table 4.7 and 4.8) summarizes the inclusion levels and chemical composition of the test ingredients and apparent digestibility coefficient.

Table 4.7: Chemical composition of the ingredients and apparent digestibility coefficient (%) of rabbits

Ingredients (%)	Test Diets					
	Chemical composition (gkg^{-1} DM)					
	T_0	T1	T2	T3	SED	P-value
DM	877	862	894	886		
CP	349.2	380.5	395.1	363.9		
NDF	246.91	412.76	489.72	271.9		
ADF	38.80	78.83	77.89	39.3		
Hem	206.64	331.82	410.56	583.42		
Ash	47.50	70	52.50	47.50		
Apparent digestibility coefficient (%) of rabbits						
DM	70.33	78.60	75.38	68.35	3.690	0.051
CP	75.67^{a}	83.58^{ab}	85.74^{b}	77.42^{a}	2.745	0.005
NDF	27.78^{a}	73.55^{b}	79.31^{b}	62.58^{b}	5.84	<.001
ADF	17.16^{a}	38.06^{ab}	37.27^{ab}	50.94^{c}	9.210	0.017
Hem	47.68^{a}	81.69^{b}	84.16^{b}	82.93^{b}	3.930	<.001
Ash	81.89^{ab}	86.25^{b}	88.75^{b}	78.01^{a}	2.303	0.001

**premix composition (g/kg): vitamin, 12, 500 IU; vitamin D3, 2500 IU; -itamin,50.00 mg; vitamin K3, 2.50 mg; vitamin B1, 3.00 mg; vitamin B2, 6.00mg;vitamin B6, 6.00 mg; niacin, 400 mg; calcium pantothenate, 10mg;biotin, 0.8 mg; vitamin B12, 0.25 mg; folic acid, 1.00 mg; chlorinechloride,300 mg; manganese, 100 mg; iron, 50 mg; zinc, 45 mg; copper,2.00 mg; iodine, 1.55 mg; cobalt, 0.25 mg; selenium, 0.10 mg; antioxidant, 200 mg. Mean with different superscript are significantly different at P<0.05, DM= Dry matter, CP=Crude protein, NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre.* ^{abc} *means with different superscript along rows differ significantly (p<0. 05). Mean with different superscript are significantly different at P<0.05, DM= Dry matter, CP=Crude protein, NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre.*

Rabbits on diet T2 had a higher DM content (894 gkg^{-1}DM) when compared to rabbits on diet T3 (886 gkg^{-1}DM), diet T0 (877 gkg^{-1}DM) and diet T1 (862 gkg^{-1}DM). Similarly, diet T2 had a higher CP value (395.1 gkg^{-1}DM) when compared to rabbits on diet T1 (380.5 gkg^{-1}DM) diet, T3 (363.9 gkg^{-1}DM) and diet T0 (349.2 gkg^{-1}DM). However, the highest ash value recorded was in diet T1 (70 gkg^{-1}DM) and the range was 47.5-70 gkg^{-1}DM.

In terms of the diet offered, rabbits on diet T3 recorded higher NDF content (661.28 gkg^{-1}DM) when compared to rabbits on diet T2 (489.72 gkg^{-1}DM), diet T1 (412.76 gkg^{-1}DM) and diet T0 (246.91 gkg^{-1}DM) (Table 4.7). On the contrary, diet T1 recorded a higher ADF value (78.83 gkg^{-1}DM) when compared to rabbits on diet T2 (77.89 gkg^{-1}DM) diet, T3 (75.91 gkg^{-1}DM) and diet T0 (38.80 gkg^{-1}DM).

Moreover, the mean hemicellulose differed considerably between rabbits on diet T0, T1, T2 and T3 which ranged from 206.64, 331.82, 410.56 and 583.42 gkg^{-1}DM respectively.

The apparent digestibility coefficient of the test ingredients were all substantially higher ($p<0.05$) (DM) (Table 4.7). The crude protein was significantly higher for the rabbits on diet T2 (85.74%) than rabbits on diet T1 (83.58%), diet T3 (77.42%) and diet T0 (75.67%). Although, dry matter digestibility was not significantly affected, but statistically rabbits fed diets T1 (78.60%), T2 (77.38%) and T0 (70.33%) were had high numerical values compared to T3 (68.35%). In addition, both NDF and ADF were significantly ($p<0.05$) inlfluenced, rabbits fed diet T2 had higher digestible of NDF ranging from 27.78-79.31% for T0 and T3 respectively. The ADF noted were higher on rabbits fed diet T3, T1, T2 and T0.

In terms of hemicellulose digestibility, higher values were observed in rabbits fed diet T2 (84.16%), T3 (82.93%) and T1 (81.69%) when compared to diet T0 T0 (47.68%). Finally, the

Ash digestibility were substantially greater in rabbits fed with diet T2 (88.75%) and had a range from 78.75-88.75% for T0-T3 respectively.

4.4 Hematological Analysis

The results of hematological values obtained for all the weaner rabbits before and after the experimental period in this study are presented in Table 4.8 and Table 4.9.

Table 4.8 Hematological Parameters of the Rabbits before Experimental trials.

Parameter	Dietary Treatment					
	T0	T1	T2	T3	SED	P-value
WBC(×10^{9}L)	5.84	5.60	4.83	6.93	1.051	0.322
Lymphocytes (%)	79.4	63.9	53.7	65.9	11.45	0.243
Monocytes (%)	6.37^{ab}	6.63^{ab}	2.42^{a}	13.08	2.155	0.007
Neutrophils (%)	11.3	16.8	40.6	20.3	11.68	0.143
Basophil (%)	0.040^{a}	0.070^{a}	0.070^{a}	0.327	0.0904	0.042
Eosinophil's (%)	0.587	0.657	0.760	0.947	0.233	0.481
RBC(×10^{12}L)	5.73	5.25	4.94	5.04	0.449	0.363
Hemoglobin (g/dL)	12.87^{c}	10.97^{ab}	10.72^{a}	11.20	0.617	0.031
PCV(pg)	41.31^{b}	34.57^{a}	34.03^{a}	34.77	1.561	0.005
MCV(fL)	66.93	66.67	67.23	69.47	2.386	0.645
MCH (pg)	21.27	21.23	21.20	22.17	0.574	0.333
MCHC(g/dL)	31.77	31.83	31.07	32.13	0.437	0.174
Platelets(× 10^9/L)	181	236	154	121	43.9	0.139
MPV (fL)	4.13	5.13	4.10	4.77	0.400	0.085
PDW (fL)	3.83	5.33	3.93	5.43	0.637	0.061

Note*: P-value, 0.05 is considered statistically significant. Abbreviations: WBC, white blood cell; monocyte, basophil, and eosinophil; RBC, red blood cell; Hgb, hemoglobin; PCV packed cell volume; MCV, mean cell volume; MCH, mean cell hemoglobin; Mchc, mean cell hemoglobin concentration; RDW, red blood cell distribution width; MPV, mean platelet volume; PDW, platelet distribution width; SD, standard deviation*.

The hematological parameters considered for the weaner rabbits adjustment period and before rabbits were allotted to the designed treatment were differed significantly (P<0.05) with the range of 2.42-13.08% (Monocytes), 0.04-0.327% (Basophil), 10.72-12.87g/dL (Hemoglobin) and 34.03-41.31% (Packed cell volume).

However, the rest of the parameters were not significantly affected, and these were ranged from 4.83-6.93 (×10^{9}L WBC), 53.7-79.4% (Lymphocytes), 0.587-0.947% (Eosinophil's), 4.94-5.73

($\times 10^{12}$L RBC), 11.3-40.6% (Neutrophils), 66.67-69.47fL (MCV), 21.20-22.17 pg (MCH), 31.07-32.13g/Dl (MCHC), 121-236 ($\times 10^{9}$/L Platelets), 4.10-4.77 fL (MPV) and 3.83-5.43 fL (PDW).

The results on the effect of the treatment diets on hematological parameters are presented in Table 4.13. The WBCs, PCV and Hgb values were significantly ($P<0.05$) influenced by the dietary test ingredients. Rabbits on diet T2, had a numerically higher WBC value (9.27×10^{9}L) when compared to rabbits on diet T1 (8.93×10^{9}L), diet T0 (6.73×10^{9}L) and diet T3, (6.67×10^{9}L). However, the MCH and MCHC values observed had a range of 20.69-22.23 pg and 29.90-32.50 g/dL. Rabbits on diet T0 had a higher PCV content (41.33%) when compared to rabbits on diet T2 (37.87%), diet T1 (35.93%) and diet T3 (35.77%). In the same way, the trend in the values observed for PCV followed a similar outline observed for Hg.

Rabbits on diet T0 had a numerically higher Hb content (13.37 g/dl) when compared to rabbits on diet T2 (12.40 g/dl), diet T3 (11.93 g/dl) and diet T1 (11.57 g/dl). However, Eosinophil's, MCH and MCHC were significantly influenced by the diets whiles platelets, PDW, MPV, MCV, Basophils, Neutrophils, lymphocytes did not differ across the test ingredients.

Table 4.9: Hematological Parameters of the Rabbits after Experimental period.

Parameter	Dietary Treatment					
	T0	T1	T2	T3	SED	P-value
WBC(×10^9L)	6.73[a]	8.93[b]	6.67[a]	6.67[a]	0.622	0.004
Lymphocytes (%)	68.7	44.4	48.9	46.1	12.98	0.287
Monocytes (%)	8.7	10.7	11.7	13	13	0.606
Neutrophils (%)	20.08	33.54	31.88	34.02	14.49	0.747
Basophil (%)	0.16	0.93	0.21	0.98	0.462	0.216
Eosinophil's (%)	2.69[b]	1.147[a]	2.353[b]	1.617[a]	0.160	<.001
RBC(×10^{12}L)	5.35	5.17	5.88	5.39	0.449	0.463
Hemoglobin (g/dL)	13.37	11.57[a]	12.40[ab]	11.93[a]	0.878	0.008
PCV(pg)	41.33[b]	35.93[a]	37.87[ab]	35.77[a]	1.129	0.004
MCV(fL)	69.50	68.33	67.20	66.74	1.985	0.541
MCH (pg)	20.69[a]	21.07[a]	20.67[a]	22.23	0.319	0.004
MCHC(g/dL)	29.90[a]	30.83[a]	30.67[a]	32.50	0.439	0.002
Platelets(× 10^9/L)	143	213	187	153	61.7	0.661
MPV (fL)	4.70	4.80	4.97	4.13	0.426	0.301
PDW (fL)	4.70	6.43	6.47	4.23	1.246	0.237

Note*: P-value, 0.05 is considered statistically significant. Abbreviations: WBC, white blood cell; monocyte, basophil, and eosinophil; RBC, red blood cell; Hgb, hemoglobin; HCT hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red blood cell distribution width; MPV, mean platelet volume; PDW, platelet distribution width; SD, standard deviation.*

CHAPTER FIVE

5.0 Discussion

5.1 *M. oleifera* biomass yield and qualitative Phytochemicals

Tropical tree and shrubs (leaf and stem) harvesting have been practiced since ancient times for being used especially as feed in rainy and summer seasons (Stür *et al.*, 1994). In the present study, the potential of *M. oleifera* whole plant (leaf, stem and petiole) for producing better biomass yield while studying the trend in the phytochemicals present was investigated considering four harvest regimes (70d, 80d, 90d and 100d).

It was observed in this study that maximum biomass production was obtained when *M. oleifera* plants were harvested at a longer regimes (90d and 100d). This finding agrees with the report by Nouman *et al.* (2013) who found that harvest regimes affects *M. oleifera* biomass production (both fresh and dry). It was also observed that as aging in *M. oleifera* increase biomass yield also increases. This study agrees with the report that longer harvest regimes increase biomass production (Guevara *et al.*, 1978; Ella *et al.*, 1989; Assefa, 1998; Barnes, 1999; Latt *et al.*, 2000; Tuwei *et al.*, 2003).The possible reason could be that climatic and environmental factors may play a significant role in *M. oleifera* biomass production (Mabapa *et al.*, 2017). It was further explained that biomass growth increase under favourable weather conditions (temperature and rainfall) even when plants were pruned (Mabapa *et al.*, 2017). This is an evidence that *M. oleifera* plants could survive and produce satisfactory yields even under conditions of water limitation such as Botanga irrigation site where the plants exposed to relatively low rainfall at the time of cultivation.

There was positive correlation (P=0.0001) on biomass production against plant height, number of leaf and stem (Table 4.2). The present study agrees with the report by Foidl *et al.* (2001) who observed a positive correlation between planting densities and *M. oleifera* production.

This finding concurs with those of Amaglo *et al.* (2006), Gadzirayi *et al.* (2013) and Mabapa *et al.* (2013) who showed that relatively harvest regimes resulted in taller plants because of competition for growth factors. The common explanations point out that appropriate field management of soil moisture and soil amendment (organic or inorganic fertilizer) is required to supply plants nutrients and moisture to reduced competition among the plants. It was also, observed in the study that *M. oleifera* plant produced more branches when compared to the leaves. Some researchers have found that higher harvest regimes produced higher dry matter yield (Hairiah *et.al*, 1992); However, this study was in disagreement with Blair and Edger (1990) who observed that in some cases, cutting height did not affect biomass production, likewise (Stür et. al. 1994), on the other hand Ella, and Curet, (1989) considered that the effect of cutting height on the growth pattern of plants is still not clear and call for more investigations as well as the number of leaves per plant on the plot.

The study also agrees with the suggestion made by Lazer (1981) that total biomass production increased as the harvest regime prolonged due to plants release more buds after harvesting, stimulate fast regrowth, and develops high leafy retention and coppicing capacity after cutting during the dry season.

However, LSR of the *M. oleifera* did not differ from each harvest regime. This study agrees with the findings by Sultana *et al.* (2014) who reported that there was no significant of leaf to stem ratio among harvesting intervals of *M. oleifera*. Statistically, there was variation in the values

and this may be attributed to plants adaptability with climatic condition, growth and canopy structures under the growing condition.

5.1.2 Chemical composition, Phytochemicals and *in vitro* digestibility of *M. oleifera* whole plant

The present study revealed the following phytochemicals in the *M. oleifera* plants; polyuronoids, tannins, flavonoids, saponins, phytosterols, triterpenes, reducing sugars and anthracenosides which are known to exhibit medicinal as well as physiological activities (Shrestha *et al.*, 2015). The plant extracts were also revealed to contain saponins which are known to inhibitory effect on inflammation (Just *et al.*,1998). Phytosterols have been reported to have antibacterial properties (Epand *et al.*, 2007) and they are very imperative compounds particularly due to their relationship with compounds such as sex hormones (Okwu, 2001) mammals or animals. Although it is feasible to securely attribute the lack of certain phytochemicals in some plant part and its presence in the other to the multiple physiological and biosynthetic reactions within the plant, the environment impact should not be neglected.

It was observed that saponins were present in plants harvested at 70d but was absent at 80d. Interestingly, saponins appeared to be present in both 90d and 100d. The possibly reason could the difference in *M. oleifera* maturity and water stress in plant development. This study agrees with the paper by Bhat *et al.* (2013) who suggested that certain phytochemicals of plants is affected by plant species, genotype and stage of maturity and may vary with plant parts (leaves, stems, seed), season of growth and other specific environmental factors. However, the presence of certain phytochemicals may limits the plant use as feed. This is because some phytochemicals could have antiparasitic properties, which depend on their structure, extent of ingestion and accessibility within the gastrointestinal tract of the animal (Athanasiadou and

Kyriazakis, 2004). These researchers suggested that the antiparasitic properties of the phytochemicals should be well assessed on plant anti-nutritional effects before they could be use as feed. This is because the consumption of phytochemical-rich plants by animals could result in reduced intake, weight loss, toxicity and death (Milgate and Roberts, 1995; Waghorn and McNabb, 2003). It have been reported that condensed tannin consumption was related with reduced feed intake, decreased digestibility and impaired rumen metabolism (Barry and McNabb, 1999; Min *et al.*, 2003).

The presence of saponins in *M. oleifera* in the study, have also been considered responsible for reduced feed intake and growth impairment (Applebaum and Birk, 1979; Milgate and Roberts, 1995). Saponins have also been related with hemolytic action and bloat in ruminants (Athanasiadou and Kyriazakis, 2004) but little is known on rabbits. Excessive consumption of alkaloids, glycosides and terpenoids can result in lesions in the nervous system (Conn, 1979; Mabry and Gill, 1979).

The lowest value of CP (151.4 g/100g DM) for the whole plant (*M. oleifera)* is well above the range of 7.0–8.0 g/100g DM suggested as critical limit below which intake of forage by ruminants and rumen microbial activity would be adversely affected (Van Soest, 1994). The high CP content in all the four harvesting regimes are an advantage to rumen microbes that depend on dietary source of nitrogen to build up their body protein. The high CP concentration observed in the whole plants harvested on the 70d compared with the 80d, 90d and 100d could be as a result of the age of the plant and this agrees with earlier studies on the effect of botanical fraction on CP levels (Tang *et al.*, 2008). As grass or plants matures, the nutritional content of the grass begins to decrease. The stems become tougher with time, more fibrous, protein and energy levels can decrease, the leaves also contain most of the energy and protein the plant has to

offer. The CP supplied by *M. oleifera* is above the protein requirement for rabbit (suggested 27 g animal protein per caput/day) (Ajayi et al., 2007).

The chemical composition (CP, Ash) of *M. oleifera* differed significantly among the harvest regimes (Table 4.7). This agrees with the report by Mabapa *et al.* (2017) who found significant differences in terms of CP and some minerals at different harvest. The possible reason for these observations may be that the *M. oleifera* were storing nutrients at a slower rate due to the low temperature and rainfall experienced at different location (Mabapa *et al.,* 2017). This finding differs from the report by Sanchez *et al.* (2006) who detected no significant differences in chemical´ composition at continuous harvests (or in the harvesting of young leaves and tender stems). Furthermore, the study disagrees with Nouman *et al.* (2013) and Moyo *et al.* (2011), reported no´ changes in the chemical composition (CP) of *M. oleifera* at different harvest. A hot rainy season enhances biomass production and the plant's nutritional content. In this study, the decline in chemical composition of the *M. oleifera* may be because of inadequate nutrients in the soil, mainly nitrogen and phosphorus. A number of factors might have influenced the concentration of chemical composition in the *M. oleifera*, like those in the soil, their accessibility to the plant, type of soil, soil pH, stage of plant growth, and weather conditions (Lukhele and Van Ryssen, 2003; Sanchez *et al.*, 2006).

NDF and ADF contents obtained in this study for the different harvest regimes of *M. oleifera* were within the range of 256-348 g kg^{-1}DM and 96.7-192.3 g kg^{-1}DM (Makkar and Becker, 1996; Foidl *et al.*, 1999; Aregheore, 2002; Al-Masri, 2003). The NDF and ADF values reported in the study compares favorably with what has been reported in the *M. oleifera* (Sanchez *et al.*, 2006; Moyo *et al.,* 2011). The present study revealed that there was a gradual increase with the ADF levels as the *M. oleifera* plants matured. The least digestible feed components, including

cellulose and lignin, ADF values are known to inversely relate to digestibility so the feed with low ADF concentrations are mostly considered as higher in energy. The ADF content is higher than the average value (120 g/kg DM) reported by Nouman *et al.* (2014) and Sánchez *et al*. (2006) except the 80d (96.7 g/kg DM). In general, the NDF content of *M. oleifera* plant is very moderate at 70d and 80d, which has an implication with regard to feed intake in rabbit production. The extent and trend of crude fibre is valuable because of the correlation that exists between it and digestibility of feed (Kundu *et al*., 2005) the quantity of it in *M. oleifera* may have limitation to some small ruminants like rabbit.

Young stems are generally of high quality, but the quality decreases faster than in leaves, because epidermis and fibrous cells change into secondary cellular wall and lignin content increases with increased age of the plant. The effects of harvest regime on CP content and IVOMD were significantly affected in the study. These results do not agreed with the report by Ventura and Pulgar (1997) in that CP content did not show differences between plant densities while total nitrogen and IVOMD (Assefa, 1998; Nygren and Cruz, 1998) declined with time but not significantly as harvesting regimes increased. It is well observed that nutritive composition of tree or shrub species may be determined by soil fertility, part of the plant (stem, leaves, fruit), age of regrowth, environmental conditions, season of the year and other factors (Lascano, 1996). As observed earlier (Table 4.4) the longest harvest interval resulted in higher total DM yield, but nutritive value generally decreases as harvest interval increases (Maass *et al.*, 1996).

The harvesting regimes did not affect SCFA significantly; statistically the values were relatively the same across all the harvesting regimes. Lower fibre fractions in *M. oleifera* relative to other forage may have resulted in the higher values for IVOMD and SCFA (Van Soest, 1994).

The ash content in the present study fell within the range of 7.43–8.45 g kg^{-1}DM for *M. oleifera* as feed (Makkar and Becker 1996; Al-Masri, 2003). Even at longer harvest regime, the CP and IVOMD were more or less stable at 80d and 100d. This can be explained by the fact that N content in the plants (leaves or young stems) generally do not drop with maturity (Miquilena *et al.,* 1995). In the present investigation, CP content and IVOMD of *M. oleifera* was within the range of 151.4-218.4 g CP kg^{-1}DM and 37.46-40.62% DM, respectively, this finding is in lined with the previous report on *M. oleifera* by other workers (Makkar and Becker, 1996; Foidl *et al.,* 1999; Aregheore, 2002; Al-Masri, 2003; Manh *et al.*, 2003).

High ME of *M. oleifera* coupled with high CP in the harvest regimes suggests that the plants may advance microbial protein synthesis as it may promote better synchronization of fermentable energy and degradable N in the rumen as concluded in the study (Olafadehan *et al.,* 2014).

However, a suitable formulation and feeding trial of the plant leaves and petiole could be use as feed for monogastrics such as rabbits while investigating its effects on the animals. It is obvious, that *M. oleifera* is an efficient source of dietary protein, presence of certainly mineral and vitamins (Table 2.3). The experiments containing protein-rich *M. oleifera* leaf, the faecal protein digestibility was found to be high (70-71%), (Adeniji *et al.,* 2012) or increased in presence of *M. oleifera*, when ranged from 65% to 80% (Nuhu, 2010; Ewuola *et al.*, 2012). This positive effect of *M. oleifera* leaf on protein and dry matter digestibility was also observed at reasonable inclusion rates as low as 3% (Djakalia *et al.,* 201 1). It was also found that when *M. oleifera* leaf contained less protein (20-23%) and more NDF 31% and ADF 26% (Safwat *et al.,* 2014), the estimated protein digestibility was approximately 50% (Lebas, 2013). The digestible energy in *M. oleifera* leaf was studied and estimated to range from 10.6-13.8 MJ/kg DM for low-protein

and high-protein meals respectively (Lebas, 2013). Therefore, it is suggested that the composition and nutritional value of *M. oleifera* is assessed or formulated as rabbits diet on a per case basis before used in balanced diets.

5.2 Growth Performance, Nutrient digestibility and Hematology of weaner rabbits

5.2.1 Growth Performance of weaner rabbits

Gain in body weight, generally expressed as a mean weight gain per day (g/day) is the popular important measure of growth in growing animals (Nsoh, 2018). Frequently, the overview of a fresh feed substance can lead to declining feed consumption and associated consequences of indigestion for most animals, especially rabbits.

In this study, however, rabbits on test ingredients voluntarily consumed feed containing both the T2 and T1 diets than those on the control diets T0 and T3. The weight gain noted in the rabbits can correspond to the enhanced consumption of feed (Shanmuganathan *et al.*, 2003). *M. oleifera* leaf meal may efficiently boost rabbit weight when correctly managed. Moreover, strategies to the professional use of feeds (*M. oleifera* plant) are much more successful if the test system matches the resources available (Preston and Leng, 1987).

This study showed a gradual increase in the average daily intake of rabbits fed with test ingredients. The total feed intake obtained was within the range of 57.5-95.1 g/day for rabbits (Federick, 2010) but higher than the reported values of 44.73-57.90 g/day (Mufwa *et al.*, 2011). The weight gain recorded was higher than 6.78-8.64g/day (Odeyinka et al., 2008). The optimal growth performance obtained in the study, particularly, diets T2 and T1, may have been the addition of *M. oleifera* leaf concentrates to the rabbit diet (Safwat *et al.*, 2014). The study is in line with the study by Dahouda *et al.* (2013) and Safwat *et al.* (2014) who reported that the inclusion of *M. oleifera* leaf meal at 30% and 40% in rabbit's diets improved their growth

performance.

However, the prime limitation of using *M. oleifera* leaf meal for feeding weaned rabbits is the dietary balance needed rather than the product itself. A number of researchers included *M. oleifera* leaf meal in diet to rabbits weaning males at an amount of 15% (Abu *et al.,* 2013), and increasing at a rate of 7.2% in substitution for soya bean meal (Ayodele *et al.,* 2014). The growth performance obtained in test ingredient T1 and T2 (thus, 40% *M. oleifera* leaf meal inclusion) was much better compared to the growth performance reported by Abu *et al.* (2013) and Ayodele *et al.* (2014). The possibly reason could be that the rabbits were able to use forage more effectively (Lukefahr and Cheeke, 1997). Also, Jabeen *et al.* (2008) reported that the efficiency of animal feed concentrates can be improved by supplementation with M. oleifera leaf meal.

In the same way, Sultana *et al.* (2014) reported that soybean meal added with *M. oleifera* leaf meal significantly influenced growth performance of birds body weight, body weight gain, better health status and feed conversion ratio (FCR). The FCR values observed in this study was higher than 2.63-3.00 reported by Okorie (2003) nevertheless was comparable to the values (4.22-5.13) reported earlier (Federick, 2010) for rabbits. The possible reason for the differences observed in the FCR in maybe difference in; age, environmental and feed composition (Shanmuganathan *et al.,* 2003).

There was an improvement in the growth rates in T2 and T1 when compared to the control T0 and T3, which shows better use of nutrients with different inclusions (Rexen, 1981; Hollister *et al.,* 1990; Singh *et al.,* 1995). In this study, the presence of each of these enzymes; cellulase, amylase, glucanase, protease, xylanase, phytase in the test ingredients may suggest that the performance response was directly linked to the effects of multi-enzymes on the digestibility of

nutrients. An increase in the dosage of amylase in monogastric feed has been reported to improve daily gain (4.5%) and feed intake (3.6 %) (Gracia *et al.*, 2003a).

The presence of enzyme may result to either reduced or increased in FCR. Although the FCR was insignificant, it is interesting to note that the two test ingredients (T2 and T3) made up of multi-enzymes had a low FCR compared to T0 and T1. This finding is consistent with the report by Shanmuganathan *et al.* (2003), they noted FCR in the test ingredients with yeast and EM inclusion level were also lower than the presence study. Singh *et al.* (1995), also revealed that feed required per unit gain was reduced by 3% in NZW rabbits fed with Saccharomyces cerevisiae. Singh and co-workers data tend to suggest again that 5% improvement in body weight gain was observed. This phenomenon has been explained earlier that diets with adequate levels of phytate have depressed body weight and FCR (Liu *et al.*, 2008).

This present study weight gain by rabbits fed diet T2 (1024 g) and T3 (654 g) were lower than the report by (Raach-Moujahed *et al.*, 2017). The researchers reported that Rovabio and Kemzyme plus Dry diets (without phytase) increase weight gain (2010.56 g) compared to control and Cibenza DP100 diets. This study concurs with the findings reported by Hollister *et al.* (1990) who observed an improved feed to gain ratio in rabbits fed on diets with Lacto-Sacc. Li et al. (1995) and Inciong (1996) have reported higher feed efficiency of poultry diets supplemented with effective microorganism.

The improved weight gain on rabbit fed test ingredients (T2 and T1) could possibly be the inclusion of *M. oleifera* leaf or the multi-enzyme in the diets. This study agrees with the report on rabbits fed with test ingredients comprising probiotics supplementation (Kritas and Morrison, 2005). It is obvious that the weight gain per day of rabbit fed diets T1(15.9 g/day) and T2 (20

g/day) was higher than (15 g/day) compared to studies conducted by Ramchurn and Raggoo (2000) and also lower than the reported values of 30 g/day for rabbits fed commercial pellet feed (Awotarowa, 1992). The possible reason for the different observations on the faster growth rate with the test ingredients may be due to enzyme supplementation caused positive effects in energy and protein digestibility of broiler chickens (Pourreza et al., 2007). The inclusion of xylanase significantly decreased the competition for nutrient usage from the gut micorbiota and it have been suggested that adequate nutrients were accessible for poultry bird (Hosseini and Afshar, 2017). The implication of the study was that the use of multi-enzyme-treated diets would be a promising innovation to facilitate the utilization of tropical forage with or without anti-nutritional factors in the rabbit production.

5.3 Chemical composition and nutrient digestibility of test ingredients

Studies on the comparative impacts of feed diets on the basis of raw material/maize have been identified as likely feed element on quantitative nutrient consumption, estimation of nutrient digestibility and the resulting growth efficiency (Dougnon *et al.*, 2012; Ansah *et al.*, 2014). The Crude protein content of the test ingredients (Table 4.7) in this study was higher than the values reported by Iyeghe-Erakpotobor *et al.* (2006), Federick (2010), Adam (2013), Ansah *et al.* (2014), Ayandiran and Odeyinka (2016) and Jimoh (2018). The variations in the diets values could be due to the concentration of protein in the residue (different ingredient) following the removal of starch due to fermentation and microbial development.

The crude protein content of test ingredients was above 160 gkg^{-1} DM suggested for optimal development of rabbits (NRC, 1977; Obinne and Okorie, 2008). The higher CP content found in test ingredients T1 and T2 could potentially be the inclusion level of *M. oleifera* leaf at 40% to

satisfy the daily protein requirements of weaned rabbits. This study agrees with the report by Ayodele *et al.* (2014) who stated that when 20% *M. oleifera* leaf meal was used as a substitute to soybean meal, the rabbits performed well on their growth characteristics than those of the control diet

Fiber is a complex carbohydrate bonded in such a way that it cannot be broken down by the animal enzymes. The bonds and digest (ferment) fiber can only be broken down by microbes residing in the rumen. For keeping ordinary rumen function, fiber is essential. Excess fiber in a ration, however, will restrict the consumption of nutrients and digestibility of ration.

The present study reported higher NDF content in rabbit fed diet T2 (489.72 gkg^{-1} DM) and T1 (412.76 gkg^{-1} DM). However, the crude fibre values obtained was higher than the values required for normal development (9% crude fiber) in rabbit diet (Champe and Maurice, 1983). The inclusion of *M. oleifera* leaf meal in the two diets have improved the crude fibre content required for rabbit's growth and development. The reason could be that the digestive capacity of the rabbit might have influence by endogenous enzyme activities, digestion by the microbial population and the rate of passage of the feed (Richard and Church, 2002).

5.3.1 Digestibility of the Nutrients

Rabbits on the test ingredients T2 and T1 were ranked the first and second in nutrient digestibility of CP (85.74%; 83.58%), DM (75.38%; 78.60%) and Ash (88.75%; 86.25%). The apparent digestibility of DM and CP were higher than the values reported by Iyeghe-Erakpotobor *et al.* (2006) who observed the range of 63-76% and 69-79% for DM and CP respectively. Similarly, substantially higher values were recorded on rabbit fed multi-enzyme treated diets (T2 and T3) in this study when compared to the values observed by Shamuganathan *et al.* (2003) for DM, CP and crude fiber nutrient digestibility.This higher consumption may be due to low toxic

phytochemical contents in the *M. oleifera* leaf according to some authors.

The significant (P<0.05) difference in nutrient digestibility observed conversely corroborates that of Ajayi *et al*. (2007) who detected significantly different values and disagree with that reported by Olabanji *et al*.(2007) who did not observed significant values for the digestibility of nutrient by rabbits. Although, the apparent digestibility of nutrients varied among the test ingredients, rabbits fed multi-enzyme treated+*M. oleifera* leaf meal and *M. oleifera* leaf meal without multi-enzymes generally performed better than the T0 and T3. Rabbit, on the other hand, is a single herbivorous stomach, its digestive system is appropriate for high cellulose diet, and the digestibility of proteins from various sources could differ greatly especially when compared diets T2 and T1 to the control (T0 and T3). The protein energy ratio in the diet is very valuable because when rearing rabbit for meat e.g. too high or too low leads to two adverse effects, one directly affects the production index, the other obviously increases the mortality rate. However, it has been underscore that, the ratio of digestible CP and digestive energy is appropriate at 10.8 in the diet reported by De Blas and Mateos (1998) for healthy development in rabbits. Gidenne (1997) proposed that low digestive ingredients (cellulose and lignin) could decrease digestive tract disorder and that the fiber demand in the fattening diet of rabbit was reported. Gidenne *et al.* (1998) indicated that lignified cellulose in the diet could decrease the digestibility obviously.

The rabbit does not vary in its ability to digest starch and sugars, which are the main nutrients in "concentrates" compared to other domestic animals (Fielding, 1991). Also, a part from some other animals, fibre constituents can only be digested in rabbits through microbial fermentation (de blais *et al.,* 1999). It could be observed that rabbits on the diets (T1 and T2) were able to utilized crude fibre but was effectively with feed containing multi-enzymes (Table

4.8). Thus, the addition of multi-enzyme increased the digestion of the crude fibre utilization (Freiria *et al.,* 2018), although the amount of multi-enzyme present in the diet in this study may have influenced the digestibility of crude fibers in the test ingredients. The low level of multi-enzyme inclusion may have resulted to low differences in the diets T2 and T1. However, adequate quantities of multi-enzymes in feed have the ability to improve in animal fiber digestion (Freiria *et al.,* 2018). The multi-enzyme treated *M. oleifera* leaf provides substantial benefits to the animal industry and could be used with great flexibility in a variety of diets for rabbits when the appropriate dosage is identified.

The integration of multi-enzymes into rabbit diets is a comparatively new model. If rabbit production costs could be cheap through biotechnological synthesis, then the practice might become universal. Some predictions on these multi-enzyme are: proteases to make protein more accessible; B-glucanase to break down complex cereal starches to glucose; cellulase to digest plant cell walls; and phytases to liberate tightly bound phosphates and others. Each of the enzymes could release specific nutrients from the test ingredient so that these are available to the animals in greater quantities. The prime objective of supplementing multi-enzyme to animal (rabbit) diets is to improve the utilization of nutrients in feedstuffs (Sheppy, 2001). The paper by Phillipes (2010) explained that, much attention have been given to protease because protein is the second most expensive item in animal diets compared to energy. Protease inclusion is thought to be beneficial particularly to young monogastric due to the fact that the proteolytic and amylolytic digestive system is not fully developed. This study agrees with the report by Moeser and van Kempen (2002) who observed that the inclusion of fiber degrading carbohydrase enhanced dry matter digestibility by 2% and energy digestibility by 3% while decreasing fecal output.

5.4 Hematology of weaner rabbits

An animal's blood acts as a means of transportation. It carries food products like glucose, fatty acids, vitamins and electrolytes from the intestinal tract to body tissues where they are used for body construction and energy. Some recognized variability reaction may be due to the contribution impacts on multi-enzyme and feed compositions in the current research with the feed additives.

Increase or decrease in body weight over a specific period is the main indicator of the efficiency of the meat animal and varies depending on the quality and, to an extent, on the quantity of feed given (Blood and Studdert, 1999). This means that weight, feed and blood are directly linked. In the present study, the final weights had a substantial difference ($P<0.05$), although the live weight gain was not influenced ($p=0.059$), statistically the numerical values (mean weight) were comparable. It have been maintained that feed components influence blood constituents (Harper et al., 1979) and hematological indices may be used to determine the effects of the test ingredient on animals physiological status (production and disease conditions) (Schalm *et al.*, 1975; Adenkola and Durotoye, 2004; Khan and Zafar, 2005; Makinde, 2017). The values reported in the present study fall within the normal range of 4.1-9.79×10^9L reported by (Mitruka *et al.*, 1977; Jain, 1986; Zimmerman *et al.*, 2010).

It was also noted that rabbits fed diets T1, T2 and T3 increased WBC by 3.33%, 4.44% and 0.27% respectively. The inclusion of *M. oleifera* leaf meal and/or multi-enzyme may have contributed or influenced the WBC values. This finding is in line with Ologboho *et al.* (1986) who proposal that a higher than normal WBC count is an implication of the existence of

inhibitory substances and foreign bodies in the body. The values of 5.17, 5.39 and 5.88x10^{12} L obtained in this study for diets T1 and T2 respectively are within the normal range of 5.135-5.394 x10^{12}L reported for rabbits fed agro-industrial by-products (Ansah *et al.*, 2014).

It was proposed that the quantity of entire unclotted blood occupied by the RBC could be helpful in identifying whether dehydration or excitation has thus happened when PCV is abnormally high or declined (Blood and Studdert, 1999). Anaemia happens when blood supply, haemoglobin content and PCV are reduced in RBC count. These three variables are therefore important in the research to determine the existence or lack of anaemia (Fraser *et al.*, 1991). These parameters hemoglobin, WBC and PCV values were used to determined nutritional anaemia for the test diets formulated. This is because the values obtained for these parameters (WBCs, PCV and Hb) are within the normal range values as reported by (Mitruka *et al.*, 1977; Jain,; Zimmerman *et al.*, 2010), it can be concluded that the dietary treatment did not cause any nutritional anaemia and can supply the needed nutrients to the rabbits.

The reduction in RBC values could be directly linked to residues of residual antinutritional factors in the test ingredients, which could result in anaemia. Swenson (1990) reported that variations in nutrition, physical activity and RBC volume could lead to anaemia. It can be concluded from the above research that under the test ingredients T1 and T2 rabbits could be grown cost-effectively without negative health effects. *M. oleifera* and multi-enzyme nutritional incorporation will gradually influenced the health and immune status of rabbits. Although, the WBC in the study was not significantly influenced when rabbits fed the test ingredients, T2 and T1 had a lower values compared to the control. The possible reason could be that the low level of WBC in the blood could be as a result of no disease condition or low production from bone marrow (Clement *et al.*, 2010).

The variation of monogastric to antioxidants fed (*M. oleifera* leaf), as well as appropriate absorption of iron from feed may explain high values for circulating red blood cells in test ingredients (T2 and T1). Therefore, at an inclusion ratio of *M. oleifera* leaf with or without multi-enzyme in this study, may have individually contributions optimally to stimulate iron-absorption, utilization and subsequent production of RBC by stem cells (Adegoke *et al.*, 2018).

CHAPTER SIX

6.0 Conclusion and Recommendation

6.1 Conclusion

The findings from the study showed that the four harvest regimes of *M. oleifera* plants were rich in crude protein and have moderate fibre level and unknown concentrations of phytochemicals. In addition, for higher quality of forage and higher total DM yield for animal feeding, *M. oleifera* should be harvested at 70 days after germination because the nutritive value of *M. oleifera* forage in terms of CP and IVOMD was better under 70d compared to the longer harvest regimes. *M. oleifera* contained high levels of CP and IVOMD, 218 g kg^{-1} DM and 40.62%, respectively.

The generally high *in vitro* degradability and estimated dry matter digestibility suggests that *M. oleifera* whole plant (at a tender age) has nutritive potential as alternative low cost sources of good protein supplements to poor quality fibers for feeding livestock especially during the dry season.

It can be concluded that CP digestibility was not compromised, with the diets with a marginal increase in CP digestibility in the *M. oleifera* leaf and *M. oleifera* leaf+multi-enzyme. The NDF digestibility was significantly enhanced in rabbits fed *M. oleifera* leaf+multi-enzyme. Feed intake and average daily weight gain was higher in rabbits fed *M. oleifera* leaf and *M. oleifera leaf*+multi-enzyme. The inclusion levels of *M. oleifera* leaf (40%) +multi-enzyme (0.03 g) could be used without any detrimental effect.

6.2 Recommendations

Therefore, future studies are highly recommended with different inclusion levels of the multi-enzyme and *M. oleifera* whole plants (petiole, stem and leaf) meal.

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APPENDIX

AP. Plates from the field

M. oleifera plants on the field and management practices images

M. oleifera plants on the field and management practices images

New Zealand Whites breeds (Rabbits), Feed and management images