TITLE PAGE

USMANU DANFODIYO UNIVERSITY, SOKOTO

(POSTGRADUATE SCHOOL)

EVALUATION OF SOME PLANT OILS IN FEEDING GROWING AND FATTENING UDA RAMS

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BY

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DEDICATION

This research work is dedicated to my parents; late Mohammed and Maryan Mohammed Kolo for all their support, prayers and encouragement.

CERTIFICATION

This thesis by Kolo, Usman Mohammed (Adm. No. 16310603003) has met the requirements for the award of the Degree of Doctor of Philosophy (Animal Science) of the Usmanu Danfodiyo University, Sokoto, and is approved for its contribution to knowledge.

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ABSTRACT

The study was conducted to determine the effect of plant oils on the performance of growing and fattened Uda rams. This study was conducted in three phases: The first phase was Influence of plant oils on *in vitro* fermentation characteristics. Four (4) dietary treatments; Treatment 1 is the control. Treatment 2, 3 and 4 each contained 30ml/kg of garlic, Soybean and groundnut oils respectively. The second phase was to determine effect of plant oils on the growth performance and blood profiles of growing lambs. The third phase was the evaluation of effect of graded levels (0, 15, 30 and 45ml/kg) of groundnut oil on the performance of fattened rams. Result of in vitro fermentation showed effect of plant oils on the concentrations of methane, 18:00, 18:1 cis9, 18:2, 18:3, total C18:0, biohydrogenation (18:2, 18:3 and total C18:0) differed significantly ($P<0.05$). The results of growing lambs showed significant differences ($P<0.05$) in the final weight and basal feed intake. The results of fattened characteristics of rams showed significant differences ($P<0.05$) in the final body weight and body weight gain. The highest mean daily live weight gain (77.38) and the estimate percentage of CH_4 reduction (66.11) in lambs fed diets containing groundnut oil. The highest mean daily live weight gain (101.19) and estimate percentage of CH_4 reduction (79.74) in fattened rams fed diets with highest groundnut oil were better compared to control. The increase inclusion of groundnut oil decreases the cost of feed per Kg. Conclusively the study showed that groundnut oil at 45 ml/kg gave better performance of fattened ram. Therefore, this study recommended the use of oils inclusion in the diet of growing and fattened rams.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

High content of Saturated Fatty Acid (SFA) in sheep products is cause by hydrolysis of lipids and the consequent biohydrogenation of the constituent Poly Unsaturated Fatty Acid (PUFA) by rumen microbes (Kim *et al*., 2009). Plant oils have the potential to manipulate carbohydrate breakdown to reduce methane production and potentially improve performance. Methane (CH4) is a by-product of carbohydrate breakdown in the reticulo-rumen. Methane production is energy inefficient, wasting 2-15% of digested energy (McCrabb and Hunter, 1999). However, methane can be produced from volatile fatty acids (VFAs) and alternative sinks for hydrogen to remove hydrogen from the rumen for healthy rumen functioning.

Supplementation of lipid rich feeds, either in the form of extracted lipids or wholeoil seeds, is another strategy for modulating ruminal biohydrogenation as well as for increasing energy contents of the diets. Hart *et al*. (2008) suggested 2 modes of action of essential oils (EO): one is EO affect the pattern of bacterial colonization of substrates, particularly starch rich substrates, as they enter the rumen. The other suggested mode of action which is the inhibition of hyper ammonia producing bacteria involved in deamination.

In addition to high PUFA contents of many lipid sources (e.g., linseed, sunflower, soybean oils and seeds, fish oil), it has been known that dietary lipids may cause antimicrobial effect on biohydrogenating bacteria especially lipids with higher degree of unsaturation. Lipids rich in poly unsaturated fatty acid could contribute to the increase of conjugated linoleic acid contents in animals (Lourenco *et al.*, 2010). Fish

oil, which is rich in long-chain poly unsaturated fatty acid, has been shown to inhibit the last step of biohydrogenation (Potu *et al.,* 2011). Administration of lipids in the form of whole seeds usually significances in a lower biohydrogenation.

The process of biohydrogenation of linoleic (C18:2 *n*-6) and linolenic (C18:3 *n*-3) acids produces a range of intermediates such as Conjugate linoleic acid and Vaccenic acid (*trans-11* 18:1) (Harfoot and Hazlewood, 1997), which have been given away to potential health benefits. The Conjugate linoleic acid (*cis-9 trans-11* 18:2 isomer) and its precursor (18:1 *trans*-11) have concerned significant research attention as anti-carcinogenic, anti-inflammatory, anti-atherogenic and anti-diabetic agents (Kennedy *et al*., 2010). Recognition of additives to reduce biohydrogenation of PUFA would reduce both the health and economic impacts of this seemingly inevitable change in consumption pattern. Although several studies examined plant oils effect on rumen fermentation (Castillejos *et al.*, 2007), but little is known about their effect on rumen biohydrogenation practice. Therefore the present studies will be conducted using locally available plant oils (garlic oil, Soybean oil and groundnut oil) to determine the best plant oil and the best level to be added to concentrate diet that would produce the best performance of sheep.

1.2 Statement of Problem

Livestock contributes to global climate change by emitting Green-house gases (GHGs) from enteric fermentation of feeds. The major GHGs from the livestock sector are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) that are produced throughout the production process. The CO_2 that is emitted from livestock is not measured as a net contributor to global warming because plants utilize it during the day for photosynthesis (Steinfeld *et al.,* 2006). Enteric fermentation activities in ruminant

production are the primary sources of emissions, representing around 39 and 45% of the of the total sector's emissions (Opio *et al.*, 2013).

Plant oils are potentially promising natural alternatives to antibiotics for manipulating ruminal fermentation due to their ability to modify cell permeability in microbes and their toxicity to some strains of rumen microorganism, particularly the Gram-negative (Conner, 1993). Ruminal biohydrogenation can be manipulated to alter the amount of intermediates, such as poly unsaturated fatty acids, and decrease the flow of *trans*-10, *cis*-12 conjugated linoleic acid (CLA). The extent of biohydrogenation is determined by the characteristics of the fat sources, passage rate of feedstuffs in the rumen, and biohydrogenation capacities of the ruminal microorganisms. Supplementation of fat not only alters metabolic activity of ruminal microorganisms and increase FA flow from the rumen, but also increases energy content of the ration with minimal changes in the plant forage to concentrate ratio, especially valuable for high producing cows (Palmquist, 1984). The inhibition on substrate incorporation of ruminal bacteria to produce microbial nitrogen by inclusion of oils is possible. However, the biohydrogenation of different FA in different feeds for sheep is not fully understood in terms of microbes and metabolic processes related to biohydrogenation.

1.3 Justification of the Study

Plant oils are thought to have a similar mode of action to antibiotics and used as alternative for antibiotics and growth promoters in ruminant; they favour propionate producing bacteria in the rumen. Plant oil, reduce methane emissions by reducing the number of rumen protozoa which can affect the methane producing bacteria and slow down protein turnover in the rumen as well as increasing transport of microbial nitrogen to the duodenum. Inhibition of methane production in the rumen might also affect biohydrogenation of unsaturated fatty acids (UFA) (Danni, 2013). Two groups of

bacteria are involved in the biohydrogenation of fatty acid. The first is group "A", including some fiber digesting bacteria (Polan *et al*., 1964), which isomerize and reduce UFA to *trans*-11 vaccenic acid (C 18:1). The other is group "B" bacteria which catalyze the rate-limiting step for biohydrogenation and reduction of *trans*-11 to 18:1 to 18:0. In co-cultures, if methane production is inhibited, the growth of group "A" bacteria might be decreased due to the accumulation of the metabolic hydrogen, which is one of the end products of fiber degradation. Therefore, the biohydrogenation might be hindered, resulting in increased *cis*-9, *trans*-11 and *trans*-11 intermediates (Danni, 2013). Garlic oil, Soybean oil and groundnut oil are the essential oils with most supporting evidence for methane reduction. To be sustainable and taken up by the industry, the feed additive would need to be effective over long periods of time, non-toxic for animals, environmental friendly, consumers safely and cheap enough for standard use in animal feeds.

1.4 General Objective

To determine the effect of plant oils on the performance of growing and fattened Uda rams.

1.4.1 Specific objectives are to compare plant oils:

to determine the effect on *in-vitro* fermentation characteristics

to determine the effect on the performance of growing Uda ram lambs.

to determine the effect on hematological and serum biochemical indices of Uda rams.

to evaluate the performance of fattened Uda rams fed graded levels of the best oil identified in the previous growing trial.

to analysis economic performance of growing and fattened Uda rams.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Rumen Ecosystem

Ruminant animals four-chambered stomach of grazing animals, are exclusive in terms of fibre digestion because of the presence of microorganisms which help in fermentation and break down of complex carbohydrate into volatile fatty acids (VFAs) (Ashiru, 2014). The stomach of ruminant animals consisted of rumen, reticulum, omasum and abomasum. The rumen is the largest of the four stomach compartments. It serves as the microbial fermentation vat of consumed feed. It is connected to oesophagus and reticulum. The rumen also known as the paunch which contains many microorganisms, composed of four muscular sacs; dorsal sac, ventral sacs, caudo-dorsal blind sacs and caudo-ventral blind sac (Ensminger, 2002). The lining of the rumen wall is covered with a small finger-like projections (approxi mately 5mm in length and 3mm in width in cattle) called papillae, which increase the absorptive surface area of the rumen wall and assist in absorbing volatile fatty acids (VFAs) and methane gases. The rumen is heterogeneous environment in terms of temperature, pH and pressure. The rumen environment is affected by many factors such as diets, water uptake and rumination. During rumination, the rumen undergoes cycles of contractions up to 1-3 times per minutes leading to changes in the pressure.

Rumen conditions are anaerobic; as a result the microbial forms found in the rumen are nearly all anaerobes or facultative anaerobes (Ensminger, 2002). Microbial activities in the rumen result in the conversion of starch and fibre of feeds to volatile fatty acids. The VFAs are absorbed through the rumen wall and provide as much as 80% of the animals total energy requirements (Ensminger, 2002).

2.1.1 Rumen microorganisms

The plant structural carbohydrates such as lignin, cellulose, and hemicellulose, which they are not able to utilize themselves because of their inability to produce respective hydrolytic enzymes. Therefore, symbiotic microorganisms are established in their alimentary tracts that can hydrolyze these compounds to generate energy for themselves, as well as for the host animal, rumen microbes require a friendly atmosphere for desirable fermentation. Rumen microbial population consists of three main groups: bacteria, protozoa and fungi. The microbes colonize the rumen either attached to food particles or free in the rumen liquor. The ruminant host provides them with a favourable environment and regular food supply which enables them to proliferate quickly. The rumen is open to the external environment, and there is a continuous flow of material into and out of the rumen. It has a dry matter content of 10–13 %, and temperature is typically regulated between 38 and 41 °C. The physiological pH range is between 5.5 and 6.9, and it is one of the most variable factors in the rumen environment.

Approximately all the rumen microorganisms are anaerobic or facultative anaerobes. It has been described that a reverse relationship between ruminal bacteria count and protozoa exist (Orskov and Ryle, 1990). Rumen microorganisms are in reciprocal interaction for digestion process and their number and ratio change depending on several factors, such as feed and nutrient content, rumen volatile fatty acids (VFAs), rumen pH, forage maturity, cutting time of forage, animal species, various processing applied to feeds. Fluctuation in the number and ratio of rumen microbes according to the rumen conditions is very important in terms of retaining higher feed efficiency and rising of the amount and quality of products. Any factor affecting rumen microorganism populations directly or indirectly affects yield and feed efficiency ratio (Hobson and

Stewart, 1997). The major type of substrate entering the ecosystem will mainly determine the population of microbe (Orpin, 1982). In return the ruminant has access to the products of microbial digestion, including those from fibrous polysaccharides which are normally not digestible by most mammals. Fungi appear to be the first organisms to invade and commence digesting the structural plant components beginning from the inside (Preston and Leng, 1987). They reduce the tensile strength of these particles. The damage to digesta particles allows bacteria to colonise the cell materials, thus they are initiators of fermentative breakdown of insoluble plant cell wall materials and their presence must reduce any lag-phase of fibre digestion (Preston and Leng, 1987).

They digest plat material by fermentation, yielding large quantities of volatile fatty acids, which are mainly absorbed through the rumen wall, and microbial cell mass which is rich in protein and vitamins for the ruminant to digest in its abomasum and small intestine. The symbiotic relationship between the ruminant and its microflora concludes with the digestion of the latter.

2.1.2 Microbial population

The diet is the main factor influential number of species of microbes in the rumen (Siddons and Paradine, 1981; Nocek, 1988). The types or number of microorganisms in the rumen, or both, vary with diet (Siddons and Paradine, 1981), any plant factor that has an encouragement on the ruminal microbial population, such as starch content of diet, could affect rate and/or extent of CP digestion in diet (Vanzant *et al.*, 1998). Cereal diets have higher proteolytic activity than dry roughage diets, probably because amylolytic bacteria tend to be more proteolytic than cellulolytic bacteria (Wallace and Cotta, 1988). Plant induced changes in rumen micro flora are accompanied by changes in rumen fermentation patterns. When a high cereal, as opposed to high roughage diets

is fed, the soluble sugars and starch are rapidly fermented, reducing rumen pH and causing a shift to a more amylolytic population at the expense of cellulolytics and protozoa (Nocek, 1988).

Level of proteolytic activity in the rumen is also likely a function of microbial numbers, which are often higher with higher cereal diets (Siddons and Paradine, 1981). This could explain why Siddons and Paradine (1981) found that levels of proteolytic and deaminase activity in the rumen were higher when a predominantly cereal diet was fed compared to a predominantly roughage diets, resulting in a higher degradability of casein, since soluble proteins such as casein are readily accessible to microbial proteases and their rate of degradation is determined by level of proteolytic activity in the rumen. However, with most feed, levels of proteolytic activity in the rumen is not as important in determining rate of degradation of CP, since only small proportion of CP is soluble in the rumen. This solubility decreases at lower pH, which could partly explain why CP degradability is lower with higher concentrate diets. The accessibility, and therefore degradability of insoluble CP to microbial proteases probably depends on the rate of digestion of non-protein OM as there is often a similar disappearance of CP and DM (Siddons and Paradine, 1981).

All major proteolytic bacteria are found in the rumen of animals fed both low and high grain diets while cellulolytic bacterial number decreases with those fed high grain diets (Loerch *et al.*, 1983). Addition of starch or sugar has resulted in decreases fibre digestibility, which is often accompanied by drop in CP degradation (Lindberg, 1985). Reduction in cellulose digestion could explain decrease degradability of plant CP supplemented with cereal diets (Siddons and Paradine, 1981).

According to Kopecny and Wallace (1982), the optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0. Ruminal pH is one of the factors influencing microbial population dynamics (Cronje, 1992) and can be a major factor in determining competition among bacteria (Grant and Mertens, 1992). The bacterial fraction of rumen microorganisms has a broad pH optimum of between 6 and 7 (Wallace and Cotta, 1988). While different bacterial specie grow in different pH ranges, individual species of bacteria vary in their sensitivity to low pH (Strobbel and Russell, 1986; Grant and Mertens, 1992). Cellulolytic bacteria are sensitive to acid pH whereas amylolytic species are more acid tolerant (Strobel and Russell, 1986; Grant and Mertens, 1992).

Growth rate and yield of rumen bacteria are depressed as pH is reduced (Strbel and Russell, 1986; Stokes *et al.*, 1991) and if pH drops below 6, there is a reduction in proteolytic activity and low count of proteolytic bacteria (Lindberg,1985). Usually, protozoa numbers also decline as pH declines, which could be partially involved in reduction in the extent of ruminal proteolysis at a low rumen pH (Owen and Zinn, 1998). The period of time that ruminal pH is below optimal may be more critical for digestion than the relationship between mean diurnal pH and optimal pH (De Veth and Kolver, 2001). De Veth and Kolver (2001) found a negative linear relationship between time at sub-optimal pH and microbial CP flow and a large diurnal variation in ruminal pH may reduce microbial growth to a larger extent than when mean ruminal pH is less variable. Variation is less when feeding more frequent, probably by avoiding drops in pH below 6 which negatively affects digestion (Madsen and Hvelpund, 1988). Microbial activity is inhibited even when exposed to short period (i.e. four hours) of sub-optimal pH (5.4) (De Veth and Kolver, 2001) which affects the microbial population with microbes being able to recover in intervals when ruminal pH is optimal.

Therefore, the depression in microbial growth is not as large when reductions in rumen pH are cyclic and of short duration (Stokes *et al.*, 1991).

2.1.2.1 Rumen bacteria

Rumen harbors unlike types of bacteria, which are most actively involved in the plant fi ber degradation, as revealed by the fact that bacteria associated In addition, most of the rumen endoglucanase and xylanase activities are contributed by bacteria, and bacteria are pivotal to the rumen digestion. These are separated into four groups on the basis of their association as free floating in the liquid phase, attached to the feed particles (fi rmly/ loose), rumen epithelium, protozoa, and fungi. Bacteria are generally the largest microbial biomass in the rumen. There are a number of distinct grouping of bacteria including: bacteria free in the liquid medium (usually 30% of the total), bacteria attached to feed particles (about 70% of the total), bacteria adhering to the epithelial lining of the rumen and bacteria attached to protozoa (mainly methanogens) (Ashiru, 2014). The number of bacteria in the liquid phase is therefore important in determining the rate of colonization and therefore the rate of fermentation of feed particles (Ashiru, 2014). The bacteria floating freely in the rumen are therefore the ones that depend on soluble nutrients but there are also those that are in transit between plant particles (Ashiru, 2014).

Several hundred species of bacteria have been found in the rumen and about 109- 1010 bacteria per ml of rumen fluid have been estimated (Ørskov and Ryle, 1990). Among the different functional groups are; cellulolytic, amylolytic and proteolytic bacteria, those which ferment cellulose are the most important. Cellulolytic and amylolytic bacteria both require ammonia (NH_3) and branched – chain fatty acids as growth factors. Urea can provide NH_3 and so promote efficient utilization of fibrous roughages, if the rumen pH does not fall below about 6.0 (Ørskov and Ryle, 1990). The most

important cellulolytic bacteria species include *Ruminococcus flave faciens, Ruminococcus albus, Bacteriode succinogenes (*or *Fibrobacter succinogenes)* and *Butyrivibrio fibrisolvens* (Cheng *et al.*, 1990). In some situations *Cillobacterium cellulosolvents* and various *Clostridium* species become prevalent (Cheng *et al.*, 1990).

Bacteria have an important function of digesting structural carbohydrates (hemicelluloses and cellulose), non- structural carbohydrates (starch, sugar and pectin) and protein to form nutrients themselves and eventually the host animals. Bacteria degradation results in the moderation of VFAs that are absorbed and used by the animals (Ashiru, 2014). The bacteria themselves are degraded in the small intestine and are absorbed. Microbial efficiency is also associated with the availability of carbohydrates contained in the fibre (Ashiru, 2014). For instance, it has been shown that tropical legumes are higher in protein and lower in fibre than grass counterparts, and thus can serve as valuable supplements to straw and stover-based rations (Van Soest, 1982).

2.1.2.2 Rumen protozoa

Rumen protozoa account to about 50 % of the viable biomass in the rumen and are dependent diet consumed by the animals. Majority are ciliates and few flagellates and are very motile. Unicellular organisms with 20–200 μm size are not attached to feed particles. Protozoa occur in rumen of sheep and cattle on fibrous diets (which are low in soluble sugar) but their population masses are low (less than 100, 00/ml), whereas on diets high in starch or sugar they can reach densities up to 4,000,000/ml of rumen fluid (Ashiru, 2014). The diet determines the species of protozoa in the rumen but little is known about the factors that determine the balance of protozaol species or their biomass. Two major group of ciliate protozoa have been isolated, the *Holotrichs* protozoa (mainly *Isotricha* specie) and *Entodineomorphs* (largely *Entodinia* specie)

(Ashiru, 2014). The cilia of these organisms are restricted to tufts located mainly near the oesophagus; their function is the propulsion of food particles into the oesophagus. The *Holotrichs* protozoa occur in animals fed sugar/fibre diets and on fresh grass pastures, which are usually a combination of soluble and insoluble carbohydrates. *Entodinium Diplodium* and *Isotricha,* which are the most common protozoa varieties, contribute to the cellulose and starch digestion (Cheng *et al.*, 1990). Some protozoa are cellulolytic but the major substrate appear to be sugars and starches, which are rapidly assimilated and stored as poly-dextrans. In this way they often buffer the pH of the rumen. Rumen protozoa can digest other microbes and ferment constituent plant materials for energy (Cheng *et al.*, 1990). Protozoa are not essential for rumen functioning. The positive effect of rumen defaunation on the digestibility of fibrous feeds and live weight gain in sheep offered straw diets has been reported (Bird, 1990).

2.1.2.3 Rumen fungi

The rumen is a depot of anaerobic fungi that are not found elsewhere and play a very main role in the degradation of lignocellulosic components of the feed particles. Anaerobic fungi have been shown to be present in the rumen of a number of animal species including sheep, goats, cattle and members of the deer family. They have also been found in the cecum of horses and elephants and in the pseudo-rumen of Kangaroos (Bauchop, 1981). Fungi appear to be the first organism to invade and commence digesting the structural plant components, beginning from inside. Fungi also possess the unique capacity to penetrate the cuticle at the plant surface and the cell walls of lignified tissues (Akin, 1989). They reduce the tensile strength of these particles and thus increase particle breakdown in the rumen. The damage to digesta particles by fungi allows bacteria to colonize the cell materials. They are thus extremely important

initiators of fermentative breakdown of insoluble plant cell wall materials and their presence reduce any lag-phase of fibre digestion.

The specie of fungi isolated from sheep's rumen includes *Neocallimastiz frontalis, Piramonas communis* and *sphaeromonas communis* (Orpin, 1982) but more are being discovered. These fungi digest some of the plant structural components. It appears to be a reasonable assumption that fungi break hemicelluloses-lignin complexes and solubilize lignin. This may allow fibre that is physically protected by lignin to be fermented by the rumen bacteria (Orpin, 1982). It has been reported that ruminal fungi have enzymes which can hydrolyze the majority of the structural polysaccharides found in plant cell wall (Dehority and Tirabasso, 2001). Rumen fungi help break down digested particles and hydrolyze ester linkages between lignin and hemicelluloses or cellulose. Most of the fungal biomass is present as rhizoids infiltrating fibrous plant tissue. Ørskov and Ryle (1990) reported that this group of microorganisms may be particularly important for the degradation of the plant structural materials which predominates in coarse roughages, although lignin does not appear to be susceptible to attack by rumen fungi.

2.1.2.4 Interaction between rumen bacteria, fungi and protozoa

Ruminal microorganisms that interact with particles can be functionally described as three distinct sub-populations. Those associated with the ruminal fluid, those loosely attached to feed particles and those firmly attached to feed particles (Cheng and McAllister, 1997). Microorganisms associated with the ruminal fluid include those newly detached from feed particles as well as those that survive on soluble feed components within the ruminal fluid and have little or no direct involvement in the digestion of soluble feed particles (Latham, 1980). This subpopulation is an integral part of the rumen ecosystem, as these microbes colonize and initiate digestion of newly

ingested feed particles by ruminal microorganisms is rapid (Craig *et al.*, 1987) occurring within five minute of feed entering the rumen (Bonhomme,1990). To investigate these interactions, studies have been made by Bird (1990) on effect of defaunation on fungal growth and digestibility of feed in the rumen. The digestibility of fibrous feeds in nylon bags in the rumen of sheep were faunated, then defaunated (and remained un faunated) and then refaunated showed that the unfaunated state of the rumen resulted in an increased rate of disappearance of straw dry matter (6-10 units/24hrs). Elimination of protozoa in the rumen leads to an increase in the number of bacteria in the liquid pool (Ashiru, 201). Any manipulations of diet must be viewed in the light of the interactions among protozoa, bacteria and fungi. For instance, feeding concentrate supplements to ruminants on roughage based diet often decrease the intake of roughage. The net effect of adding concentrates (and molasses blocks) to a roughage diet may be to increase protozoal number. The interactions are obviously complex and the result of any research into manipulation of the rumen that does not measure responses in the biomasses of protozoa, bacteria and fungi will be difficult to explain (Ashiru, 2014).

2.1.3 Rumen fluid

Rumen liquor is the fluid left when the rumen content is filtered and large particles are discarded. It is known that ruminal fluids contain a large amount of microorganisms. Because of the microbial enzymes, ruminants can utilize feedstuffs (cellulose, hemicelluloses and non-rotein nitrogen) that provide little or no nutritional benefit to non-ruminants (Ashiru, 2014).

Adeyemi and Sipe (2004) studied the effect of fermenting cassava root using rumen filtrate; they found an increase in CP concentration when fermented with rumen filtrate with or without ammonium sulphate as the source of nitrogen. Songsak and Sirilak

(2009) studied the nutritive value of cassava starch industry by-product using fermentation method. An increase in CP was recorded and a decrease in DM contents of the naturally and microorganisms fermented cassava by-products. It was concluded from their studies that cassava starch by-product fermented with rumen micro-organisms is a potential useful feed material for monogastric feeding.

Another study using rumen filtrate was reported by Adeyemi *et al*. (2007) who recorded an improvement in the nutrient composition of whole cassava root-meal upon fermentation with rumen filtrate. The results showed that protein yield on a steep rise up to day 3 and crude fiber was significantly reduced by fermentation. Ashiru (2014) reported 237.8 % increase in the crude protein value of whole cassava root meal fermented with rumen filtrate when caged layer waste was used as a source of nitrogen.

Abouheif *et al.* (1999) studied the nutritive value of rumen content and Barley (*Avena sativa*) mixtures for growing lambs and reported that there is no evidence of pathological effect in lambs, and did not show any palatability problems. Average daily DM intakes were higher in lambs fed rumen content barley meal diets than those fed the control diets. The DM requirement per Kilogram live weight gain increased with replacement of increasing levels of control diet by rumen content and Barley mixtures.

EL-Deek *et al*. (1984) showed that rabbits fed on diets containing dried rumen contents tended to gain weight slower than control animals. On the other hand, Khattab *et al.* (1996) found that DM intake and daily body weight gain in lambs fed on the 25 and 50 % rumen content-supplemented diets were higher than in control group. Abasiekong (1991) observed an improvement in the feeding value of spent sorghum when fermented with a stock culture of some rumen microorganisms and reported direct

fermentation of spent sorghum with rumen fluid produced similar results that could be utilized on farm.

Adeyemi *et al.* (2007) studied the effect of fermenting whole cassava root meal using caged layers waste and pig excreta as nitrogen sources for different time duration using rumen filtrate as inoculum. The authours reported an improvement in the crude protein and ether extract of whole cassava root meal with increased fermentation time, while dry matter, crude fibre, hydrogen cyanide, Ash, calcium and phosphorus, were reduced. However, the highest crude protein content was recorded for cassava fermented with additional source of nitrogen. In another study, fermentation of sago pith and rumen content mixture was able to reduce the crude fibre content by 33 % and increase crude protein content by 42 % (Wizna *et al.*,2008). Adeyemi and Familade (2003) studied the replacement of maize by rumen filtrate fermented corn-cob in layer diets and indicated that fermentation with rumen filtrate increased the crude protein content three folds while crude fiber decreased from 42.46 % to 28.94 %, and concluded that fermentation enhanced the nutritional value of this feed-stuff.

Jovanovic and Cuperlovic (1977) studied the nutritive value of rumen contents for animals and reported that an average sample of rumen content contained 21 % crude protein, 30.3 % crude fiber, 6.1 % fat and 11.5 % ash. Shebata *et al*. (1984) evaluated rumen liquor as a feed ingredient for poultry and reported that biological and chemical analyses for dried rumen liquor indicated a true metabolizable energy value of 2470kcal/kg, 25.92 % crude protein and high amount of minerals. Adeyemi *et al*. (2008) suggested that cassava enhanced with dried cage layer waste and fermented with rumen filtrate is a potentially useful feed material for monogastric animals.

2.1.4 Rumen pH

Ruminal pH is an important parameter of digestion and rumen health. It has relationship with rumen fermentation and animal production. An ideal rumen pH provides an optimal rumen environment for digestion (Wales *et al.*, 2004). De Veth and Kolver (1999) established in their study that dry matter digestibility of pasture was optimized at pH 6.35. Wales *et al.* (2004) suggested large gains in productivity could be achieved by increasing average daily ruminal pH from 5.6 to 6.1. When ruminal pH decreases to a certain level, the digestion of food ingested could be affected. De Veth and Kolver (1999) suggested a large reduction in digestion occurred when ruminal pH was less than 5.8. Beauchemin (2007) suggested that pH of 5.8 caused decrease in fibre digestion and feed efficiency. The digestion of food causes pH decrease and the heat of fermentation causes a temperature increase. The rumen provides an environment that is favorable for the growth of microbes as the ruminal pH ranges from 5.5 to 7 and temperature ranges from 39 to 41^{o} C (Erfle *et al.*, 1989).

2.2 Factors Affecting Rumen

Ruminal pH is affected by fibre concentration of the diet i.e. neutral detergent fibre (NDF) and the balance between production of fermentation acids and secretion of salivary buffers (Krause and Oetzel, 2006). The pH of the rumen can vary from more than 7.0 on a roughage diet to less than 5.0 on a high grain diet (Russell and Dombrowski, 1980) even though the rumen is well buffered by bicarbonates, phosphates and protein (Russell and Dombrowski, 1980).

Decreasing the diet crude fibre (CF), NDF or ADF level generally decreases rumen pH (Erdman, 1988) while increasing the amount of concentrate or non-structural carbohydrates (NSC) in the ration generally decreases ruminal pH (Ørskov, 1992; Ørskov, 1994). Readily fermentable carbohydrates decreased rumen pH (Strobel and

Russell, 1986) due to rapid microbial fermentation and less rumination and salivation (Mould and Ørskov, 1983). Reduction of ruminal pH due to rapid fermentation of grain-based diet with high starch content occurs to a lesser extent if cows are adapted to the high grain diet (Clayton *et al.*, 1999), Rumen pH is largely a function of the VFAs concentration (Erdman, 1988; Stokes *et al.*, 1991) and pH will drop if there is a reduced rate of VFAs absorption (Owens *et al.*, 1988). In a diet with high non-structural carbohydrates and readily digestible proteins, VFAs concentrations are high and ruminal pH is low (Zhao *et al.*, 1993). Russell (1998) found that cows fed 90% concentrate had lower ruminal pH than cows fed forage only, and concluded that as much as 25% of the decrease in acetate propionate ratio could be explained by pH alone.

Feed intake and salivary secretion affects pH in the rumen (Maekawa *et al.*, 2002). At a higher level of feed or DM intake, the pH of the rumen is lower (Robinson *et al.*, 1986; Madsen and Hvelplund 1988; Zhao *et al.*, 1993). Haaland *et al.* (1982) found pH at maintenance to be 6.57 and at two times maintenance to be 6.35. Ruminal pH declines following a meal, the drop depending partly on the initial pH (Maekawa *et al.*, 2002; Nocek *et al.*, 2002). Ruminal pH generally continues to decline hours after feeding with the lowest rumen pH generally occurring 4-6 hours after feeding (Lindberg, 1981; Madsen and Hveplund, 1988). The duration of low pH generally increases with increasing grain levels in the diet (Nocek *et al.*, 2002) although Maekawa *et al.* (2002) found no effect of roughage to concentrate ratio on ruminal pH. Infrequent feeding is conducive to large fluctuations in the rumen environment, including pH (Vanzant *et al.*, 1998). Variation in pH is smaller when feed intake is more frequent, which can be achieved by feeding restricted amount of feeds several times a day (Madsen and Hvelplund, 1988; Ørskov, 1994; Le Liboux and Peyraud, 1999). As meal frequency decreases the fluctuation in ruminal pH production of VFAs is more pronounced and

correlated to a larger fluctuation in ruminal pH. The minimum meal frequency needed to maintain a steady rumen pH increases as plant NDF decreases and *vice versa* (Pitt and Pell, 1997). Pitt and Pell (1997) predicted, with the use of metabolic models, that DM intake and body weight, as well as use of plant buffers at levels less than 1% of DM, have relatively small effect on magnitude of ruminal pH fluctuations.

Feeds with rapid ruminal rates of starch degradation may result in low ruminal pH (Batajoo and Shaver, 1998) and so diet with higher levels of rapidly fermented carbohydrates tend to exaggerate diurnal fluctuations in pH (Robinson *et al.*, 1986). Martin-Orou *et al.* (2000) fed diets with differing ratios of maize and barley grains concentrate portion of the diet. Ruminal pH was slightly lower (6.46 *vs* 6.46) for cattle offered a higher proportion of barley than those with a higher proportion of maize because barley grains is more fermentable in the rumen. However, ruminal pH never decreased below 5.50, and there was no difference in effective rumen degradability between the two diets. Increasing plant CP increases pH of the rumen (Haaland *et al.*, 1982). Acids that are produced during fermentation of grains keep the rumen pH slightly below 7.0 or at 7.0 (NRC, 1985). The mechanism of the pH drop below neutral levels depends on how much acid is produced, the rate at which it is absorbed from the rumen and the amount of salivary secretion which is released to neutralize the acids (NRC, 1985). When ruminant are fed high forage diets, the bacterial population shifts away from lactate producers (NRC, 1985). The optimum ruminal pH range for maximal cellulose digestion is between 6.4 and 6.8 (Erdman, 1988) and fibre digestion rate (cellulolysis) decreases when ruminal pH declines below about 6.0 – 6.2 (Ørskov, 1994; Mabjeesh *et al.*, 1997; Pitt and Pell, 1997) as cellulolytic bacteria are sensitive to rumen pH and their growth is inhibited.

Physical disintegration and heat processing of cereals increases their rate of fermentation and thereby reduces rumen pH (Lindberg, 1985). The amount of barley grain needed to depress rumen pH depends on the degree of processing (Mould *et al.*, 1983). Krause *et al.* (2002) found that decreasing forage particle size, or increasing the level of rumen fermentable carbohydrates, decreased ruminal pH and minimum diurnal ruminal pH below pH 5.80. Le Liboux and Peyraud (1999) found that if Lucerne was ground and pelletized, as opposed to chopped, mean diurnal pH was lower and range of pH and time when it was low, were higher.

Methods of sampling also have an effect on rumen pH as rumen fluids sampled by stomach tube tend to have higher pH than those taken via a rumen fistula (Erdman, 1988). Site of sampling and time post feeding probably have more extensive effects on rumen pH than molar proportions of VFAs (Erdman, 1988).

There are many factors affecting pH and although some metabolic models have attempted to predict pH fluctuations, limited information is available on the effects of these fluctuations on microbial fermentation and nutrient digestion (Calsamiglia *et al.*, 2002).

2.2.1 Ruminal acidosis

Acidosis is a pathological condition resulting from accumulation of acids or depletion of the alkaline reserves in the blood and body tissues and characterized by an increase in hydrogen ion concentration (Bramley *et al.*, 2003). Rumen acidosis is a syndrome related to a metabolic disorder of the rumen causing decrease in pH in the rumen. There are two types of acidosis: acute and subacute acidosis.Acute acidosis is severe and less common in the field. The affected animals are depressed, dehydrated, show anorexia,

diarrhea, abdominal pain, lethargy, staggering, recumbency and may die (Bramley *et al.*, 2003; Krause and Oetzel, 2006).

Subacute ruminal acidosis (SARA) is less severe and more common compared to acute acidosis. It has a greater economic impact to the herd as it is harder to detect and causes long term health issues such as whole herd lameness problem (Bramely *et al.*, 2003). Subacute acidosis is defined as periods of moderately depressed ruminal pH. The threshold ruminal pH varied in different studies depending on the measuring technology. For example, ruminal pH 5.5 was suggested by Garrett *et al.* (1990) using rumenocentesis to collect ruminal fluid samples, while ruminal pH 6.0 was suggested by Plazier (2004) using stomach tube approximately 4 hours after feeding. Beauchemin (2007) used a threshold value 5.8 because of cellulolytic ruminal bacteria do not grow below pH 6.0, which causes a decrease in fibre digestion and feed conversion efficiency. Subacute acidosis occurs when VFAs production from fermented diets exceeds the ability of the rumen environment to neutralize or absorb. Absorption of VFAs occurs passively across the ruminal wall and is enhanced by papillae, which provides a large surface area for VFAs absorption. Passive VFAs absorption through the rumen papillae was also increased when rumen pH drop to threshold value (pH 5.5) because ruminal VFAs are rapidly shifted towards the dissociated form and VFAs are passively absorbed only in the dissociated form. Besides increasing VFAS absorption, cattle could maintain ruminal pH by regulating their food intake, increasing buffer production i.e. saliva production, and through microbial adaptation. All these responses are short term responses, If the amount of VFAs produced is still excessive, ruminal pH decrease to a dangerous level (pH 5.5) and Subacute ruminal acidosis occurs (Krause and Oetzel, 2006). When ruminal pH is below 5.5, ruminal glucose from structural and non-structural carbohydrates (cellulose and starch) is fermented to lactic acids instead

of VFAS by *Streptococcus bovies.* Lactate has a lower absorption rate than VFAs in low pH because is less dissociated than VFAs. For example, at pH 5.0, lactate is 5.2 times less dissociated than VFAs. Lactate then remains in the rumen causing more reduction in pH. When pH drops under 5.0, growth of lactate-utilizing bacteria like *Megasphaera eldenii* is inhibited causing further decrease in pH (Krause and Oetzel, 2006).

2.3 Manipulation of Rumen

Rumen manipulation have attracted great interest in animal nutrition. Besides their contribution to the texture and quality of animal products and to the amount of plant energy, different fatty acid components in the ruminant product, such as short- and medium-chain, mono- and polyunsaturated, *cis* and *trans*, conjugated FA, etc. exert potentially positive or negative effects on the health of consumers (Parodi, 2004).

Ruminal biohydrogenation is the saturation of plant UFA in the rumen. The *trans*FA are produced by the ruminal biohydrogenation of linoleic and linolenic acids, and VA is the predominant isomer produced. The biohydrogenation limits the availability of PUFA, especially 18:2n-6, which serve as the major source of CLA in food derived from ruminants, with about 70 and 25% coming from products and red meat, respectively (Ritzenthaler *et al*., 2001). The major CLA isomer of intermediates of biohydrogenation, *cis*-9, *trans*-11 CLA, or rumenic acid, represents about 75 to 90% of the total CLA in ruminant products, such as milk, meat, and butter (Bauman and Griinari, 2003). Attempts have been made to increase the amount of *cis*-9, *trans*-11 CLA in ruminant fat because of their reported health benefits to reduce the risk 6 for cardiovascular diseases (Kritchevsky *et al*., 2004). Another intermediate of biohydrogenation, *trans*-10, *cis*-12 CLA, was first identified as a potent inhibitor of rumensynthesis by Bauman and Griinari (2001). This accomplishment has helped

people to better modify the biohydrogenation pathway to avoid *trans*-10, *cis*-12 CLA production and increase the *cis*-9, *trans*-11 CLA in the ruminant.

Lipolysis releases the non-esterified fatty acids that form the substrates for biohydrogenation. Ruminal pH affects the biohydrogenation. Feeding rations with low forage to ruminants can decrease lipolysis of triglycerides and biohydrogenation due to the decreased ruminal pH (Harfoot and Hazlewood, 1988; Van Nevel and Demeyer, 1996). The liberation of PUFA by lipolysis, however, seemed to be susceptible to inhibition as pH declined below 6.3 (Van Nevel and Demeyer, 1996). Consistently, Qiu *et al*. (2004) reported that low pH may increase intermediates of biohydrogenation, such as CLA. Changing the diet from a high to a low forage type can hinder lipolytic and biohydrogenation activities *in vitro* and decrease the number of ruminal lipolytic bacteria (Latham *et al*., 1972). However, Van Nevel and Demeyer (1996) reported that lipolytic activity was not affected by the presence of hay. They reported an inhibited lipolysis at pH $\leq$ 6.0, and the inhibition became stronger with decreasing pH. Incubations with 80 mg of soybean oil, at the same pH, had a stronger inhibition of lipolysis than with 40 mg. Griinari *et al*. (1998) showed that a low fiber diet decreased the rumen pH and increased proportion of propionate. They also reported that the low fiber diet and UFA increased concentration of *trans*-10 18:1 in milk fat. Lee (2013) showed that biohydrogenation pathways of linolenic acid partially switched under different pH conditions, with a strong influence on the *cis*-*cis* CLA at low pH.

The extent of biohydrogenation is determined by the characteristics of the fat sources, passage rate of feedstuffs in the rumen, and biohydrogenation capacities of the ruminal microorganisms. Supplementation of fat not only alters metabolic activity of ruminal microorganisms and increase FA flow from the rumen, but also increases energy content of the ration with minimal changes in the plant forage to concentrate ratio

(Mattias, 1982; Palmquist, 1984). However, unprotected fat, especially UFA added at high levels can negatively affect fiber digestion (Devendra and Lewis, 1974) Remarkable increases in *trans*-10 18:1 concentration have been reported in ewes' product when the diet was supplemented with high levels of unprotected oil (Mele *et al*., 2006). The increased concentration of *trans*-10 18:1 in milk fat is generally attributed to a shift in the biohydrogenation pathways, due the altered ruminal environment as a result of plant changes (Chilliard and Ferlay, 2004; Palmquist *et al*., 2005).

Efforts have been focused on variable effects of fat sources on biohydrogenation. One factor to cause the differences in biohydrogenation is degree of saturation. The toxic effect of PUFA on biohydrogenation bacteria (dominated by *Butyrivibrio fibrisolvens* and related taxa) and many other ruminal bacteria increased as the degree of UFA increased (Zhang *et al*., 2008). Thus, the toxicity of non-esterifed PUFA (Maia *et al*., 2007) released by lipase in the rumen would have to be removed by biohydrogenation, which seems to be a defense mechanism of the microbes. Also, having a free carboxyl group is critical for inhibition. Lipolysis is a prerequisite for biohydrogenation to proceed because the isomerase that catalyzes the initial step to form the *trans*-11 isomer is not functional unless the FA has a free carboxyl group. Feeding of protected lipids, such as calcium salts of LCFA, fatty alcohols, fatty acyl amides, and triglycerides, is a common practice to achieve greater passage of UFA to the duodenum (Jenkins and Palmquist, 1984). Lundy *et al*. (2004) showed that calcium salts and amide derivatives of FA were both effective in enhancing omasal flow of UFA in lactating dairy cows, and amides were more effective than calcium salts for increasing the postruminal flow of oleic acid.

Duckett and Gillis (2010) suggested that fish oil addition altered ruminal formation of biohydrogenation intermediates that is dependent on oil source supplemented in the diet. They observed that fish oil inclusion increased the outflow of n-3 FA, *trans*-10 18:1, and the majority of CLA isomers, including *cis*-9, *trans*-11. *Trans*-9 18:1and *trans*-11 18:1 flows to the duodenum were increased when fish oil was included in the canola oil-supplemented diet; however, no changes were observed when fish oil was included in the corn oil-supplemented diet. The higher biohydrogenation of C18:2 for raw soybeans than for extruded and roasted soybeans reported in Reddy *et al*. (1994) is similar to the result presented by Troegeler-Meynadier *et al*. (2006). There are limited reports comparing the effects of particle size of soybeans on biohydrogenation. Tice *et al*. (1994) reported minor effects of particle size of roasted soybeans on utilization of FA, but Tice *et al*. (1994) suggested larger particles may limit availability of C18 FA for uptake by bacteria because concentrations of 12:0 and 16:0 in bacteria linearly decreased and concentration of 18:1 in bacteria tended to increase as particle size of soybean was reduced. Results of the experiment *in vitro* by Whitney *et al*. (2000) showed that among treatments of corn, soybean meal, soybean oil, and soybean, 18:3 was the only FA completely biohydrogenation, therefore the biohydrogenation of 18:1 and 18:2 was not complete.

The milk fat from cows receiving coconut cake contained less 18:1 n-9 and more C12 and C14 than typical (Hilditch and Jasperson, 1943). Storry *et al*. (1971) demonstrated that the contents and yields of fat in milk tended to be higher on the coconut oil supplemented rations when compared with those on the basal ration. Another aspect with supplementing fat is protozoal suppression, which probably depends on plant conditions. Firkins (1996) showed that the suppression was enhanced more in high

grain rations than with high forage, which agrees with similar trends in more recent publications (Machmüller and Kreuzer, 1999; Eugene *et al*., 2004).

Essential oils (EO) are volatile, aromatic compounds that can be extracted from plants by steam distillation. As the potential feed additive to manipulate bacterial populations involved in ruminal biohydrogenation (Calsamiglia *et al*., 2007) due to its inhibition on methanogenesis and biohydrogenation of long-chain PUFA, EO have antimicrobial activity against gram-positive and gram-negative bacteria, protozoa, and fungi (McIntosh *et al*., 2003). The components of EO vary remarkably due to different ways of extraction and plant sources. The EO has been widely studied mostly *in vitro* with fairly inconsistent results, especially rumen methanogenesis (Benchaar and Greathead, 2011). The direct effect of EO on ruminal long-chain PUFA metabolism has not been extensively studied (Calsamiglia *et al*., 2007). Ethanolic extracts of EO from Australian plants selectively inhibited the growth of pure cultures of *Clostridium proteoclasticum* involved in the terminal step in the biohydrogenation of linoleic acid, resulting in the accumulation of intermediates, such as CLA and VA in batch culture incubations (Durmic *et al*., 2008).

Using a continuous culture fermenter system, Lourenço *et al*. (2008) found that cinnamaldehyde decreased apparent biohydrogenation of 18:2n-6 and 18:3n-3 and shifted the biohydrogenation away from the *trans*-11 pathway toward the secondary biohydrogenation pathway of 18:2, leading to the accumulation of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA. This *trans*-10 "shift" has been documented to cause MFD *in vivo* (Jenkins *et al*., 2008). However, in a recent study (Benchaar and Chouinard, 2009), supplementation of TMR for lactating dairy cows with cinnamaldehyde (1 g/d) did not modify the FA profile of milk fat. The difference between the effects seen in continuous culture versus *in vivo* could be due to the absence of protozoa in continuous culture.

Protozoa do not contribute to biohydrogenation directly, but they could affect activity or populations of lipid-metabolizing bacteria by selective predation (Karnati *et al*., 2009a).

2.4 Plant Oils in Ruminant Nutrition

2.4.1 Plant sources of lipids for ruminants

The main plant sources of lipids for ruminants are forage and oilseeds. The lipid content and fatty acid composition are significantly different among the different sources of plant lipids.

2.4.2 Oil seeds

These concentrate feeds which are processed to prevent negative effect on digestion of fibre in the rumen, contain large amounts of crushed cereal grains, crushed oil seeds or their extracts (Palmquist, 1988). The addition of concentrate supplements to ruminant feed can also help to manipulate the digestion and absorption of nutrients, in addition to increasing the energy value of the diet (Palmquist, 1984; Palmquist, 1988). Rumen acidosis or reduced milk fat content due to low fibre diet or high carbohydrate diets can be limited by addition of fats to the diet (Palmquist, 1984). In addition, the fatty acid composition of ruminant food products can be manipulated to increase their desirability by food industries and for human consumption through addition of concentrates (Grummer, 1991). Although grass and forages contain high concentration of C18:3 *n*-3 as a proportion of the total fatty acids, the content of C18:2 *n*-6 is higher in most commonly used oil seeds (Gurr and Harwood, 1996). However, a significant concentration of C18:3 *n*-3 is found in a few number of oil seeds such as linseed oil, where C18:3 *n*-3 accounts for more than 50% of the total fatty acids (Gurr and Harwood, 1996).

Polyunsaturated fatty acids in plant fats can be naturally protected from ruminal biohydrogenation through the supplementation of whole oilseeds with intact seed coat in ruminant diets instead of oils. This has been observed to elevate the concentration of USFA in muscle tissue (Solomon *et al*., 1991; Ekeren *et al*., 1992). However, the efficiency of this technique can be reduced during processing and mastication, which disrupts the intact seed coat. For efficient post-ruminal digestion, it has been generally accepted that oilseeds or grains for ewes and cattle should be rolled or coarse ground before feeding. This form of processing would disrupt the seed coat consequently exposing the lipids to microbial transformation in the rumen. Orskov *et al*. (1974) suggested that there is no advantage in processing oil seeds because sheep masticate their feed efficiently.

In summary, comparison of the ruminal fatty acid protection potential for some of the available protection technologies was made by Lundy *et al*. (2004). In that study, the authors offered three equal amounts of soybean fatty acids to cows as amides, unprotected and as calcium salts. The C18:2 ruminal disappearance as a proportion of intake was 92.4%, 94.8 and 92.2 for the amides, the unprotected oil and the calcium salts, respectively. This clearly demonstrates a non-substantial difference between the control and the two protection technologies. In addition, De Veth *et al*. (2005) conducted a study to evaluate the effect of formaldehyde and calcium salts protected forms of a *trans*-10, *cis*-12 CLA. It was observed that the mean transfer of the CLA isomer to milk fat was 3.2 and 7.0 for the calcium salts and formaldehyde treatments, respectively. This compared with approximately 20% transfer effectiveness for abomasal infusion of the same CLA. This indicates less rumen protection efficiency by both calcium salts and formaldehyde treatments. However, protection was better with formaldehyde treatment than calcium salts.

As earlier discussed, inhibition of ruminal biohydrogenation is not consistent with any of the available protection technology and none of these technologies meets all the essential features of any supplement to protect ruminal biohydrogenation of PUFA. Therefore, in the recent times, considerable attention has been given to evaluation of plant secondary metabolites and how they influence ruminal biohydrogenation of PUFA. These compounds which possess antimicrobial activities are thought to inhibit lipases involved in hydrolysis of fat.

2.5 Plant Extracts and Rumen Biohydrogenation

Plant metabolites refer to a wide range of chemical compounds which are produced by plants but are not a requirement for the primary biochemical processes involved in growth, reproduction and development of the plant (Calsamiglia *et al*., 2007; Patra, 2012). The primary functions of these plant secondary metabolites in plants are that they act as protective agents against invasion from a wide range of foreign particles such as pathogenic microorganisms (Calsamiglia *et al*., 2007).

The use of plant bioactive compounds (PBC) such as tannins, saponins and essential oils to modify ruminal biohydrogenation is quite a recent development. Over many decades in the past, the main aim of evaluating plants (trees, bushes or forages) which are high in secondary compounds was to ascertain their suitability as feeds in livestock farming such as their effects on digestion of feeds, performance and growth, reproduction and health of animals (Vasta and Bessa, 2012). But, the interest in evaluating the influence of PBC on the fatty acid composition of meat was developed only in the last few years (Vasta *et al*., 2013). In this review, only the effects of essential oils on rumen fermentation and biohydrogenation would be considered. However, the effects of other PBC on rumen fermentation and biohydrogenation of PUFA can be found in the recent review by Patra (2012). The effects of essential oils or their

constituent compounds on VFA and methane production and ammonia nitrogen metabolism have been adequately investigated and recently reviewed (Patra, 2011; Vasta and Bessa, 2012). However, there is a scarcity of information on the potential of EOs or EOCs as modifiers of rumen biohydrogenation of PUFA and concentrations of biohydrogenation intermediates such as CLA and VA.

2.6 Methods to Study Ruminal Fermentation and Methane Production

A microbial system can be described based on (1) the types and populations of organisms concerned, (2) identifying what they do, and (3) observing how fast they do it (Hungate *et al*., 1971). The rates of many rumen activities have been measured in pursuance of step 3. Dual flow continuous culture systems have been used in such an analysis, which facilitates the study of the digestion of nutrients by the ruminal microorganisms, as well as their interrelationships and interactions. In the fermenters, a filtrate pump with a multi-stage filter system, described in Karnati *et al*. (2009b), can be used to achieve different flow rates for solids and liquids to remove only liquid and small particles from the vessel. The filter system retains most protozoa from passing with the filtrate so that they pass primarily with the overflow. This increases the residence time for the solids that pass out only in the overflow from the fermenter, which resembles the differential passages of solids and liquids and turnover rates of the materials in the rumen. The measurement of microbial activity and their integration with the amount and type of materials disappearing during the passage of feed have shown the fermenter's resemblance to the physical environment of the rumen.

Compared with batch culture *in vitro* and experiments *in vivo*, dual flow continuous culture systems allow researchers to measure volatile fatty acids (VFA) and methane production in millimoles per day, rather than concentration in millimoles per liter, which helps to better describe the ruminal fermentation. Different *in vitro* and *in vivo*

techniques have been developed to estimate the efficiency of strategies in mitigating ruminal methane formation, including the rumen simulation technique (RUSITEC) developed by Czerkawski and Breckenridge (1977) and modified by Machmüller *et al*. (2002) to reproduce the ruminal environment in a culture vessel with a stable microbial community and similar fermentation pattern and product yield. RUSITEC is a semi-continuous culture system, but different from the continuous culture fermenters with lower feeding rates, absence of separate solid and liquid outflows, and lack of pH control.

The tracer technique (Johnson and Johnson, 1995) allows animals to move and graze and does not require sampling directly from an animal's rumen or throat. It is useful under controlled conditions but is limited under production situations. A small permeation tube that releases an inert tracer gas, sulfur hexafluoride (SF6), at a known rate is inserted into rumen. This method assumes that the dilution rate of SF6 by the ambient air when it exits the animal is exactly the same as that of the methane. Methane emission rate is calculated from the release rate of the SF6 and the ratio of methane to SF6 concentration in the sample. The estimate of methane includes both the portion of ruminal methane and methane produced in hindgut that is absorbed into blood and respired. The major concern of this method is that SF6 might not mix exactly the same with the ambient air as methane when they exit the animal.

The Tunnel system offers measurement of methane production under grazing conditions and can be portable or fixed (Murray *et al*., 2007). Respiration chambers allow direct measurement of methane but it is more expensive to maintain and a limited number of animals can be tested at a time. The animals are more restrained than those in the tunnel system. Estimates of methane from the chambers tended to be higher than

the tunnels (31.7 ± 0.35 vs 26.9 ±0.46 L/kg DM intake, respectively) because the different degree of restraining might affect the animal's behavior (Murray *et al*., 1999).

Another method is predicting methane production from measurement of VFA production using stoichiometric equations (Hegarty and Nolan, 2007). However, the limitations of this method make it the least appropriate measurement among those mentioned because of the requirement of cannulated animals and errors with assumption of constant rumen conditions over the experimental period.

A newly patented system called GreenFeedTM system (C-lock Inc., USA) has been proposed for estimating CH4 and CO2 emissions from free-ranging ruminants (Storm *et al*., 2012). The system installed with wheels is combined with an automatic feeding system. Animals fitted with a radio frequency identification device in their ear tags entering the system are recognized, and samples of CH4 and CO2 emitted during eating are taken and measured with high resolution. Airflow rates and other environmental measurements are calibrated with a propane sensor to estimate production of CH4 and CO2. Using the sensor information, a volumetric flux (liter per minute) of gases emitted by the animal is directly calculated. Once the volumetric flux is known, the mass flux in (grams per minute) can be calculated using the ideal gas law. This system can be used inside automatic milking systems, in tie-stalls, and for grazing animals. However, the methane measurement requires an animal's head to be in the feeder during eating. Therefore, correlations with whole-day emissions must be examined thoroughly.

2.7 Microorganisms Involved in lipolysis and Biohydrogenation

Lipases responsible for lipolysis are from both microbial and plant origin. However, the actual contribution of plant and microbial lipases to these processes is not clear (Lourenco *et al*., 2010). About 74 strains of microbial lipases in the rumen have

been reported, all with varied lipolytic activity (Fay *et al*., 1990). The lipolytic activity of *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica* has been extensively studied and reported (Harfoot, 1978). Hespell and O'Bryan-Shah (1988) examined the lipolytic activities of 30 strains of *Butyrivibrio fibrisolvens* and observed that there is wide variation among strains of *Butyrivibrio fibrisolvens. Anaerovibrio lipolytica* hydrolysed triglycerides less rapidly than diglycerides; however, they did not hydrolyse galactolipids and phospholipids (Henderson, 1971). The production of saturated fatty acids require first, the hydrolysis of plant esterified lipids by plant (Lee *et al*., 2004) and microbial (Harfoot, 1978) lipases to release the constituent fatty acids. Galactolipids and phospholipids are not however, affected by these lipases (Kim *et al*., 2009). Latham *et al*. (1972) identified bacteria with the same morphology like those of the genus *Butyrivibrio* having the capacity to hydrolyse triglycerides. A *Butyrivibrio* strain and *Butyrivibrio fibrisolvens* have also been observed to carry out phospholipase activity (Hazlewood and Dawson, 1975).

Hydrolysis of ingested esterified plant lipids has also been linked with the activity of ciliated rumen protozoa. In the early 1960s and 1970s; the activity of protozoa in lipolysis was reported (Wright, 1961; Latham *et al*., 1972). Wright (1961) suggested that protozoa, particularly, *Epidinium spp*.could contribute about 40% of the total lipolysis occurring in the rumen. This observation emerged after it was observed that ruminal lipolysis was reduced when cultures were treated with penicillin. Harfoot and Hazlewood (1988) proposed that the engulfment of lipases in the chloroplasts by protozoa could be responsible for the lipolytic activity in protozoa. The ruminal fungi have not been linked with the hydrolysis of esterified lipids in the rumen; however, there is evidence that they are involved in biohydrogenation (Nam and Garnsworthy, 2007). Biohydrogenation of FFA has been exclusively attributed to rumen bacteria

(Lourenco *et al*., 2010). The main microbes which are responsible for biohydrogenating PUFA are the surface-associated bacteria (Lough, 1970). This is because the released PUFA are absorbed to the surface of plant material by means of hydrophobic interactions (Harfoot *et al*., 1973). Initially, the biohydrogenating bacteria were grouped into group A and group B (Kemp and Lander, 1984). The group A bacteria are generally seen as bacteria with the ability to reduce PUFA to form vaccenic acid, whilst, group B bacteria have the ability to biohydrogenate UFA to stearic acid. The main group A bacteria have been recognized as *Butyrivibriofibrisolvens*, whereas *Fusocillus* spp has been known as the main group B bacteria (Kemp *et al*., 1975; Harfoot and Hazlewood, 1997). More recent studies have shown that the C18:0 producers are clustered together and strains are so similar to *Clostridium proteoclasticum* (van de Vossenberg and Joblin, 2003; Wallace *et al*., 2006). These biohydrogenating bacteria are generally recognized as cellulolytic bacteria (Kepler and Tove, 1967), particularly, the *Butyrivibrio* group which are the most active group (Durmic *et al*., 2008). Although the bacterium that was called *Clostridium proteoclasticum* (Kemp *et al*., 1975) which is now re-classified as *Butyrivibrio proteoclasticus* (Moon *et al*., 2008), is the onlybacteria capable of converting VA to C18:0, all bacteria in the *Butyrivibrio* group are capable of producing CLA from linoleic acid. Hudson *et al*. (1998) observed that *Streptococcus bovis* has the ability to cause hydration of linoleic acid to 13-hydroxy-9-octadecenoic acid, hence diverting it from the biohydrogenation course. This early study provide evidence that other facultative ruminal bacteria including *Lactobacillus,Staphyloccous*, *Pediococcus* and *Enterococcus* have the ability to hydrate linoleic acid (Hudson *et al*., 2000). In addition, a concurrent increase in *Megasphera elsdenii* within the rumen has been identified with increased concentrate feeding (Counotte *et al*., 1981).

Biohydrogenation of linoleic acid to the *trans*-10, *cis*-12 CLA has been linked to the activity of *Megasphera elsdenii* (Kim *et al*., 2009). There is limited evidence whether there are many more bacteria involved in biohydrogentaion due to the time-consuming nature and the high cost of isolating such bacteria (Huws *et al*., 2006). Available evidence suggests that other bacteria such as ruminococcaceae, *Anaerovoax,Prevotella* as well as other clostridiales that have not been identified could also play a role in biohydrogenation pathways (Huws *et al*., 2006, Huws *et al*., 2011).

Although biohydrogenation of PUFA has been exclusively attributed to bacterial and protozoal fractions of rumen contents (Wright, 1960). This suggests a potential role of protozoa in the saturation of PUFA. However, further study suggests that the rumen protozoa act as hosts for bacteria in a commensal relationship (Dawson and Kemp, 1969). Therefore, the role of protozoa in biohydrogenation was at this time questioned. An experiment conducted at the Rowett Research Institute shows that the concentration of CLA in protozoa was higher than in bacteria (Devillard *et al*., 2006). Protozoa were not shown to have delta-9 desaturase activity, suggesting that they preferentially incorporate VA and CLA produced by bacteria. Or-Rashid *et al*. (2007) also carried out an *in vivo* study and the data showed that the concentrations of PUFA and CLA in protozoa were higher than their concentrations in bacteria. Nam and Garnsworthy (2007) reported that fungi also have the potential to biohydrogenate PUFA, but at a rate lower than bacteria, even though there are not known to be involved in lipolysis.

2.8 Blood Parameters

Blood which is a important special circulatory tissue is composed of cells in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis (Isaac *et al.*, 2013) thereby maintaining homeostasis of the internal environment

(Bentrick, 1974). Blood composition of animal might be influenced by certain factors such as nutrition, management, and great of animals, sex, age, diseases and stress factors that might affect blood values (Schalm *et al.*, 1975). Blood is an important indicator of physiological and pathological changes in an organism (Mitruka and Rawnshey, 1977). The major function of the blood is to transport oxygen to body cells (Duke, 1975). Significant varriation in serum biochemical and haematological parameters are seen status of living which these can assist in the diagnosis (Huff *et al.*, 1986). The haemotological and biochemical components (Akinmutimi, 2004). Haematological tests have been widely used for the diagnosis of various diseases and nutritional status of animal. The information gained from the blood parameters would substantiate the physical examination and together with medical history provide excellent basis for medical judgment (Schalm *et al.*, 1975). In addition, it would help determine the extent of tissue and organ damage, the response of defence mechanism of the patient and aid in the diagnosing the type of possible anemia (Schalm, 1975). Hematology and serum biochemistry are an index and reflection of the effects of dietary treatment on the animals in terms of the type and amount of feed ingested and were available for the animals to meet its physiological, geochemical and metabolically necessities (Ewuola *et al.*, 2004). A quantifiable variation was reported in blood parameters due to altitude, management, feeding level, age, sex, breed, health status, method of blood collection, hematological techniques used, diurnal and seasonal variation, ambient temperature and physiological status (excrement, muscular exercise, pregnancy, estrus, parturition, time of sampling, water balance and transportation.(Schalm *et al.*, 1975; Ewuola *et al.*, 2004).

Physiologic and pathological changes can be best evaluated when normal blood values are available for comparison. Even though considerable information is available on the

normal blood parameters of domestic animals, the values are that of exotic breeds kept under different environment and management conditions (Tibbo *et al.,* 2004). The blood values are very important in concept the physiological, pathological and nutritional status of an animal. Harmful effect in animals may be manifested by decreased serum concentrations uric acid (Oguz *et al.,* 2000). Haematological parameter is an important and reliable medium used to monitor and elevate health and nutrition status of animals (Gupta *et al*., 2007). Haematological values could serve as baseline information for comparison in conditions of nutrient deficiency, physiology and health status of farm animals (Daramola *et al.,* 2005). Haematological components, which consist of red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring the health status of farm animals (Oyawoye and Ogunkunle, 2004). Swenson (1970) and Jain (1986) pointed out that the MCHC values may reflect an anaemic condition and the capacity of the bone marrow to produce RBC of normal size and metabolic capacity. The MCV, MCH and MCHC have been referred to as RBC indices. Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Johnston and Morris, 1996; Chineke, *et al.*,, 2006).) According to Isaac *et al.* (2013) red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Isaac *et al.,* 2013).

2.8.1 Hematological parameters in ruminant

Hemoglobin is a complex molecule formed from four heme units attached to four globins (two α and two β globins). Iron is added in the last step by the ferrochelatase

enzyme. Hemoglobin has the physiological functions of transporting oxygen to tissues of the animal for oxidation of food or other body functions and also transportation of heme or globin leads to anemia (Merck, 1998). Hemoglobin also known as enthrocyte functions majorly in three (3) ways: transportation and release of oxygen and carbon dioxide (gas exchange), contributes to blood volume and thereby participating in the dynamics of blood circulation and also participate in the blood clotting mechanism (Coles, 1986).

Packed cell volume (PCV) or Hematocrit/ Erythrocyte Volume Fraction refers to the percentage of blood volume that is occupied by red blood cells (erythrocytes) in an animal, it provides an accurate practical evaluation of red blood cells (RBC) status (Merck, 1998) therefore, and an increase in its percentages shows a better transportation of oxygen and absorbed nutrients. Low PCV indicates anemia while an increase PCV is most often caused by dehydration (Merck, 1998). It is considered an integral part of an animal's complete blood count results, along with hemoglobin concentration, white blood cell count and platelet count. It has been established that a reduction in the levels of nutrients in feed results in a decreased PCV and hemoglobin (Hb) of animals (Hawkey and Dennett, 1989). Red blood cells also known as RBCs, erythrocytes red blood corpuscles, hematids or erythroid cells are the most common type of blood cells and the vertebrates organism's principal means of delivering oxygen to the body tissues via the blood flow through the circulatory system. The function of RBC is to carry oxygen to the tissue at pressures sufficient to permit rapid diffusion of oxygen (Merck, 1998). This is done by carrier molecule hemoglobin; a vehicle (RBC) capable of bringing the intact hemoglobin to the cellular level; and a metabolism geared to protect both hemoglobin and RBC from damage. Interference with synthesis or release hemoglobin production or survival of RBC, or metabolism causes disease (Merck,

1998). Swenson (1990) reported that nutritional status of animals can affect its RBC count. Red blood are responsible for the transportation of oxygen and carbon dioxide in the blood as well as manufacture of Hg hence higher values indicates a greater potential for this function and better state of health (Merck, 1998).

Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC) and Mean corpuscular hemoglobin are red blood cell indices used to further characterize and classify anemia. Mean corpuscular volume is an indication of RBC size and is measured in femtoliters (fl) = (PCV * 10 / RBC). Anemia with a high MCV is classified as a macrocytic anemia while anemia with a low MCV is classified as microcytic anemia. A low MCV in an anemic adult animal indicates iron deficiency from slow loss of blood (usually gastro intestinal or renal) (Merck, 1998). Mean corpuscular hemoglobin concentration indicates the concentration of hemoglobin (g/dL) = (Hemoglobin * 100) / PCV and it provides similar information as the MCH but is considered to be more accurate (Merck, 1998). The MCH calculated in pictograms (pg) = (hemoglobin * 10) / RBC. A low MCHC accompanying macrocytosis is indicative of a regenerative anemia. A low MCHC accompanying microcytosis is seen in iron deficiency while an increased MCHC indicates hemolysis (Merck, 1998).

White blood cells (WBC) also known as leukocytes consist of the phagocytes and lymphocytes. The principal function of phagocytes is to defend the body against the invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory responses. Phagocytes are of two types- mononuclear phagocytes and granulocytes. Mononuclear phagocytes arise primarily from the marrow and are released into the blood as monocytes (Merck, 1998). Granulocytes on the other hand have a segmented nucleus and are classified according to their staining characteristics

as neutrophils, oesinophils, or basophils. Monocytes form a link to the specific immune system by processing antigen for presentation to lymphocytes and by producing substances like interleukin-1, which initiates fever and lymphocyte activation and stimulate early hemaopoietic progenitors (Merck, 1998). Eosinophils, while having a role as phagocytes, also has more specific functions that include providing a defense against parasites and modulating the inflammatory process. They respond chemotactically to histamine, immune complexes and oesinophil chemotactic factor of anaphylaxix, a substance released by degranulating mast celss (Merck, 1998). Basophils are not true phagocytes but contain large amount of histamine as well as other mediators of inflammation (Merck, 1998). Depressed leukocyte (WBC), lymphocytes and monocytes counts resulted in leucopenia, lymphopenia and monocytopenia. Leucopenic, lymphopenic and monocytopenic humans and animals are more susceptible to disease causing agents, less resistance to disease process and hence less productive (Coles, 1986).

Hemoglobin and PCV mean values of 8.55g/dL and 27.32% respectively were reported for five healthy goats on wheat straw mixed with starch and urea (Sandhu *et al.*, 2001). The low levels of hemoglobin and PCV was ascribed to the fact that wheat straw, starch and urea are deficient in iron and cupper. Non supplementation of iron in the diet might have led to the decline in the hemoglobin and PCV with time. Aruwayo *et al.* (2009) reported initial values for PCV, Hb, RBC and WBC as 30.50 – 33.50%, 6.53 – 10.20g/dL, 4.85 – 6.20 $\times 10^{12}$/L and 5.45 – 6.85 $\times 10^{9}$/L respectively when growing lamb were fed diets containing fore – stomach digesta and poultry litter wastes. The authors obtained similar values for the control and the treated groups signifying non deleterious effects of the treatments on the animals. Similarly, Lukden and Finangwa (2013) reported some hematological values of Hb (8.90 – 10.50b/dL), PCV (26.00 –

30.50%) and RBC (3.25 – 3.80%) for Yankasa rams fed urea treated Acha (*Digiteria exilis*) straw. Girgiri *et al.* (2013) obtained the following values 9.30 – 11.30/dL, 28.00 – 34.00, 3.10 – 4.00, 3.50 – 7.50, 25.00 – 30.00, 0.75 – 0.90ft and 3321.40 – 3333.30g/dL for Hb ,PCV, RBC, WBC, MCH, MCV AND MCHC respectively for Yankasa rams fed varying levels of Doum palm (*Hyphaene thebaica L*) meal.

Samanta *et al.* (1995) reported on the influence of macro and micro elements on anemia related to production and reproduction of grazing cattle. Spontaneous cases of anemia were recorded in grazing cattle. The anemic cattle with loss of milk production (group 2) and anoestrus (group 3) showed a decrease in the levels of hemoglobin (8.18 – 9.18%), PCV (24.12 and 27.60%), blood glucose (35.14 and 33.58mg %) and total protein (7.94 and 7.16g %) respectively compared to the control (group 1) (hemoglobin 11.68%; PCV 33.46%; blood glucose 42.78mg % and total protein 8.24%). However, supplementation to the animals of both groups showed satisfactory responses to improve body condition, milk yield and reproductive performances. This further proves that rearing livestock by grazing alone or without supplementation of certain minerals can rarely satisfy the mineral requirements of cattle, and such cattle may likely suffer from various problems. Echoche *et al.* (1990) reported a decline in PCV, hemoglobin and TP values of Zebu bulls during mid (PCV 26.7 and 26.1%; hemoglobin 8.9 and 8.6gmg/dL and TP 7.1 and 6.9mg/dL) and final (PCV 26.5 and 25.9%; hemoglobin 9.0 and 8.8mg/dL and TP 6.9 and 6.8mg/dL) feeding periods on low and medium planes of nutrition respectively, compared to bulls on high plane of nutrition whose PCV, hemoglobin and TP for mid and final feeding periods were 28.0 and 28.1%; 9.1 and 9.8mg/dL; 7.4 and 7.5mg/dL respectively. Also plasma urea nitrogen levels were significantly lower during feed restriction and first half of the full feeding periods in

animals on low and medium plane of nutrition than in animals on high plane of nutrition.

Knowledge on blood parameters is of value in the diagnosis of some diseases. Howlader and Huq (1997) reported significantly lower hemoglobin and PCV values in infected goats than non – infected goats. Hemoglobin and PCV values of 9.17mg% and 26.60% respectively for infected goats and 10.51mg% and 32.2% respectively for non-infected goats were reported. The lower hemoglobin and PCV values were attributed to a considerable amount of iron lost through the processes of absorption and excretion of iron by the *Fasciola gigantic* flukes. It was thus, concluded that chronic *Fasciola gigantic infections* significantly lower the hemoglobin and PCV values in goats. Romney *et al.* (1993) reported a decline in PCV values of infected N'dama heifers. Similarly, hemoglobin and PCV values of does infected with *Haemonchus contortus* were significantly lower than that of non-infected does (Howlader and Huq, 1997).

2.8.2 Serum biochemical parameters in ruminant

Serum biochemical analyses are used to determine the level of heart attack, liver damage and to evaluate protein quality and amino acids utilization in animals (Harper *et al.*, 1999). The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood in the form of urea and a measure of renal function. Urea is a by-product of metabolism of proteins synthesized by liver from ammonia; it is freely filtered by the glomerulus of the kidney, but about half the filtered urea passively reabsorbed during passage through the renal tubules. The liver produces urea in the urea cycle as a waste product of protein digestion. Blood urea nitrogen and creatinine are the substance in blood most often used to assess renal function (Duncan *et al.*, 1994; Merck, 1998). Normal range for BUN in sheep is 3.7 – 9.3mmol/L (Merck, 1998). An increase in BUN level is known as azotenia. An elevated BUN may be caused by impaired renal function,

congestive heart failure as a result of poor renal perfusion, dehydration, shock, hemorrhage into the intestinal tract, acute mycocardial infraction, stress and excessive protein intake or protein metabolism (Duncan *et al.*, 1994). Similarly, decreased BUN may be seen in liver failure, malnutrition, anabolic steroid use, over hydration, pregnancy (due to increased plasma volume), impaired nutrients absorption and syndrome of inappropriate anti-diuretic secretion (Duncan *et al.*, 1994). High protein diets and increased protein catabolism from fever or tissue can cause mild increases in BUN. In ruminants, BUN concentration tends to be lower due to a diet relatively low in protein and to metabolism of urea by rumen flora, decreased BUN levels can occur in severe liver diseases.

Total protein (TP), globulin, and albumin are responsive to total protein intake (Whitaker, 1998). An increased plasma protein concentration is most often due to dehydration or to hyperglobulinemia seen in immune response (Merck, 1998). Albumin (Latin: albus, white) refers generally to any protein that is water soluble, which is moderately soluble in concentrated salt solutions and experiences heat denaturation. They are commonly found in blood plasma and are unique to other plasma protein in that they are not glycosylated (ability to add a saccharide unit to a protein). Albumin is a protein made by the liver. A serum albumin test measures the amount of this protein in the clear liquid portion of the blood.

Aspartate aminotranferase (AST) is associated with mitochondria and cytoplasm, alteration in activity could imply alteration in the cystosolic content. The mitochondrion is regarded as the engine house of the cell and exposure of this organelle to assault of any form could imply cell death. The AST is often used to detect liver disease in large animals, but this enzyme is not liver-specific because it is also increased in mycocardial and skeletal muscle disease (Merck, 1998). Alanine aminotransferase (ALT) is a liver-

specific hepatocellular enzyme in some animals measuring hepatic necrosis (Cornnelius, 1986). Both AST and ALT are present in many tissue cells, especially liver and heart cells therefore increased values indicate necrosis of cells (Kaneko, 1980). Transaminases are the most commonly used indicators of cellular necrosis and as mentioned high level in serum may indicate liver malfunctioning. They occupy a central position in amino acids metabolism therefore increased in their activities in the serum could have a consequential effect on the amino acids metabolism in these tissues.

Furthermore, it may indicate some sort of injury to the organs. Such damages may cause the enzymes to leak from the injured organs to the blood stream. An increase in serum AST and ALT above the normal range has been reported to signify necrosis and mycocardial infection or response to the presence of a number of toxin factors (Merck, 1998). Increase in serum values of AST is associated with cell necrosis of many tissues (Merck, 1998). Normal ALT levels indicate that the activities of osteoblast (a cell from which bone develops) were not affected because the blood levels of ALT is usually a good indicator of the rate of bone formation (Guyton, 1991). Alkaline phosphatase (ALP) is a sensitive indicator of chondroblast, odteoblast, the hapatobiliary system, renal tubules, gastrointestinal mucusa and placenta (Duncan *et al.*, 1994; Merck, 1998). Active growth causes ALP levels to be 2-4 times higher in young animals than in adults due to increased production of the bone isoenzyme. But the intestinal, renal and placental isoenzymes are not usually important sources of increased ALP activity (Merck, 1998). Therefore, increases in liver enzymes should be interpreted in conjunction with other clinical signs and therapeutic history because production of enzymes such as ALT, AST and ALP can be induced by drugs including glucocorticoids.

Bilirubin (formerly referred to as hematoidin) is the yellow breakdown product of normal heme catabolism. Heme is found in hemoglobin, a principal component of red blood cells. Bilirubin is excreted in the bile and urine and elevated levels may indicate certain diseases (Merck, 1998). Normal bilirubin values buttressed the presence of hemolysis (destruction of red blood cells and the release of the hemoglobin they conatain) since bilirubin is an insoluble molecule derived from the breakdown of hemoglobin in the spleen (Sirois, 1995). Total bilirubin is made up of conjugated (direct) and unconjugated (indirect) bilirubin. A predominance of unconjugated bilirubin is suggestive of hemolytic jaundice while that of conjugated bilirubin is generally associated with hepatic diseases and extrahepatic biliary obstruction. Increase in the levels of both proportions of bilirubin indicates hepatocellular diseases (diseases relating to liver cells). In addition, damaged cells often impair circulation within the liver so that conjugated bilirubin is not released into the bile duct. Bilirubin is responsible for the yellow coloe of bruises, the yellow color of urine (via its reduced breakdown product, urobilin), the brown color of feaces (via its conversion to stercobilin) and yellow discoloration in jaundice (Sirois, 1995).

Several authors have reported on serum biochemical indices of ruminants. Aliyu *et al.* (2012) studied the blood profile of Yankasa sheep fed on treated *Pennisetum pedicellatum* (Kyasuwa). The authors did not encounter any ill-health as a result of feeding treated Kyasuwa. Garba and Abubakar (2012) found that the concentration of total protein or its fractions as albumin and globulin were not affected in Yankasa sheep fed graded levels of *Tamarindus indica* (tamarind) leaves. Aruwayo *et al.* (2009) reported that urea concentration, total protein, albumin and globulin values in blood plasma were 4.67 – 6.34mmol/L, 4.74 – 5.27g/dL, 3.44 – 3.82g/dL and 1.05 – 1.82g/dL, respectively at the beginning ot trial in growing lambs fed fore-stomach digesta and

poultry litter waste. Slight increases and decreases were observed in their final values. Girgiri *et al.* (2013) reported on the hemato-biochemical indices of Yankasa rams fed varying levels of Doum palm *(Hyphaene thebaica L)* meal. Total bilirubin was 11.00 – 18.00mmol/L, which were all within the normal range (Girgiri *et al.*, 2013).

Total protein, glucose and urea nitrogen mean values of 7.0g/dL, 54.29mg/dL and 17.99mg/dL respectively were reported for five goats on wheat straw mixed with starch and urea (Sandhu *et al.*, 2001). The authors reported a decline in total plasma protein, glucose and urea nitrogen levels as compared to the base value. The decrease in plasma protein and urea nitrogen levels was attributed by the authors to the fact that the animals were being fed on wheat straw which is a poor source of crude protein. Higher levels of urea in blood could be attributed to the presence of some anti-nutritional factors which might have lowered the quality of the protein, indicating imbalance of amino acids in the diets and caused elevated blood urea concentration (Sandhu *et al.*, 2001).

2.9 Techniques for Assessing the Nutritive Value of Ruminant Feeds

Literature reports that feed evaluation techniques (*in vivo*, *in situ* and *in vitro*) can be employed in several applications, as: i) the prediction of voluntary dry matter intake (Van Soest, 1994); ii) the study of the effects of lipids on microbial activity and rumen fermentation (Getachew *et al*., 2004); iii) the study of the effects of different additives on rumen fermentation (Busquet *et al*., 2006); iv) the evaluation of associative effects between ruminant feeds. However, the main efforts are currently focused on the accurate estimation of energy value of feeds and on the determination of rumen nitrogen balance, in order to evaluate the efficiency of rumen fermentation process.

The accurate evaluation of energy value of ruminant feeds represents a great challenge in order to formulate well-balanced diets, which can meet animal requirements and

support their performance. Over the years, different energy systems have been developed for estimating energy content of ruminant feeds, and different equations have been proposed. Predict energy content of ruminant feeds from their chemical composition, but the main shortcoming of this approach is that it is a theoretical method and it does not take into account neither the animal nor the feed characteristics (Kitessa *et al*., 1999). Among the alternative approaches proposed to estimate energy value of feeds is the *in vitro* GP at 24 h of incubation (Menke and Steingass, 1988). These alternate approaches present significant differences, as they involve the use of values obtained from different procedures of analysis (*invivo*, *in situ* or *in vitro*). To this regard, NRC (2001) simply indicated that *invitro* techniques should be preferred as more standardized. The equations based on *in situ* or *in vitro* NDFD (NRC, 2001) usually consider 48 h as reference incubation time, whereas those based on *in vitro* GP use 24 h (Menke and Steingass, 1988).

The nutritional evaluation of ruminant feeds and the formulation of well-balanced diets which can meet requirements of animals represent important challenges in livestock production (Adesogan, 2002). The ability of a feed to sustain animal performance depends mainly on its digestibility. Feed digestibility is influenced by its chemical (carbohydrate, proteinand fat content) and physical (feed particles size) characteristics, as these properties affect capability of digestive enzymes to colonize and digest the feed particles (Kitessa *et al*., 1999). The digestibility of ruminant feeds can be evaluated using different techniques. It can be estimated by biological methods, which simulate the digestion process. The three major digestion techniques currently available to determine the nutritive value of ruminant feeds are (1) digestion with rumen microorganisms as in the work of Tilley and Terry (1963) or gas method (Menke *et al.*,

1979) (2) cell free fungal cellulase and (3) *in situ* incubation of samples in nylon bag in the rumen (Getachew *et al.,* 2004).

2.9.1 The use of *In vitro* gas production technique for evaluation feeds

According to Bergman (1990) in the rumen forage is exposed to a great population of microbes that initiates the fermentation and digestion of plant cell walls into carbohydrates and sugars. Rumen microbes use the carbohydrates with ammonia and amino acids in feeds to grow. Microbes ferment sugars to produce volatile fatty acid – acetate, propionate and butyrate; other gases produced are methane, hydrogen sulfide, carbondioxide. Proper feeding of ruminants requires that the quantity and quality of forage offered to them and the proportion of this consumed by them be correctly estimated. Improved performance of ruminants will be achieved when the nutritive value and intake are used to estimate what should be offered to them as supplementary feeds (Blummel and Ørskov, 1993).

Several gas measuring techniques and *in vitro* gas methods are in use by several groups. Differently from the digestibility techniques which aim to evaluate the substrate disappearance, gas production (GP) method measures the appearance of fermentation products (gases, volatile fatty acids, NH_3) (Blummel and Ørskov, 1993) and is a powerful tool for studies on degradability of complex substrates such as grasses, legumes and other whole crop forages. It is used to get additional information about nutrient utilization and the efficiency by which an animal utilizes feed nutrients. The *in vitro* gas method based on syringes (Menke *et al*., 1979; Blummel *et al*., 1997) appears to be the most suitable for use in developing countries and has been modified by Blummel and Ørskov (1993), Cone *et al*. (1999). The *in vitro* gas method is more efficient in the sense that it can be used in evaluating the effects of tannins or other anti-nutritional factors. The gas production techniques in combination with

polyvinypolyueriolidone (PVP) was reported to have a good potential for providing better insight into the effects of phenol related anti-nutritive factors in biological systems (Khazaal *et al*., 1993). In addition, the *in vitro* gas method can better monitor nutrient-anti-nutrient and anti-nutrient-anti-nutrient interactions (Makkar, 2000) and detects and provides useful data on fermentation kinetics of both soluble cell contents and non-soluble fractions of feedstuffs (Getachew *et al.,* 1997). According to Getachew *et al.* (2004) an animal's feed intake, and how well that feed is digested, determines the feed's production performance.

The *in-vitro* gas-production technique of Menke *et al.* (1979) is commonly used to determine the amount of gas produced over a 24-hour incubation period. The amount of gas released when a feed is incubated *in vitro* with rumen fluid is closely related to the digestibility of the feed. The gas produced is read either at a fixed incubation time, 24 hours, or at a series of incubation times (sequential incubation), mainly 6, 12, 24, 48, 72 and 96 hours. Sometimes, 3 and 120 hours of incubation are included if the asymptote of the gas production curve is to be clearly defined. Both fresh and dry samples can be assayed and the gas produced from 200 mg DM of both samples is determined for comparison (Osuji *et al.*, 1993).

In vitro methods have the advantage not only of being less expensive and less time consuming but they allow to maintain experimental conditions more precisely than do *in vivo* trials (Makkar, 2000). The *in vitro* gas production technique does not require sophisticated equipment and is a relatively simple method for evaluating feeds, and can analyse large number of samples at one time, making the analytical capacity high (Cone *et al*., 1999). The most negative aspect with this method is that it requires donor animals to supply rumen fluid, which is an economic question where the costs for the animals are high (Kitessa *et al*., 1999). This method has been successfully applied in the aspects

of feed evaluation, including organic matter digestibility (OMD), metabolizable energy (ME) (Menke *et al*., 1979) net energy for lactation (NEL) Menke and Steingass, 1988), short chain fatty acid (SCFA), kinetics of fermentation (Ørskov and McDonald, 1979). Bogoro *et al*. (1994) measured gas production of sorghum stover at different periods – 3, 6, 12, 24, 48, 72 and 96 hours, and got the following results – 3.16, 5.50, 11.00, 19.40, 28.77, 33.43 and 37.20mls respectively. According to Khazaal *et al.* (1993), gas production from 8 browse plants studied for 24 and 48 hours varied and the gas produced ranged from 14.8 to 35.1ml in 24hours and 26.60 to 38.70mls in 48 hours. Khazaal *et al*. (1993) observed that a strong relationship ($R2 = 0.84$: $P < 0.01$) exist between the increase in percentage (%) in gas produced and the change in total VFAs.

Beuvink (1993), described the curve of gas production on dry matter disappearance as a sigmoid curve with 3 phases viz: slow phase, involving hydration, microbial attachment and colonization. The second phase (exponential) represents enzyme degradation and the third phase (Asymptotic phase) when gas production rate decreases and falls to zero. Blummel and Ørskov (1993) observed a correlation between dry matter degradability and gas production at 48 hours, but they did not obtain a relationship between the rate of dry matter degradation and gas production.

2.9.2 Determination of *in vitro* fermentation characteristics of feeds

Fermentation characteristics of feedstuffs in buffered rumen fluid can be studied using *in vitro* techniques using syringes (Menke *et al*., 1979; Blummel *et al.,* 1997). These techniques simulate the rumen fermentation process and they have been used to evaluate the potential of feed to supply nutrients to ruminants. Moreover, the *in vitro* gas production method can be used to examine animal waste components that impact the environment and develop appropriate mitigations. When a feed is degraded in the rumen, the degraded matter is partitioned to yield microbial protein, volatile fatty acids

(VFA) and gases. The sum of microbial protein and VFA represents the amount of feed energy which can be potentially used by the animals to meet their requirements, whereas gases are the proportion of energy which is lost by the animals.

Volatile fatty acids (VFAs), also known as short-chain fatty acids, are produced in the GIT by microbial fermentation of carbohydrates and endogenous substrates such as mucus. According to Bergman (1990) they can be of great advantage to the animal since no digestive enzyme exists for breaking down cellulose or other complex carbohydrates. VFAs are produced by both herbivorous animals and other species including man at different sections of their gastrointestinal tract (GIT) and are the main source of energy to ruminants (Bergman 1990). The greatest quantity of VFA is produced in the rumen of ruminants and in the lower digestive tract of man and other species. According to Bergman (1990), volatile fatty acids represent a major product of ruminant digestion, account for 70-80% of absorbed energy and their proportions affect animal production. The major VFA in either the rumen or the large intestine are acetate, propionate and butyric acids. They are produced in a ratio varying from approximately 75:15:10 to 40:40:20 (Bergman, 1990). For example, high proportions of propionate can cause milk fat depression but when acetate supply exceeds the energy needs it is stored as fat. Although nutritive value is indicated by forage chemical composition, digestion by the rumen microflora affects the composition of nutrients absorbed by the host. Microbial activity is regulated to some extent by the host animal and is affected by substrate availability, especially DM release from ruptured plant cells and particulate surface area available for bacterial colonisation. The production and absorption of VFA has a very significant effect on epithelial cell growth, blood flow and the normal secretory and absorptive functions of the large intestine, caecum and rumen. Also the absorption of VFA and sodium seem to be interdependent, and release of bicarbonate

usually occurs during VFA absorption. Increasing the plant fibre of species may be recommended, because this will lead to increase in the production of VFA; and this will increase the contribution of VFA to the energy supply to the body.

The proportion of acetic acid usually increase with increases cellulosic roughages in the diet while grain or starch usually ferment to yield a higher proportion of propionic acid. Ørskov, (1994) observed that feeding whole rather than rolled barley, wheat, oats and maize had a very large influence on the type of fermentation. Therefore chemical composition of feedstuffs alone may not be enough as a basis for determining the type of fermentation to expect. According to Ørskov (1994), the type of fermentation depends on rumen environment, especially the pH and the degradation rate of substrate. About 80% of the volatile fatty acids produced in the rumen are absorbed through its wall, the surface area of which is greatly enlarged by numerous papillae. These papillae grow and regress in response to changes in food consumption and VFA concentration. Other remaining VFA is absorbed in the omasum and the abomasum (true stomach).

2.9.3 The effect of quality of rumen fluid for *in vitro* gas production

Many different factors can potentially influence or alter digestibility and GP measures. It is largely recognized that a common source of variation of *in vivo*, *in situ* and *in vitro* techniques is represented by the quality of rumen fluid (Mould *et al*., 2005). Literature indicates that the quality of rumen fluid, in terms of number and kind of microbial population, is mostly influenced by diet composition and by frequency of feeding (Thorley *et al*., 1968). *In vivo* and *in situ* trials should be preferably conducted in animals consuming the feeds or the diets of interest, to limit the diet effects and to achieve a rumen fluid "ideal" in terms of microbial population (Vanzant *et al*., 1998; Kitessa *et al.,* 1999). Cronje (1992) reported that *in situ* digestion of forages was higher

in rumens of animals usually fed forage-based diets compared to that of animals receiving high-concentrate diets.

Among forage diets, the kind of forage offered to the animals was found to influence the rate and extent of *in situ* digestion (Vanzant *et al*., 1998). When rumen fluid is taken from intact cows, the greatest challenge is to achieve a representative sample in terms of microbial population and concentration (Mould *et al*., 2005). It is largely accepted that a correct sampling procedure should avoid oxygen and saliva contamination of rumen fluid (Mould *et al*., 2005). Saliva contamination could modify pH and alter fermentation patterns, whereas oxygen contamination could reduce or completely inhibit activity and growth of anaerobic rumen microorganisms (Hungate, 1966). It was reported that *in vitro* digestibility of forages decreased when rumen fluid was collected by donor animals consuming high-concentrate diets (Russell and Wilson, 1996; Tejido *et al*., 2002) and was observed that high-concentrate diets significantly affects the microbial count of rumen fluid.

Nagadi *et al*. (2000) argued that the real challenge should be feeding diets which allow to attain the minimal microbial activity to ensure GP, because the diet fed to donor animals was found to exert small effects on GP kinetics (Menke and Steingass, 1988; Cone *et al.*, 1996; Nagadi *et al.*, 2000). Cone *et al*. (1996) found that microbial activity of rumen fluid was significantly affected by the timing of collection, as they noted a greater microbial activity in rumen fluid collected after feeding. Menke and Steingass (1988) hypothesized that the minimum microbial activity should be ensured by using rumen fluid collected from animals fed within the previous 16h. However, the same Authors suggested to use rumen fluid collected before feeding, as it could have a less variable composition compared to that collected after feeding.

2.10 Digestibility of Nutrients

According to Coles *et al*. (2005), digestibility is simply the measure of the availability of nutrients and when combined with intake, one can make an accurate prediction of overall nutritive value of animal feeds. In animal nutrition, digestibility is defined as the percentage of the feed or the single nutrient in the feed that is acted upon in the digestive tract, absorbed and made available for use by the body's cells. Digestibility analysis is essential when developing or reformulating animal's feeds (Lowman *et al*., 1999), as it allows the manufacturer to pass on vital feeding recommendations to customers. Digestibility information is also important economically to the consumer because accurate feeding guidelines reduce overfeeding and waste (Coles *et al*., 2005). Frappe (2004) highlighted that nutrient composition and digestibility can differ within a single ingredient and batch of processed feed. Also manufactures and distributors do benefit economically when ingredient digestibility and cost are considered together before producing a diet.

The nutritive value, or energy content of an animal feed is determined predominantly by its digestibility, which affects intake, or how much the animal will eat (DePeters *et al*., 2003). Digestibility and intake, in turn determines the feeds productive performance such as to support milk synthesis or muscle growth. Studies with live animals to determine the digestibility of feeds is known as *in vivo* methods. The digestibility of feeds can also be estimated by biological methods known as *in vitro* techniques, which are conducted outside of the animals system but simulate the digestion process. Generally, *in vitro* techniques are those based on measuring either fermentation residues or products (Getachew *et al*., 1999).

In a study reported by Oba and Allen (2000) where the relationship between fibre digestibility and animal performance was evaluated using 45 sets of treatment means

from 27 articles published in the journal of dairy science. These articles had reported significant differences in NDF digestibility *in vivo, in situ,* or *in vitro.* Experiment with cows averaging less than 100 days in milk production indicated 5.2% increase in fibre digestibility of the diets for the early cows and a 9% unit increase in digestibility for mid-lactation cow. Grant (2004) fed dairy cows diets containing forage and obtains 58% NDF digestibility.

According to Tamminga (1993), the process of fibre digestion consist of hydrolysis of polysacharrides and the conversion of monosaccharides to VFA, fermentation gases and heat. The rate of hydrolysis is limited by penetration of the enzymes that degrade the cell wall deep into lignin-polysacharides complexes. Varga and Kolver (1997) highlighted that the rate of hydrolysis is generally the limiting factor in fibre digestion in the rumen.

The effect of starch on fibre digestion does vary with starch source. According to Herrara-Saldana *et al*. (1990) replacing corn with barley has been shown to have a negative effect on digestibility. When the starch sources cassava, barley and corn were studied, cassava and barley starch sources had more pronounced effect on the amount of fibre in the rumen over time after feeding (Tamminga, 1993). Apparent digestibility of fibre were 55.1, 56.3% and 63.6% for barley, cassava and corn containing diets respectively. Adding 2.4 – 7.2% total sugar as dried molasses to diets containing 60% forage on a dry matter basis (65% corn silage and 35% alfalfa haylage) resulted in a 4% unit increase in total tract NDF digestibility in Holstein dairy cows (Broderick and Radloff, 2004). When liquid molasses was added to provide 2.6 to 10% total sugars to corn silage an 8% increase in total tract digestibility was observed. Sugar addition to the diet has been shown to enhance fibre digestibility, especially for poorer quality forages (Varga, 2003). Digestibility of the straw is dependent on the de-polymerization

of its structural carbohydrates. Enzymatic degradation of these macro molecules in the straw will result in degradation and increase in digestibility and availability of carbohydrates (Fazaeli *et al*., 2014).

Bacteria and protozoa may degrade protein entering the rumen to produce proteolytic enzymes, and is therefore not greatly affected by changes in the composition of the animal's diet (Getachew *et al*., 1999). Free amino acids appearing in the rumen are rapidly deaminated to keto-acids and ammonia, rather than taken up by bacteria, despite an obligatory requirement for amino acids by many species (Tamminga, 1993). Non-protein nitrogen is rapidly degraded largely to ammonia, which may be taken up by bacteria for protein synthesis (Varga and Kolver, 1997). Depending on diet, 60-90% of the daily nitrogen intake of the ruminant may be converted to ammonia (Tamminga, 1993) and according to Frappe (2004), from 50-70% of bacterial nitrogen may be derived from ammonia.

Careful treatment of plant protein to prevent proteolysis in the rumen will increase the amount of bypass protein that arrives at the duodenum (Varga and Kolver, 1997). Passage of nitrogen from the rumen between 60 and 85% of the total amino acid nitrogen entering the small intestine is microbial protein. Protein is not materially affected by passage through the omasum, although both urea and ammonia may be absorbed (Grant, 2004). Firkins (1997) reported that 50-75% of microbial protein may be synthesized from the ruminal ammonia pool; about 30-50% of this protein may be hydrolysed within the rumen and returned to the ammonia pool.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site:

The studies were carried out at the Livestock Teaching and Research Farm, Department of Animal Science, Main Campus, Usmanu Danfodiyo University, Sokoto. Sokoto State is located in North-western Nigeria between latitudes $11^0$30' and $14^0$00'N and longitudes $4^0$00' and $6^0$40'E. The state covers a total land area of 32,000 km^2 (Mamman *et al*., 2000) and at an altitude of 350m above sea level (Mamman *et al*., 2000). The State falls within the Sahel savannah vegetation zone. The hot dry spell extends from March to May and sometime to June in the extreme northern part. A short, cool, dry period (harmattan) occurs between October and February (Malami *et al*., 2001). The annual rainfall is about 700 mm. The rainy season start from June to early October with a peak in August, potential evapotranspiration has been reported to be 162mm. maximum temperature of 41^{o}C has been reported in April and minimum of 13.2^{o}C in January (SEPP, 1996). The State is one of the largest livestock producing area in Nigeria. Among the livestock produced in the area are cattle, sheep, goats, donkey, poultry and camels.

3.2 Experiment I: Influence of Plant Oils on *In vitro* Fermentation Characteristics

3.2.1 Experimental procedure

3.2.1.1 Sources of the oils:

The soybean and groundnut oils used in this experiment were bought from Gerewa oil mills while garlic oil was extracted locally in Kano State.

3.2.1.2 Experimental diets

Four (4) dietary treatments were formulated (Treatment 1 (T1) is the control diet with no oil additive. Treatment 2 (T2) contained 30ml/kg garlic oil, Treatment 3 (T3) had 30 ml/kg Soybean oil while 30 ml/kg groundnut oil) was added in treatment 4 and the diets were evaluated via *in-vitro* techniques (Menke and Steingass, 1988).

Table 1 Composition of Experimental Diet Supplemented with Different types of Plant Oils

Ingredients	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)
Maize	14	14	14	14
Soybean Meal	15	15	15	15
Cowpea husk	30	30	30	30
Wheat offal	25	25	25	25
Rice Milling Waste	15	15	15	15
Salt	1.0	1.0	1.0	1.0
Total	100	100	100	100
Calculated analysis				
ME Mcal/kg	2181	2181	2181	2181
Crude Protein %	16.00	16.00	16.00	16.00
Crude Fibre %	19.40	19.40	19.4	19.40
Ether extract (%)	4.20	4.20	4.2	4.20

GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil, ME== Metabolizable Energy

3.2.1.3 Rumen liquor collection

3.2.1.3.1 Animal management, feeding and collection of rumen fluid

The rumen fluid were collected from two donor sheep which were offered water and sorghum husk *ad libitum* as well as supplemented with additional 400 g/sheep/day of concentrate as presented in Table 1. The sheep were placed on the experimental feed for 7 days, before slaughter. Feed were withdrawn from the ram at 18.00 hours on the evening before the day of slaughter. Whole rumens were then collected and immediately sealed in tough plastic containers to prevent oxygen entry and transported in insulated boxes to maintain rumen temperature to the Biochemistry Laboratory in Yobe State University. The rumens were incised with a scalpel and rumen contents were scooped and the liquor strained through 2 layers of cheesecloth.

3.2.1.3.2 Incubation

In vitro incubations: 100 ml syringes were fitted with rubber stoppers to prevent escape of fermentation gases (Wang *et al.,* 2000). The bottles were placed in an incubator at temperature of 39±0.5°C and were affix to a rotary shaker platform at 120 oscillations/min. On the day before incubation, 0.25 g DM of each diet samples were weighed into each bottle (n = 3 for each treatment). On the day of incubation, bottles containing substrate were warmed to 39°C in the incubator for 60 min, and gassed with N_2 before adding 20 ml of a 4:1 mixture of buffer: rumen fluid saturated with CO_2 and 0.5 ml cysteine sulphide reducing agent (Wang *et al.*, 2000).

3.2.2 Data collection

Gas samples were measured at 3, 6, 12 and 24 hrs of incubation. The procedures for sampling bottles were: 15 bottles (3 replicates× 4treatments+3 blanks) were removed from the incubator and gas productions from each bottle as immediately determined using a water displacement apparatus (Fedorak and Hrudey, 1983).

3.2.2.1 Determination of Methane Production

Methane (CH_4) determination; 10 ml of headspace gas were collected from the bottles at 24 h by inserting a 20 ml syringe through the septum and immediately injecting from syringes into gas chromatography. 3 ml subsamples of gas were analyzed for CH_4 by gas chromatography as described by Chaves *et al*. (2006). Methane were express as mg CH_4/g digested DM and total net gas production in ml/g DM incubated (Chaves *et al.*, 2006).

3.2.2.2 Determination of volatile fatty acids (VFA)

At 24 h of incubation; incubation bottles (n = 3) of each diets were withdrawn from the incubator and put in ice to stop fermentation, and then processed for determination of volatile fatty acids (VFA), ammonia and *in vitro* DM digestibility (IVDMD) as described by Wang *et al*. (2008).

3.2.2.3 In vitro dry matter digestibility (IVDMD):

After 24h digestion, the samples were transferred into test tubes and centrifuged for 1hour in order to obtain the residue which was then filtered using Whatman No 4 filter paper by gravity and the residues placed in crucible for drying at 65^0C for 24h. The dried residues were weighed and digestibility calculated using the equation as follows:

$$\text{IVDMD}(\%) = \frac{\text{Initial DM Input} - \text{DM residue} - \text{Blank}}{\text{Initial DM InputW}} \text{ X } \frac{100}{1}$$

3.2.2.4 Fatty acid determination

The diet and rumen fluid were strained through four layers of cheesecloth. Lipids in the strained rumen fluid were extracted by the method of Bligh and Dyer (1959) with purification of samples with 20% of HCl. Extracted lipids in tubes were dissolved in 1 ml of hexane and 1 ml of internal standard (tridecanoic acid; Fluka, Sigma – Aldrich). 2 ml of transesterification reagent (1N methanolic sodium methoxide) (Baše, 1978) were added to the mixture. The mixture were held in a water bath at 50°C for 30 min.

After addition of 3 ml of 3N methanolic HCl (Supelco, USA) the mixture were incubated in the water bath at 50°C for 1 h, to separate the hexane layer in the mixture, 1 ml of hexane and 1 ml of distilled water were added. Finally, the mixtures were centrifuged at 200 g for 5 min. The hexane layers were used for the determination of fatty acid methyl esters by a gas chromatography. The samples were injected with a split less injector into a Perkin-Elmer Clarus 500 gas chromatograph (Perkin-Elmer, Inc. Shelton, Connecticut, USA).

The gas chromatograph were equipped with a capillary column DB-23 (60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies) and a flame ionization detector (constant flow, hydrogen 40 ml/min, air 400 ml, 260°C). The analyses of fatty acids (0.5 μl methyl esters in hexane injected at a 30:1 split ratio) were carried out under a temperature gradient (130°C for 1 min; 130–170°C at program rate 6.5°C/min; 170–206°C at program rate 1°C/min; 206–240°C at program rate 34°C/min) with hydrogen as a carrier gas (flow 1.8 ml/min, velocity 44 cm/s, pressure 23.2 psi). The fatty acid methyl ester peaks were identified with a commercial mixture (Supelco 37 component FAME MIX, Supelco, Bellefonte, PA, USA; *trans*11-vaccenic (TVA) methyl ester, Supelco, Bellefonte, PA, USA; *cis*9, *trans*11 conjugated linoleic acid (c9,t11-CLA), Matreya, PA, USA) and quantified by an internal standard (tridecanoic acid C13:0, Supelco, Bellefonte, PA, USA). The values of fatty acids are expressed as g/100 g of total fatty acids (g/100 g FA).

Rumen biohydrogenation was calculated as the change in the proportion of individual Fatty Acid such as 18:3 *n*- and 18:3*n*-2, in the feed relative to the amount left in incubation vessels at a given time as follows:

$$\text{Biohydrogenation (g/100g)} = \frac{\text{Initial amount of unsaturated d FA} - \text{amount after incubation}}{\text{Initial amount of unsaturated FA}} \text{ X } \frac{100}{1}$$

3.2.2.5 Microbial count of the fluid of in vitro

The microbial analysis was conducted using by serial dilution following the procedure of Adams and Moss (2007). Sterilization: All glass wares were washed with detergent rinsed with water and sterilized using hot air oven at 160^{o}C for 1 hour. While all the liquid media were sterilized in an autoclave at 121^{o}C for 15 minutes.

Nutrient agar medium: Twenty-eight (28) g of nutrient agar powder was weighed and dissolved into conical flask containing 1000 ml of distilled water, after plugging with non-absorbent cotton wool and cover with aluminium foil. This was heated and agitated gently for about 10 minutes in order to dissolve the nutrient agar which was letter autoclave at 121^{o}C for 15 minutes in order to achieve sterility. The agar formed was allowed to cool to about 45^{o}C, and then dispensed into sterile petri dishes. The agar was then left to solidify and was refrigerated at 4^{o}C for further use (Prescott *et al*., 2005).

Serial dilution: One (1) gram each of muscles and meat samples were weighed and dissolved in a test tube containing 9 ml of sterile distilled water (10^{-1}) dilution. This was shaken to obtain a good suspension. The suspension was then serially diluted to 7 tubes (10^{-7}). This process of transfer from preceding tube continued till 1:7000 dilutions is achieved using a fresh siring tip for each dilution. Furthermore, 10^{-4} and 10^{-7} test tubes were used for inoculating media. Colony count was performed by using colony count metre as described by Prescott *et al*. (2005). $Bacterial\ load = Total\ num.\ of\ colonies\ in\ a\ plate \times reciprocal\ of\ the\ dilution\ factor$

3.2.2.5.1 Bacterial species identification

Subculture for pure culture isolation: Using a sterile wire loop of the correct size dip into the enrichment culture and pick a single colony and inoculate into a small area of a plate containing fresh nutrient agar and spread by using a sterile wire loop, which was incubated for 24 hour and observed the growth. The pure isolates of each of the colony

was obtained and transferred into a sterile slant bottles containing fresh nutrient agar and refrigerated at 4^{o}C for further use (Prescott *et al*., 2005).

Gram staining: Gram staining was carried out according to Prescott *et al*. (2005) method. Smear of bacterial isolates were made on clean glass slide using drop of water with sterile wire loop. It was then allowed to air dry and then passed over a flame in order to fix the smear. After fixation the smear was covered with gentian violet for 60 seconds and washed. Iodine was then poured to cover the smear, allowed for 60 seconds and then washed. Ethyl alcohol (ethanol) was used to decolourize the smear and washed immediately with the distilled water, then follow by the application of safranin and left for 60 seconds, and later washed with distilled water. Back of the slide was cleaned with cotton and allowed to air dry. The slide was examined under electrical microscope using oil immersion x100 objectives.

3.2.2.5.2 Biochemical identification of the isolated bacterial

The biochemical test was carried out according to the description of Prescott *et al*. (2005) and Willey *et al*. (2011).

3.2.2.5.3 Triple sugar iron

The triple sugar iron was put in a slants test tube and was inoculated with the isolates using a sterile transfer needle. Using the needle the butt was stabbed then the needle was withdrawn and the slant test tube was streaked. The test tubes were incubated at 37^{o}C for 24 hours, which was examined for gas production, hydrogen sulphide production, glucose production, lactose production, sucrose production and motility (Prescott *et al*., 2005).

3.2.2.5.4 Urease production

Slants of urease medium in universal bottles were inoculated with loopfull of isolates by streaking. These were incubated for 4 days at 37^{o}C with daily examination. Change

of colouration of the media from brown to red indicates presence of urease (Prescott *et al*., 2005).

3.2.2.5.5 Methyl red production

To prepare glucose phosphate medium in a test tube, a loop full of isolates was inoculated and incubated for 2 days at 37^{o}C. Drops of methyl red solution were added to the 2 days old culture, shacked and examined. Appearance of red colour at the surface of the reagent layer showed positive methyl red production (Prescott *et al*., 2005).

3.2.2.5.6 Indole production

A loop full of the isolates was inoculated in a sterile nutrient broth at 37^{o}C for 48 hours. After incubation, 0.5 ml of Kovacs reagent was added and shacked. This was examined after one minute. A red colour in the reagent layer indicates positive indole production (Prescott *et al*., 2005).

3.2.2.5.7 Citrate production

To a sterile simons citrate medium a loop full of 24 hours culture isolate was inoculated aseptically, at 37^{o}C for 24 hours after which it was examined for turbidity daily for a period of 3 days. Turbidity indicated citrate utilization (Prescott *et al*., 2005).

3.2.2.5.8 Hydrogen sulphide production

This test detects the ability of bacterial species to produce hydrogen sulphide, e.g. by reduction of sulphur from the metabolism of sulphur containing amino acids to hydrogen sulphide. A speck of each isolate was inoculated into triple sugar iron agar and incubated at 37^{o}C for 24 hours. Evolution on blackening of the medium indicates positive hydrogen sulphide (Prescott *et al*., 2005).

3.2.2.5.9 Motility test

Motility test was carried out according to Willey *et al*. (2011), motility can sometime be inferred from the way an organism grew on solid media. Motile species tend to spread outward from the inoculated area as organism swim in the layer of surface moisture. A bit of each isolate was stab onto triple sugar iron agar and incubated at 37^{o}C for 24 hours. Motility observe is by spread of the organisms outward from the stab area.

3.2.2.6 Fungal counts

For fungal counts one milliliter of the sample was added to the fermentation tube to give a final concentration of 2,000 U of penicillin and 130 U of streptomycin per ml to inhibit bacterial growth. The antibiotic and fungicide were dissolved in distilled water previously gassed with oxygen free carbon dioxide, sterilized by passage through a 0.2µm pore diameter polysulfone membrane filter, anaerobically and aseptically to the individual tubes prior to inocubation. Then serial dilutions up to 1×10^{-6} were made on clean glass slide and calculation were done by following: Ruminal fungi /ml of supernatant = Average number of fungi per field × microscopic factor (1000) × dilution (10^{6}) (Broudiscou *et al.,* 1997).

3.2.2.7 Protozoal counts

The protozoal counts were used to calculate the protozoal generic distribution and generation time of protozoa according to the following formula: generation time in hours = total protozoal counts in fermenter/flow of protozoa in effluent per hour (Sylvester *et al*., 2004). The microorganism were isolated and identified by conventional method with grams staining for bacterial, fungal and protozoan species.

3.3 Experiment II: Effect of Plant oils on the Performance and Blood Profiles of Growing Lambs

3.3.1 Experimental animals and their management

Sixteen (16) Uda ram lambs were used for this study. The experimental animals were sourced from Achida Livestock Market in Sokoto. The animals were dewormed with Ivermitin 5% against internal and external parasites at 1ml/50kg body weight before commencement of the experiment. Also Oxytetracycline (a broad-spectrum antibiotic) long acting base were given at 1m/10kg body weight and multivitamin injection were also given at 1ml/10kg body weight for three days to reduce stress. Before commencement of the experiment the animals were quarantine for two weeks during which groundnut haulms were given to the animals *ad libitum*. The animals were housed in pens which have wide windows for adequate ventilation.

3.3.2 Experimental diets

Four (4) dietary treatments were formulated (Treatment 1 (T1) is the control diet with no oil additive. Treatment 2 (T2) contained 30ml/kg garlic oil, Treatment 3 (T3) had 30 ml/kg Soybean oil while 30 ml/kg groundnut oil) were added in treatment 4 and the diets were evaluated via in vivo techniques. The gross composition of the experimental diets for experiment II: were Maize 14%, Soybean Meal 15%, Cowpea husk 30%, Wheat offal 25%, Rice Milling Waste 15% and salt 1%. Containing 2181 kcal/kg ME give energy of 16 % CP, 19.4% CF and 4.2% Ether Extract. Plant oils were added to the concentrate diet (30 ml/kg DM). The experimental animals were fed at the rate of 2% body weight while sorghum husk were used as basal diet which was fed *ad libitun*. The gross composition is shown in table I (experiment I)

3.3.3 Experimental design and feeding

The animals were allotted to 4 treatments; four (4) animals were randomly allocated to each treatment in a Complete Randomized Design. The animals were housed in individual pens with adequate ventilation. The animals were balanced for weight before commencement of the experiment and weighed weekly thereafter throughout the experimental period. The experimental diets were offered at 2% of body weight while sorghum husk were fed *ad libitum* as basal diet. Feed intake were determined as the difference of the amounts of feed offered and the refusals. Water was offered *ad libitum* and the experiment lasted for 84 days (12 weeks).

3.3.4 Data collection

3.3.4.1 Productive parameters

The performance parameters measured are; feed intake, body weight, body weight gain and feed conversion ratio.

3.3.4.2 Feed consumption/intake

Feed consumption from each treatment was measured on daily basis by subtracting left-over from feed served the previous day per group. Adequate measures were taken to guard against spillage and related wastage. The mean daily feed intake per animal was taken.

3.3.4.3 Body weight

Lambs were weighed on weekly basis using a weighing balance to determine the body weight gain. The mean live weight of each treatment group was determined by total weight of animals divided by the total number of animals in each replicate group. The animals were fasted for 6 hours before weighing to avoid errors due to gut fill.

3.3.4.4 Body weight gain/week

The body weight gain of each of the treatment group was obtained by calculating the difference between the mean live weights of the current week from the mean live weight of the preceding week. Body weight gain was determined on weekly basis.

3.3.4.5 Feed conversion ratio

This was obtained on a weekly basis. It was measured by dividing the mean feed intake per animals in grams by the mean live weight gain in grams per animal for each replicate.

$$\text{Feed conversion ratio} = \frac{\text{Mean feed intake}}{\text{Mean body weight gain}}$$

3.3.4.6 Methane determination

Kirchgessner *et al.* (1994) Methane (g/d) = 63 + 79 × CF + 10 × NFE + 26 × CP –212 × Cfat (kg/d)

3.3.5 Blood sample collection for haematological parameters and serum biochemistry determination

At the end of the experiment, three (3) animals from each treatment were selected and fasted overnight (12 hours) and bled in the morning (7:00 – 8:00am) to avoid excessive bleeding (Kolo *et al*., 2017). The fasting of animals is to avoid the temporary elevation of blood metabolites following feeding (Jain, 1986). Blood samples were collected from each animals via jagular vein using sterilized disposable 5ml syringe. Two separate sample bottles, in which one contains ethylene diamine tetra – acetic acid (EDTA) and the other one blank were used. The sample bloods coll ected in EDTA were used for haematological studies while the samples in plain bottles were used for serum biochemistry analyses. The samples in the test tubes were centrifuged for five minutes at 1400/rpm so as to separate the serum from the blood for serum biochemical analysis.

3.3.5.1 Haematological parameters

Packed cell volume (PCV), red blood cells (RBC) count, white blood cells (WBC) counts, Leucocytes differential counts and haemoglobin concentration (HB) were determined in accordance with the methods outlined by Bush (1991).

Erythrocyte indices which include the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed in accordance with the standard formulae of Schalm *et al.* (1975) and Jain (1986) as shown below:

$$\text{MCV} = \frac{\text{PCV}}{\text{RBC Count in } 10^6 \text{ /mm3}} \text{ X } \frac{10}{1}$$

$$\text{MCH} = \frac{\text{Hb(g/dl}}{\text{RBC in } 10^6\text{/mm3}} \text{ X } \frac{10}{1}$$

$$\text{MCHC} = \frac{\text{Hb(g/dl)}}{\text{PCV (\%)}} \text{ X } \frac{10}{1}$$

3.3.5.2 Serum biochemical analysis

The blood urea concentrations were estimated by Nessler's reaction (Tanis and Naylor, 1968). Serum total proteins were estimated by the Biuret Method as described by Kohen and Allen (1995). Albumins were determined by Bromo Cresol Green (BCG) method (Peter *et al*., 1982), while globulin concentrations were determined by the difference between total protein and albumin. Aspirate amino transferase (AST or SGOT), Alanine Amino transferase ALT or SGPT), Alkaline Phosphate (ALP) activities were determined using spectrophotometric method, as described by Rej and Hoder (1983). Total bilirubin were determined using orbital techniques as described by Stone (1954).

3.4. Experiment III: Effect of Graded Levels of Groundnut oil on the Performance of Fattening Rams

3.4.1 Experimental animal and their management

Sixteen (16) Uda rams were used for this study. The experimental animals were from the previous experiment. The animals were allowed to rest for 3-4 weeks before the commencement of the trial. The animals received prophylactic treatment against internal and external parasites.

3.4.2 Experimental diets

The gross composition of experimental diet (presented in Table 2). Graded levels of the best plant oil identified from experiment II were used at 0, 15, 30 and 45ml/kg for T1, T2, T3, and T4 respectively. The experimental animals were fed at the rate of 2% body weight while sorghum husk were used as basal diet which was fed *ad libitum*

Table 2 Composition of Experimental Diet supplemented with graded level of groundnut oil

Ingredients (%)	T1 (Control) 0	T2 (oil) 15ml/kg	T3 (oil) 30ml/kg	T4 (oil) 45ml/kg
Maize	20	20	20	20
Soybean	10	10	10	10
Cowpea husk	28	28	28	28
Wheat offal	25	25	25	25
Rice milling waste	16	16	16	16
Salt	1.0	1.0	1.0	1.0
	100	100	100	100
Calculated ME Mcal/kg	2234	2234	2234	2234
Calculated Crude Protein %	14.2	14.2	14.2	14.2
Calculated crude Fibre %	20	20	20	20
Calculated Ether extract (%)	4.1	4.1	4.1	4.1

ME== Metabolizable Energy

3.4.3 Experimental design and feeding

The animals were allotted to 4 treatments; four (4) animals were randomly allocated to each treatment. The animals were housed in individual pens with adequate ventilation. The animals were balanced for weight before commencement of the experiment and weighed weekly thereafter throughout the experimental period. An experimental diet was offered at 2% of body weight while sorghum straws were fed *ad libitum* as basal diet for a period of 84 days (12 weeks). Feed intake were determined as the difference of the amounts of feed offered and the refusals. Water was given *ad libitum*.

3.4.4 Measurements of productive parameters

3.4.4.1 Productive parameters

The performance parameters measured are; feed intake, body weight, body weight gain, feed conversion ratio and cost of feed consumed. Mortality was recorded throughout the experimental period, and post mortem examination was carried to ascertain the cause of death.

3.4.4.2 Feed consumption/intake

Feed consumption from each treatment was measured on daily basis by subtracting left-over from feed served the previous day per group. Adequate measures were taken to guard against spillage and related wastage. The mean daily feed intake per animal was calculated by dividing the amount consumed by the number of animals in each replicate group.

3.4.4.3 Body weight

Rams were weighed on weekly basis using a weighing balance to determine the body weight gain. The mean liveweight of each treatment group was determined by total weight of rams divided by the total number of animals in each replicate group. The animals were fasted for 6 hours before weighing to avoid errors due to gut fill.

3.4.4.4 Body weight gain/week

The body weight gain of each of the treatment group was obtained by calculating the difference between the mean live weights of the current week from the mean live weight of the preceding week. Body weight gain was determined on weekly basis.

3.4.4.5 Feed Conversion Ratio

This was obtained on a weekly basis. It was measured by dividing the mean feed intake per animal in grams by the mean live weight gain in grams per ram for each replicate.

$$\text{Feed conversion ratio} = \frac{\text{Mean feed intake}}{\text{Mean body weight gain}}$$

3.4.4.6 Methane determination

Kirchgessner *et al.* (1994) Methane (g/d) = 63 + 79 × CF + 10 × NFE + 26 × CP –212 × Cfat (kg/d)

3.4.5 Blood sample collection for haematological parameters and serum biochemistry determination

At the end of the experiment, three (3) animals from each treatment were selected and fasted overnight (12 hours) and bled in the morning (7:00 – 8:00am) to avoid excessive bleeding (Kolo *et al*., 2017). The fasting of animals is to avoid the temporary elevation of blood metabolites following feeding (Jain, 1986). Blood samples were collected from each animals jugular vein using sterilized disposable (5ml) syringe. Two separate samples bottle, in which one contains ethylene diamine tetra – acetic acid (EDTA) and

the other one blank were used. The sample bloods collected in EDTA were used for haematological studies while the samples in plain bottles were used for serum biochemistry analyses. The samples in the test tubes were centrifuged for five minutes at 1400/rpm so as to separate the serum from the blood for serum biochemical analysis.

3.4.5.1 Haematological parameters

Packed cell volume (PCV), red blood cells (RBC) count, white blood cells (WBC) counts, Leucocytes differential counts and haemoglobin concentration (HB) were determined in accordance with the methods outlined by Bush (1991).

Erythrocyte indices which include the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed in accordance with the standard formulae of Schalm *et al.* (1975) and Jain (1986) as shown below:

$$\mathrm{MCV} = \frac{\mathrm{PCV}}{\mathrm{RBC\ Count\ in}\ 10^6\ /\mathrm{mm3}}\ \mathrm{X}\ \frac{10}{1}$$

$$\mathrm{MCH} = \frac{\mathrm{Hb(g/dl}}{\mathrm{RBC\ in}\ 10^6/\mathrm{mm3}}\ \mathrm{X}\ \frac{10}{1}$$

$$\mathrm{MCHC} = \frac{\mathrm{Hb(g/dl)}}{\mathrm{PCV}\ (\%)}\ \mathrm{X}\ \frac{10}{1}$$

3.4.5.2 Serum biochemical analysis

The blood urea concentrations were estimated by Nessler's reaction (Tanis and Naylor, 1968). Serum total proteins were estimated by the biuret method as described by Kohen and Allen (1995). Albumins were determined by Bromo Cresol Green (BCG) method (Peter *et al*., 1982), while globulin concentrations were determined by difference

between total protein and albumin. Albumin/globulin ratio were calculated by dividing albumin value by the calculated globulin value. Aspirate amino transferase (AST or SGOT), Alanine Amino transferase ALT or SGPT), alkaline phosphate (ALP) activities were determined using spectrophotometric method, as described by Rej and Hoder (1983). Total bilirubin was determined using orbital techniques as described by Stone (1954).

3.4.6 Rumen liquor and deadenal collection

Rumen liquor and deadenal content were collected. Exactly 10 ml of each rumen liquor and deadenal content were then collected and poured into a sterile container whose pH were immediately recorded. The deadenal content and experimental diets were taken to the Laboratory for determination of fatty acid as outlined in experiment I. The microbial counts of Rumen liquor were also taken as outlined in experiment I.

3.5 Digestibility Trial

At the end of each feeding trial, digestibility trial was conducted using three (3) animals from each treatment. The animals were fed the same experimental diets used in the feeding trial. The digestibility trials lasted for two weeks (one week for the adaptation and one week for total feacal sample collection). Feacal collection bags were used in the sample collection. These were fitted on the first day of adaptation. During the collection period, daily feed intake and total Feacal output from each animal were recorded. After thorough mixing, 5% of the feacal sample were sampled and oven dried at 60^{o}C for dry matter determination and proximate analysis (Coles *et al*., 2005; Rubianti *et al*., 2007).

3.6 Proximate Analysis

Experimental diets and feacal samples from all the experiment were analyzed using Standard analytical methods as outlined by AOAC (1990)

Samples of each diets were analysed for neutral detergent fibre (NDF) with a heat stable α-amylase and sodium sulphite (Mertens, 2002) and for acid detergent fibre (ADF) using method AOAC (AOAC, 1990). Lignins were computed on ADF residues by solubilisation with sulphuric acid (Robertson and Van Soest, 1981). Ash was determined after 2 h of oxidation at 600°C in a muffle furnace using method 942 (AOAC, 1990).

3.7 Statistical Analysis

Data obtained from the three (3) experiments were subjected to Analysis of Variance using Completely Randomized Design (CRD) and significant difference between treatment means were separated using the Duncan`s Multiple Range Test at 5% level. Data on *In-vitro* Gas Production at different periods of time were in line graph chart and data on methane expressed in percentage.

$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$

Where;

Y_{ij}= observation on the i^{th} treatment of the j^{th} random error,

μ = overall mean,

τ_i = fixed effect of dietary treatment,

ε_{ij} = random error

CHAPTER FOUR

4.0 RESULTS

4.1 Experiment I

4.1.1 Proximate composition of the experimental Diets

The Proximate composition of the experimental diets is presented in Table 3. The results of the proximate analysis, showed no significant difference ($P>0.05$) among the parameters measured, except in Ether Extract and Ash whose values were significantly different ($P<0.05$) between the treatment groups.

The ether extract of the diets ranged from 5.78 to 8.16% for T1 and T4 respectively. The highest ether extract content was recoded in T4 diet with groundnut oil inclusion, but treatment 4 differed significantly ($P<0.05$) from treatment 1, diet without oil supplementation, was also similar to treatments supplemented with garlic oil and Soybean.

The ash content ranged between 8.09 in T1 (Control) and 10.11% in T3 (SBO) diets. The control diet (diet without oil inclusion) (T1) had the lowest value of ash while other diets recorded higher and similar values.

Table 3. Proximate composition (%) of the experimental diets containing different plant oil supplementation

Ingredients	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM
DM	88.67	88.19	87.83	87.72	0.23
CP	16.68	17.18	17.39	17.25	0.26
CF	21.25	20.47	20.31	20.18	0.25
EE	5.78^{b}	6.55 ^{ab}	7.32^{ab}	8.16^{a}	0.39
ASH	8.09 ^{b}	10.00^{a}	10.11^{a}	9.99^{a}	0.34
NFE	48.57	45.79	44.87	44.41	0.95
ADF	25.11	24.67	23.44	24.20	0.34
NDF	36.28	35.05	34.32	34.45	0.36
ADL	10.53	10.08	9.79	9.40	0.28
Hemicellulose	10.25	9.97	9.87	9.13	0.34
Cellulose	15.21	15.53	15.67	15.99	0.21
ME (MJ/kg)	11.66	11.64	11.75	11.95	-

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly (P<0.05); DM= Dry matter, CP= Crude protein, CF= Crude fibre, EE= Ether extract, NFE= Nitrogen free extract, ADF = Acid detergent fibre and NDF = Neutral detergent fibre, ADL= Acid detergent lignin,.GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil *ME = Metabolizable Energy Calculated according to the formula of Pauzenga (1985): ME = 37 x % CP + 81 x % EE + 35.5 x % NFE

4.1.2 *In-vitro* gas production at different periods of time in diets supplemented with different plant oils

The *In-vitro* Gas Production at different periods of time in the diets supplemented with different plant oils are presented in Table 4. There were significant differences (P<0.05) between treatment groups at the periods of 12 and 24 hours while no significant difference (*P*>0.05) was observed at 3 and 6 hours. As can be seen from table 4, addition of plant oils consistently decreased the gas production at all incubation times. The *In-vitro* Gas Productions were similar among the treatments, which ranged from 0.37 in T_3, T_4 to 0.70% in T_1 (Control) at 6 hours. The gas production result showed that there was a steady increase over a period of 24h.

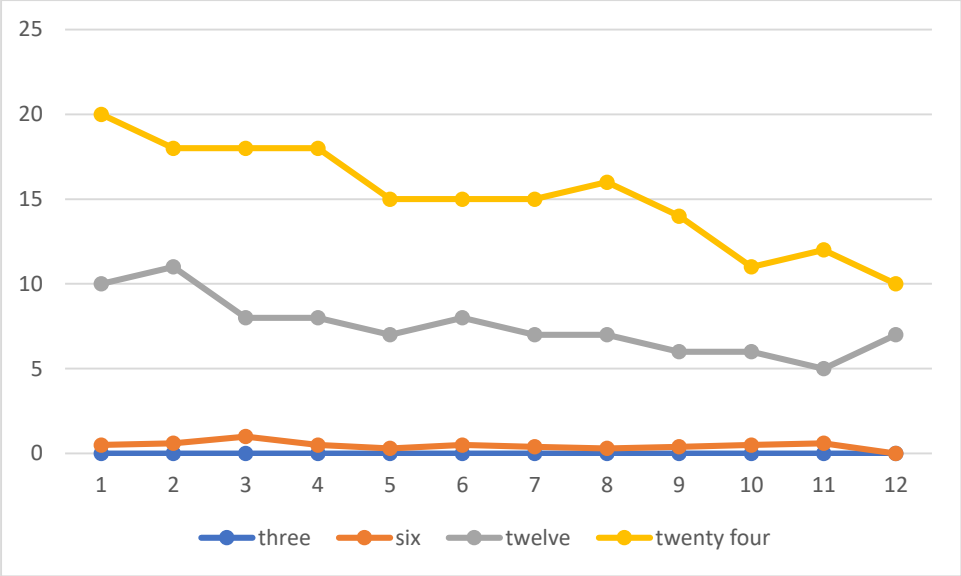

Figure 1: *In-vitro* gas production at different periods of time in diets supplemented with different plant oils

4.1.3 Total *in vitro* gas production and dry matter digestibility in diets supplemented with different plant oils

The Total *in-vitro* gas production, *in vitro* dry matter digestibility and pH of diets supplemented with different plant oils are presented in Table 5. There were significant differences (P<0.05) among the different treatments in terms of total gas production and rumen pH while no significant differences (P<0.05) were observed in the rate of gas production and *in vitro* dry matter digestibility. The Total *in-vitro* gas production ranged from 11.00 to18.67 % in T_4 and T_1 (Control) respectively. The result showed steady decrease in the gas production for diets containing Garlic oil, Soya bean oil and Groundnut oil respectively as compared to the control treatment. The highest total gas production was obtained from the control treatment. The *In-vitro* dry matter digestibility ranged between 47.72 and 48.67 % in T_4 and T_1 respectively. The pH values ranged between 6.00 and 6.47 % in T_4 and T_1 (Control).

Table 4: in *in vitro* gas production and dry matter digestibility of supplemented with different plant oils

Parameters	Treatments				
	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM
Total GP (mL)					
	18.67^{a}	16.00^{b}	15.00^{b}	11.00^{c}	0.89
Rate GP (mL^{-h})	0.78	0.67	0.63	0.45	0.03
IVDMD	48.67	48.19	47.83	47.72	0.22
pH (unit)	6.47^{a}	6.10^{b}	6.10^{b}	6.07^{c}	0.07

SEM = Standard error of means, a, b, c = Means on the same row bearing different superscripts differ significantly (P<0.05); IVDMD = *in vitro* dry matter digestibility, Rate GP = gas production, GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil

4.1.4 *In vitro* fermentation of volatile fatty acid and methane in diets supplemented with different plant oils

The effect of *in vitro* fermentation parameters of diets supplemented with different plant oils is presented in Table 6. The *in vitro* fermentation characteristics measured were not significantly (P>0.05) different on volatile fatty acid (acetate, propionate and butyrate) production while significant (P< 0.05) effect was observed on methane production. The acetate, propionate and butyrate contents were similar among the treatments which ranged between 56.67 in T_4 and 61.00 % in T_1 (Control), 19.00 in T_4 and 20.17 mmol 100 $mmol^{-1}$ in T_1 (Control) and 11.30 in T_1 (Control) and 11.99 mmol 100 $mmol^{-1}$ in T_3 respectively. The VFA (acetate and propionate) production decreased with inclusion of different plant oils (garlic, soybean and groundnut oils) respectively while increased the VFA (butyrate) production. Acetate and propionate ratio were similar among the treatments which ranged between 2.95 in T_3 and 3.03 mmol 100 $mmol^{-1}$ in T_1 (Control).

The CH_4 production differed significantly (P<0.05) between treatment means which ranged between 27.35 and 31.91 mmol in T_4 andT_1 (Control) respectively. The concentration of methane was reduced by the inclusion of oils in the diets. The treatment 4 diet with groundnut oil recorded the lowest methane concentration (27.35 mmol).

Table 5: The effect of *in vitro* fermentation on volatile fatty acid and methane production in diets supplemented different plant oils (mmol 100 $mmol^{-1}$ VFA)

Parameters	Treatments				
	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM
Acetic acid	61.00	59.00	57.67	56.67	0.72
Propionic acid	20.17	20.00	19.67	19.00	0.36
Butyric acid	11.30	11.51	11.99	11.54	0.34
A:P	3.03	2.96	2.95	2.99	0.06
Methane (mmol)	31.91^{a}	29.59^{ab}	28.58^{b}	27.35^{b}	0.50
Percentage of Methane reduction	00.00	7.27	10.44	14.29	-

SEM = Standard error of means, a, b= Means on the same row bearing different superscripts differ significantly (P<0.05); GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil; VFA== volatile fatty acid; A:P = Acetic acid and Propionic acid ratio

4.1.5 Morphological identification of isolated micro-organism in *in vitro* techniques in diets of sheep supplemented with different plant oils

The Morphological identification of isolated micro-organisms in the diets supplemented with different plant oils using invitro fermentation were shown in Table 7. The result showed isolated microbes in liquid were made up of gram positive and gram negative bacteria which comprises cellulolytic, proteolytic, amylolytic and lactic acid bacteria. Morphological identification of isolated micro-organism in *in vitro* techniques supplemented all treatment have these species *Ruminococcus albus Escherichia coli, Streptococcus foecolis, Corynebacterium spp Aspergillus niger, Saccharomyces cerevisiae, Entodinium Diplodium* and *Isotricha* while treatment with plant oils were *Escherichia coli, Streptococcus foecolis, Corynebacterium spp,*

Rhizopus stolonifera, Aspergillus niger, Bacteriode succinogenes, Butyrivibrio fibrisolvens, Lactobacillus acidophilus, Aspergillus favus Saccharomyces cerevisiae, Entodinium Diplodium and *Isotricha* micro-organism are present diets supplemented with different plant oils (garlic oil, soybean oil and groundnut oil diets) and control with exception of *Bacteriode succinogenes, Butyrivibrio fibrisolvens, Lactobacillus acidophilus, Aspergillus favus* (diet without oil supplementation).

Table 6: Micro-organisms isolated from the rumen liquor of sheep fed diets supplemented with different plant oils

Treatments	Micro-organism isolation in rumen liquid
T1 (Control)	*Fibrobacter succinogenes, , Rhizopus stolonifera,*
T2 (GLO)	*Bacteriode succinogenes, Butyrivibrio fibrisolvens, and Aspergillus favus,*
T3 (SBO)	*Lactobacillus acidophilus, Bacteriode succinogenes, Butyrivibrio fibrisolvens, Bacillus subtilis, Rhizopus stolonifera and Aspergillus favus*
T4 (GNO)	*Lactobacillus acidophilus, Bacteriode succinogenes, Butyrivibrio fibrisolvens, Entodinium Diplodium* and *Isotricha, Bacillus subtilis,Proteus Mirabilis, Rhizopus stolonifera, and Aspergillus favus,*

GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil

4.1.6 Effect of different plant oils supplementation on the microbial populations in the rumen of sheep

The Effect of different plant oils supplementation on the microbial population of sheep was given in Table 8. The Effect of different Plant oils supplementation on microbial population had no significant ($P>0.05$) effect among the treatment groups. The bacteria counts ranged between 9.56 and 10.09 in T_4 andT_1 (Control) respectively. The Protozoa counts ranged between 3.88 and 4.16 in T_4 and T_1 (Control) respectively. The Fungi counts ranged between 0.76 and 1.18 in T_3 and T_4 respectively.

Table 7: Effect of different plant oils supplementation on rumen microbial population in sheep

Parameters	Treatments				
	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM
Total bacteria (ll CFU 10^9)	10.09	9.91	9.84	9.56	0.11
Protozoa (log Cell ml^{-1})	4.15	4.05	3.84	3.88	0.12
Fungi (log Cell ml^{-1})	1.17	0.97	0.76	1.18	0.12

SEM = Standard error of means, a, b = Means on the same row bearing different superscripts differ significantly (P<0.05); GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil

4.1.7 Fatty acid (FA) composition of the diets supplemented with different plant oils

The effect of Fatty acid (FA) composition of the diets supplemented with different plant oils is presented in Table 9. The effects of plant oils on the concentrations of total FA, 18:1 cis11, 18:1 cis9 and trans18 values were not significantly ($P > 0.05$) different between the means, while other parameters measured were significantly different (P<0.05) among the treatments. The different plant oils, had significant (P<0.05) effects on 18:00, 18:2, 18:3, total C18:0 concentrations. The 18:00 concentration was low in diets supplemented with plant oils while Linolenic acid (18:3), Linoleic acid (18:2) and

Total C18:0 concentration was high in the diets supplemented with plant oils (garlic oil, soybean oil and groundnut oil diets) respectively.

Table 8: Fatty acid composition of the dietary treatments with different plant oils

Time(mL hours)	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM
Total FA, % of DM	2.03	2.09	2.10	2.12	0.01
Individual FA, g/00 g FA					
Stearic acid (18:00)	10.20[a]	8.57[b]	8.45[b]	8.39[b]	0.23
Vaccenic acid 18:1 cis11	1.46	1.43	1.33	1.26	0.05
Oleic acid 18:1 cis9	20.53	19.60	19.57	19.26	0.24
trans18	0.57	0.61	0.70	0.72	0.05
linoleic (C18:2 *n*-6) acid	37.26[b]	38.96[ab]	39.90[a]	40.30[a]	0.43
linolenic (C18:3 *n*-3) acids	9.63[b]	10.23[a]	10.70[a]	10.74[a]	0.15
Total c18	46.90[b]	49.20[a]	50.60[a]	51.04[a]	0.56

SEM = Standard error of means, a, b = Means on the same row bearing different superscripts differ significantly ($P<0.05$); GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil

4.1.8 Effect of Plant oils on fatty acid metabolism

The effect of flow fatty acid and biohydrogenation of long-chain fatty acids (FA) in diets supplemented with different plant oils was presemted in Table 10. The effects of plant oils on the concentrations of 18:1 cis11, trans18, CLA and BH 18:1cis were not significantly ($P > 0.05$) different in experiment diets, while 18:00, 18:1 cis9, 18:2, 18:3, total C18:0, bh18:2, bh18:3 and bh total C18:0 concentrations differed significantly ($P<0.05$) among the treatments. The 18:00, 18:1 cis9, 18:2, 18:3 and total C18:0 were all increased across diets supplemented with plant oils (garlic oil, soybean oil and groundnut oil diets) respectively. Biohydrogenation concentration was highest in diet without oil supplementation.

Table 9: Flow of fatty acid and biohydrogenation of long-chain fatty acids in diets supplemented with different plant oils

Time(mL hours)	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM
Flow, mg/d					
Stearic acid (18:00)	502.00^{b}	558.66^{a}	545.33^{ab}	589.66^{a}	11.31
Vaccenic acid (18:1 cis11)	182.66	204.66	221.00	218.33	9.25
Oleic acid 18:1 cis9	154.33^{b}	167.66^{a}	170.00^{a}	175.66^{a}	2.60
trans18	14.26	14.57	14.60	14.86	0.23
linoleic (C18:2) acid	160.33^{b}	178.66^{a}	179.66^{a}	189.66^{a}	3.53
linolenic (C18:3) acids	61.93^{b}	85.96^{a}	89.56^{a}	90.96^{a}	3.95
Total c18	222.26^{c}	264.63^{b}	269.23^{ab}	280.63^{a}	6.87
Conjugate linoleic acid	15.26	15.57	15.60	15.86	0.23
Biohydrogenation					
Vaccenic acid (18:1cis)	67.07	66.38	65.91	65.57	0.34
linoleic (C18:2) acid	65.82^{a}	63.66^{ab}	64.49^{ab}	62.82^{b}	0.45
linolenic (C18:3) acids	86.73^{a}	82.51^{b}	82.29^{b}	82.17^{b}	0.71
Total C18	52.55^{a}	46.17^{b}	46.78^{b}	45.00^{b}	1.02

SEM = Standard error of means; a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil, BH = Biohydrogenation, calculated as percentage of C18 FA that disappeared between intake and effluent flow (Tice *et al*., 1994); total C18 FA were weighted for the number of unsaturated bonds.

4.2 Experiment II

4.2.1 Growth performance characteristics of Uda lamb supplemented with diet containing different plant oils

Result of the growth performance of Uda lamb supplemented with different plant oils is shown in Table 11. The results of growth performance showed that there was significant difference ($P<0.05$) between treatment means in final weight, basal feed intake and dry matter intake as % Body weight, feed conversion ratio while average daily gain, dry matter intake, supplement feed intake and methane were not significantly (P>0.05) different between the treatments groups.

Body weight

Final body weight of rams supplemented with different plant oils was significantly different (P<0.05) among the treatments groups. The final body weight of the animals for T2 diet with garlic oil, T3 diet with soybean oil and T4 diet with groundnut (24.37, 25.37 and 27.50 kg) were statistically similar (P>0.05) with all the treatments supplemented with garlic oil, Soybean oil and Groundnut oil respectively. However, T4 diet with groundnut differed significantly (P<0.05) from T1 control diet.

Feed Intake

Basal feed intake (kg DM/week) of lambs fed the control diet (without oil supplementation) (2.30 kg) and treatment 3 and 4 diets with Soybean oil and Groundnut oil were similar (P>0.05) but treatment 1 control diet with were significantly differed (P<0.05) compared to diet with garlic oil supplemented i.e treatment 2 (2.77 kg). Basal feed intake of lambs on the diet with garlic oil supplemented was not difference significantly from the feed treatment 3 diets with Soybean oil and treatment 4 diets with groundnut oil (P>0.05).

Dry matter intake as % body weight of ram-lambs supplemented with different plant oils significantly differed ($P<0.05$) among the treatments groups. Treatment 2 diets with garlic oil (3.33%) was higher for dry matter intake as % body weight compared to those in treatment 1 control diet (3.18 %) and then treatment 3 diets with soybean oil (3.12 %) while the lowest intake as % body weight value was recorded in treatment 4 diets with groundnut oil (2.97%).

Feed conversion ratio of lambs supplemented with different plant oils were 12.88, 12.35, 10.62 and 6.95 for T1 control diet, T2 diets with garlic oil, T3 diets with soybean oil and T4 diets with groundnut oil respectively.

Percentage of CH_4 reduction of ram-lambs supplemented with different plant oils. Treatment 2 diets with garlic oil (30.29%) and then treatment 3 and 4 diets with soybean and groundnut oils (55.95 and 66.11 %).

Table 10: Effects of Supplementing With Different Plant Oils on Growth Performance of Growing Lambs

Parameters	**T1 (Control)**	**T2 (GLO)**	**T3 (SBO)**	**T4 (GNO)**	**SEM**
Initial weight (kg)	18.75	18.75	19.12	18.50	0.44
Final weight (kg)	23.75^{b}	24.37^{ab}	25.37^{ab}	27.50^{a}	0.61
Average daily gain (g/day)	59.52	66.96	66.96	77.38	7.36
Basal intake (g/day)	328.86^{b}	396.42^{a}	358.33^{ab}	355.05^{ab}	10.62
Supplement intake (g/day)	426.45	415.40	430.42	464.50	13.19
Total Dry matter intake (g/day)	755.31	811.82	788.75	819.55	18.48
Dry matter intake as % Body weight	3.18^{ab}	3.33^{a}	3.12^{ab}	2.97^{b}	0.06
Feed convention ratio (feed:gain)	12.88^{a}	12.35^{b}	10.62^{c}	6.95^{d}	0.69
CH_4 (KJ/day)	535.50^{a}	373.28^{b}	236.16^{c}	181.49^{d}	41.27
Percentage of CH_4 reduction	0.00	30.29	55.95	66.11	--

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil; CH_4= methane

4.2.2 Economic production of growing lambs fed diet containing different plant oils

Economic production of growing lambs fed diet containing different plant oils is presented in Table 11. Cost of feed consumed was 35.67, 45.15, 41.24 and 43.06N/day for lambs fed diets 1, 2, 3 and 4, respectively. Cost of feed per body weight gain The

inclusion of plant oils decrease the cost of feed per Kg. it was more expensive to compound diet control diet (667.00 N/Kg) followed by diet 2 (645.00 N/Kg), diet 3 (589.13N/Kg), and diet 4 (391.45N/Kg).

Table 11: Economic production of growing lambs fed diet containig different plant oils

	Treatments/Diets			
Parameters	1	2	3	4
Initial body weight (kg)	18.75	18.75	19.12	18.50
Final body weight (kg)	23.75	24.37	25.37	27.50
Supplement intake (kg/day)	0.43	0.42	0.43	0.46
Basal intake (kg/day)	0 .33	0.40	0.36	0.36
Cost of supplement diet (₦/kg)	67.46	88.46	79.16	77.96
Cost of basal diet (₦/kg)	20.00	20.00	20.00	20.00
Total weight gain (kg)	5.00	5.62	6.25	9.00
Total weight gain (kg/day)	0.06	0.07	0.07	0.11
supplement diet cost (₦/kg/day)	29.01	37.15	34.04	35.86
basal diet cost (₦/kg/day)	6.66	8.00	7.20	7.20
Total Feed cost (₦/kg/day)	35.67	45.15	41.24	43.06
Cost per Kg gain (₦/kg)	667.00	645.00	589.13	391.45

Key: T1= control, T2=garlic oil, T3= Soya bean oil and T4= Groundnut meal oil
* Cost per kilogram of the various ingredients used in compounding the experimental diets: soghum husk ₦ 20.00, Maize ₦ 84.00; soya bean, ₦180.00; wheat offal ₦ 60.00; cowpea husk, ₦ 40.00; rice milling waste, ₦10.00; salt, ₦20.00, garlic oil, ₦ 0.70,soybean oil, ₦ 0.39, groundnut oil, ₦ 0.35,

4.2.3 Heamatological parameters

The heamatological parameters of growing lambs supplemented with diets containing different plant oils is shown in Table 12. There were significant (P<0.05) differences among the treatments in Packed Cell Volume (PCV) and Haemoglobin (Hb), while Red Blood Cell value (RBC), White Blood Cell count (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean

Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell differential (with the exception of Monocytes (%)) count whose values were not significantly influenced ($P>0.05$) by treatment. However, all the parameters measured are within the reference values with the exception of MCH, which is slightly higher than reference value.

The PCV value of the growing lambs supplemented with diet containing different plant oils ranged from 31.66 to 35.33%. The Hb values of the growing lambs supplemented with different plant oils ranged from 10.55 to 11.78 g/dl. The highest Hb values of the sheep was recorded in T4 diets with groundnut oil while T1 control diet recorded the lowest values. The RBC count ranged from 8.66 to 9.00g/dl. The RBC results from all treatment were comparable to the control.

The Monocytes values was only recorded (0.66%) on lambs fed diets supplemented with garlic oil (T2) while other treatments were 0.00 %.

Table 12 Influence of plant oils supplementation on hematological indices of growing lambs

Parameters	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM	Reference values* *
Packed Cell Volume (%)	35.33[a]	33.00[ab]	32.66[ab]	31.66[b]	0.56	27-45
Haemoglobin g/dl	11.78[a]	11.00[ab]	10.89[ab]	10.55[b]	0.18	9-15
Red Blood Cell g/dl	9.00	9.00	8.66	9.00	0.22	9-15
White Blood Cells 10^9/L	8.53	8.33	7.90	8.00	0.58	4-12
MCV (fl)	39.54	36.93	37.91	32.22	1.22	28-40
MCH (pg)	13.18	12.24	12.64	11.74	0.31	8-12
MCHC (%)	32.22	33.33	33.13	33.33	0.61	31-34
WBC Differentials						
Lymphocytes (%)	62.3	59.00	68.33	59.66	3.54	2-90
Neutrophils (%)	37.66	39.33	30.66	40.00	3.41	
Monocytes (%)	0.00[b]	0.66[a]	0.00[b]	0.00[b]	0.11	0-0.8
Eosinophils (%)	0.00	0.66	0.00	0.66	0.22	0-1.0
Basophils (%)	0.33	0.00	0.33	1.00	0.19	0-3

SEM = Standard error of means; a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Haemoglobin; MCHC=Mean Corpuscular Haemoglobin Concentration; * *source = (Elmhurst *et al*., 2002)

4.2.4 The serum biochemistry

The serum biochemistry of growing lambs supplemented with different plant oils in diets are presented in Table 13. The Conjugated Bilirubin, blood urea, Creatinine, Aspartate Aminotransferase (AST), Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein (HDL) of growing lambs supplemented with diet containing different plant oils were significantly ($P<0.05$) different among the treatment groups. However, Total Bilirubin, Total protein, Albumin, Glucose, Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP) and Low Density Lipoprotein (LDL) were not statistically ($P>0.05$) different among the treatment groups. All the parameters measured were within the reference values (presented in table 13) with the exception of total protein, ALT and TG.

The Conjugated Bilirubin values of the growing lambs ranged from 0.05 to 0.13 mg/100ml. However T4 diets in which Groundnut oil supplementation was lowest of all other treatment groups was significantly lower than diets supplementated with soybean oil.

The blood urea values of the growing lambs supplemented with diets containing different plant oils ranged from 5.40 to 6.83 mg/dl. The highest Blood urea values was recorded in T1 control diet while the lowest was obtained in T2 diet with garlic oil.

The Creatinine values of the growing lambs ranged from 0.90 to 1.43 mg/dl. The values for treatment 1 (diet without oil) was significantly differed ($P<0.05$) among the treatment group with treatment 2 and 4 diets with garlic and groundnut oil supplemented.

The AST values of the growing lambs supplemented ranged from 109.33 to 134.66 iu/L. The Total Cholesterol value of the growing lamb supplemented with diet

containing different plant oils ranged from 1.38 to 1.76mmol/L for T1 control diet and T4 diet with groundnut oil respectively. The Triglyceride of the growing lambs supplemented with different plant oils ranged from 0.06 to 0.14mmol/L. The highest Triglyceride values was obtained in T3 and then followed by those in 4, 2 and then 1 treatments for diets supplemented with Soybean oil, Groundnut oil, garlic oil and control respectively. The HDL value of the growing lambs supplemented with different plant oils ranged from 0.40 to 1.21mmol/L. HDL values of sheep was lower for those in treatment 1 control diet compared to the highest value which was recorded in T4 diet groundnut oil.

Table 13 Influence of Plant oils supplementation on Serum biochemical parameters of growing lambs

Parameters	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM	Reference values**
Total Bilirubin (mg/100ml)	0.95	1.15	1.00	0.73	0.07	1.71-8.55
Conjugated Bilirubin (mg/100ml)	0.11^{ab}	0.10^{ab}	0.13^{a}	0.05^{b}	0.01	0 - 4.61
Total protein (g/dl)	5.66	5.53	5.53	6.10	0.15	6-7.9
Albumin (g/dl)	2.43	2.56	2.50	2.86	0.08	2.4-3
Glucose (mg/dl)	3.20	3.40	4.83	3.20	0.37	1.7-3.6
UREA (mmol/l)	6.83^{a}	5.40^{b}	6.66^{ab}	6.10^{ab}	0.21	3-10
Creatinine (mg/dl)	1.43^{a}	0.90^{b}	1.20^{ab}	0.90b	0.08	0.79-1.19
AST (iu/l)	109.33^{b}	134.66^{a}	116.33^{ab}	130.66^{ab}	4.26	60-280
ALT (iu/l)	19.66	22.00	19.66	21.33	0.93	22-38
ALP (iu/l)	53.66	48.66	69.66	57.33	4.82	70-390
TC mmol/l	1.38^{b}	1.68^{a}	1.72^{a}	1.76^{a}	0.05	1.05-1.5
TG mmol/l	0.06^{b}	0.11^{ab}	0.14^{a}	0.13^{a}	0.01	0.5-2.8
HDL mmol/l	0.40^{b}	0.81^{ab}	0.94^{ab}	1.21^{a}	0.12	0.8-2.6
LDL mmol/l	0.60	0.63	0.76	0.69	0.03	0.5-4.3

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil, AST – Aspartate Aminotransferase, ALT – Alanine Aminotransferase. ALP- Alkaline phosphatase TC= Total cholesterol TG= Trigleceride HDL-high density lipoprotein, LDL- low density lipoprotein * *source = (Elmhurst *et al*., 2002)

4.2.5 Nutrient digestibility of lambs Fed different plant oils

The results of nutrient digestibility of lambs supplemented with diet containing different Plant oils are shown in Table 14. Result shows that there was no significant (P>0.05) difference in Dry matter, Ash and Acid detergent lignin digestibilities between treatment means, while Crude protein, Crude fibre, Ether extract, Acid detergent fibre, Neutral detergent fibre, Hemicellulose and Cellulose digestibilities were significantly influenced (*P*<0.05) by treatments (garlic, soybean and groundnut oils).

The CP digestibility in T4 diet with groundnut oil recorded the highest value of 82.80%, while T1 control diet had the lowest value of 76.40%. The CP digestibility tended to increase in sequential order from T1 control diet to T4 diet with groundnut oil reflecting an increase in the level of DM in the diets. The CP digestibility values obtained in this study followed the same trend with DM digestibility.

The CF digestibility increased progressively across treatment groups which ranged from 36.36 to 54.38 % for T1 to T4 respetively. The CF digestibilities of diets with oils supplementation were higher than the control group.

The EE digestibility ranged from 97.21 % to 98.21 % with T2 having the highest value and is comparable to treatments 1 and 3 but significantly (*P*<0.05) different from treatment 4. Treatment 4 was similar (P<0.05) to treatments 1 and 3.

The ADF digestibility showed significant differences (P < 0.05) among treatments. Lambs fed on the T1 control diet recorded the lowest value of 29.41. The NDF digestibility ranged from 42.81 % to 60.92 % for T1 control diet and T4 diet with groundnut oil respectively. Treatment T4 diet with groundnut oil recorded the highest value. It was observed that NDF digestibility was higher with oils supplementation in the diet. The Hemicellulose digestibility T4 diet with groundnut oil recorded the highest

value of 71.87 % which contained groundnut oil supplementation, while T1 control diet had the lowest value of 57.06 %. The Cellulose digestibility in T4 diet with groundnut oil recorded the highest value of 63.23 % which contained groundnut oil supplementation, while T1 control diet had the lowest value of 40.32b %. The oil supplementation treatments were higher and comparably the same ($P > 0.05$) among treatment groups than control treatment groups. The Cellulose digestibility values obtained in this study followed the same pattern with Hemicellulose digestibility.

Table 14 Influence of plant oils supplementation on the nutrient digestibility in growing lambs

Parameters	T1 (Control)	T2 (GLO)	T3 (SBO)	T4 (GNO)	SEM
DM	63.49	70.94	70.39	71.00	1.49
CP	76.40^{b}	82.31^{a}	80.61^{ab}	82.80^{a}	1.04
CF	36.36^{b}	44.74^{ab}	48.05^{ab}	54.35^{a}	2.71
EE	97.86^{ab}	98.19^{a}	97.66^{ab}	97.21^{b}	0.13
ASH	79.87	82.41	82.41	81.12	0.85
ADF	29.41^{b}	44.73^{ab}	44.99^{ab}	52.71^{a}	3.28
NDF	42.81^{b}	53.56^{ab}	54.61^{ab}	60.92^{a}	2.60
ADL	49.98	61.66	61.84	61.44	2.17
HCL	57.06^{b}	68.68^{a}	70.44^{a}	71.87^{a}	2.17
Cellulose	40.32^{b}	55.89^{a}	57.00^{a}	63.23^{a}	3.10

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly (P<0.05); DM= Dry matter, CP= Crude protein, CF= Crude fibre, EE= Ether extract, ADF = Acid detergent fibre and NDF = Neutral detergent fibre, ADL= Acid detergent lignin, HCL=Hemicellulose, CELL= Cellulose.GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil

4.3 Experiment III

4.3.1 Proximate composition (%) of the experimental diets containing graded levels of groundnut oils

The Proximate composition of the experimental diets for fattening Uda rams are presented in Table 15. The results of dry matter (DM), crude fibre (CF), ether extract (EE) Nitrogen free extract (NFE) and Calculated Metabolizable Energy (ME) content were significantly different ($P<0.05$) between treatment means, while the other parameters measured were not significantly different ($P>0.05$) among the treatment groups.

The dry matter content ranged between 89.12 in T_3 diet with 30ml/kg of groundnut oil and 89.53 % in T_1 control diet. The dry matter content of diets without oils supplementation was higher compared to the control treatment group while those with oils supplementation were similar ($P>0.05$) among the treatments. The Crude Fibre content varied from 26.15 to 27.18 %. The lowest CF content was recorded in treatment 4 diet with 45ml/kg of groundnut oil while T_1 control diet had the highest value. The CF content of the diets decreased as the level of supplemention with groundnut oils. The ether extract of the diets ranged from 5.65 to 8.13%.

Table 15 Proximate composition (%) of the experimental diets with graded levels of groundnut oil fed to fattened Uda rams.

Parameters	T1 (Control)	T2 (15)	T3 (30)	T4 (45)	SEM
DM	89.53[a]	89.22[b]	89.12[b]	89.30[b]	0.054
CP	13.99	14.15	14.25	14.37	0.07
CF	27.18[a]	26.34[b]	26.31[b]	26.15[b]	0.15
EE	5.65[d]	7.20[c]	7.75[b]	8.13[a]	0.28
ASH	9.24	9.40	9.80	10.12	0.25
NFE	43.92[a]	42.90[ab]	41.87[b]	41.22[b]	0.39
ADF	36.57	36.44	36.18	35.70	0.14
NDF	48.66	48.61	48.56	48.12	0.17
ADL	11.24	11.25	11.28	11.32	0.01
Hemicellulose	8.35	8.38	8.41	8.41	0.05
Cellulose	25.53	25.59	25.76	26.18	0.22
ME (MJ/kg)	10.53	10.92	10.97	11.03	-

SEM = Standard error of means; a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); DM= Dry matter, CP= Crude protein, CF= Crude fibre, EE= Ether extract, NFE= Nitrogen free extract, ADF = Acid detergent fibre and NDF = Neutral detergent fibre, ADL= Acid detergent lignin, GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil *ME = Metabolizable Energy Calculated according to the formula of Pauzenga (1985): ME = 37 x % CP + 81 x % EE + 35.5 x % NFE

4.3.2 Performance characteristics of fattened Uda rams on diets containing graded levels of groundnut oil

The result of the performance characteristics of fattened Uda ram supplemented with diet containing different level of groundnut oil are shown in Table 16. The results showed there was significant difference ($P<0.05$) between treatment means in the final body weight, average daily gain and dry matter intake as % of body weight, while total dry matter intake, basal feed intake Supplement feed intake and methane were not significantly ($P>0.05$) different among the treatments group.

The final body weight of the fattened Uda rams ranged from 29.30 to 33.00 for treatments 1 control diet and 4 diet with 45ml/kg of groundnut oil respectively. Treatment T2 diet with 15ml/kg, 3 diet with 30ml/kg and 4 diet with 30ml/kg of groundnut oil were similar but treatment 4 diet with 45ml/kg of groundnut oil differed significantly ($P<0.05$) from treatment 1 (diet without oil supplementation), however treatment 1 (diet without oil supplementation) was also similar ($P>0.05$) to treatments 2 diet with 15ml/kg and 3 diet with 30ml/kg. The Average daily gain of the Uda rams for treatments 4 diet with 45ml/kg (101.19 g/day) recorded significantly ($P<0.05$) the highest, while lowest average daily gain was observed on treatment 1 control diet (58.82g/day). The Dry matter intake as % Body weight of Uda rams ranged from 3.19 to 3.41 % for treatment T4 diet with 45ml/kg and T1 control diet respectively. Feed conversion ratio values were 16.11, 14.94, 12.96 and 10.51 for treatment 1 control diet, 2 diet with 15ml/kg, 3 diet with 30ml/kg and 4 diet with 45ml/kg respectively. The Percentage of CH_4 reduction of Uda rams were 49.77, 67.24 and 79.74 % for treatment T2, 3 and 4 diet with 15, 30 and 45ml/kg respectively

Table 16: Effect of supplementing graded levels of groundnut oil on fattened performance Uda rams.

Parameters	T1 (Control)	T2 (15ml)	T3 (30ml)	T4 (45ml)	SEM
Initial weight (kg)	24.37	24.62	24.50	24.50	0.37
Final weight (kg)	29.30^{b}	30.50^{ab}	31.25^{ab}	33.00^{a}	0.71
Average daily gain (g/day)	58.82^{c}	69.94^{b}	80.35^{ab}	101.19^{a}	7.14
Basal intake (g/day)	522.50	552.50	565.00	582.50	11.93
Supplement intake (g/day)	447.50	472.5	472.50	470.00	13.32
Total Dry matter intake (g/day)	998.12	1025.00	1037.50	1052.5	20.79
Dry matter intakeas % Body weight	3.41^{ab}	3.36^{a}	3.32^{ab}	3.19^{b}	0.06
Feed convention ratio (feed:gain)	16.11	14.94	12.96	10.51	0.64
CH_4 (KJ/day)	718.16^{a}	360.76^{b}	235.29^{c}	145.49^{d}	65.67
Percentage of CH_4 reduction	0.00	49.77	67.24	79.74	--

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); CH_4= methane

4.3.3 Economic production of fattened rams fed diet containing graded groundnut oils

Economic production of growing lambs fed diet containing different plant oils is presented in Table 11. Cost of feed consumed were 38.66, 42.98, 45.55 and 48.51 N/day for lambs fed diets 1, 2, 3 and 4, respectively. Cost of feed per body weight gain The increase in inclusion of groundnut oil decrease the cost of feed per Kg. it was more expensive to compound diet control diet (644.33 N/Kg) followed by diet 2 (614.00 N/Kg), diet 3 (569.38 N/Kg), and diet 4 (485.10N/Kg).

Table 17: Economic production of fattened rams fed diet containing graded levels of groundnut oil

	Treatments/Diets			
Parameters	1	2	3	4
Initial body weight (kg)	24.37	24.62	24.50	24.50
Final body weight (kg)	29.30	30.50	31.25	33.00
Supplement intake (kg/day)	0.45	0.47	0.47	0.47
Basal intake (kg/day)	0.52	0.55	0.57	0.58
Cost of supplement diet (₦/kg)	62.80	68.05	73.30	78.55
Cost of basal diet (₦/kg)	20.00	20.00	20.00	20.00
Total weight gain (kg)	4.93	5.88	6.25	8.50
Total weight gain (kg/day)	0.06	0.07	0.08	0.10
supplement diet cost (₦/kg/day)	28.26	31.98	34.45	36.91
basal diet cost (₦/kg/day)	10.4	11.00	11.40	11.60
Total Feed cost (₦/kg/day)	38.66	42.98	45.55	48.51
Cost per Kg gain (₦/kg)	644.33	614.00	569.38	485.10

Key: T1= control, T2=15ml, T3= 30ml and T4= 45ml of Groundnut meal oil

* Cost per kilogram of the various ingredients used in compounding the experimental diets: Soghum husk ₦ 20.00, Maize ₦ 84.00; soya bean, ₦180.00; Wheat offal ₦ 60.00; Cowpea husk, ₦ 40.00; Rice milling waste, ₦10.00; salt, ₦20.00; Groundnut oil, ₦ 0.35 per ml,

4.3.3 Heamatological parameters of Uda rams fed diets containing graded levels of groundnut oil

The heamatological parameters of fattened Uda rams supplemented with different levels of groundnut oils are shown in Table 17. There were significant (P<0.05) differences among the rams fed graded levels of groundnut oil in Packed Cell Volume (PCV) values, Haemoglobin (Hb) values, Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) while Red Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell differential count values were not significantly influenced (P>0.05) by levels of groundnut oil treatments and all the parameters measured are within the reference values.

The PCV value of the growing fattening Uda rams supplemented with different levels of groundnut oil ranged from 31.16 to 35.50 % for T4 diet with 45ml/kg and T1 control diet respectively.

The Hb values of the fattening Uda rams supplemented with different levels of groundnut oils were ranged from 10.50 to 11.83 g/dl. The highest value was recorded rams on treatment 1control diet. The haemoglobin values differed significantly (P<0.05).among treatment groups while treatment 3 diet with 30 ml/kg and 4 diet with 45ml/kg were similar (P>0.05).

The Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) values ranged from 29.87 to 38.13 (fl) of MCV, 9.95 to 12.71 (pg) MCH for fattening Uda rams supplemented with different levels of groundnut oil.

Table 18: Influence of graded levels of Groundnut Oil Supplementation on Hematological Indices of fattened Uda rams

Parameters	T1 (Control)	T2 (15ml)	T3 (30ml)	T4 (45ml)	SEM	Reference values**
Packed Cell Volume (%)	35.50^{a}	33.00^{b}	31.16^{c}	31.50^{c}	0.53	27-45
Haemoglobin g/dl	11.83^{a}	11.00^{b}	10.50^{c}	10.50^{c}	0.17	9-15
Red Blood Cell g/dl	9.33	9.50	10.55	10.50	0.21	9-15
White Blood Cells x 10^{9}/L	9.00	9.33	9.66	9.10	0.35	4-12
MCV (fl)	38.13^{a}	33.26^{b}	29.87^{b}	30.03^{b}	1.17	28-40
MCH (pg)	12.71^{a}	11.61^{a}	9.95^{b}	10.01^{b}	0.38	8-12
MCHC (%)	33.33	33.33	33.33	32.22	0.27	31-34
WBC Differentials						
Lymphocytes (%)	34.33	39.00	44.66	46.66	2.46	2-90
Neutrophils (%)	63.33	58.66	53.00	51.33	2.23	
Monocytes (%)	0.66	0.66	0.00	0.00	0.26	0-0.8
Eosinophils (%)	0.00	0.00	0.00	0.33	0.08	0-1.0
Basophils (%)	0.33	0.66	1.00	0.00	0.14	0-3

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Haemoglobin; MCHC=Mean Corpuscular Haemoglobin Concentration; * *source = (Elmhurst *et al*., 2002)

4.3.4 The serum biochemistry of fattened Uda rams fed diet containing graded levels of groundnut oil

The serum biochemistry of fattened Uda rams supplemented with different levels of groundnut oils are shown in Table 18. The results indicated that high density lipoprotein (HDL), Total Cholesterol and Triglyceride were significantly ($P<0.05$) different among the rams on treatment groups while all the other parameters measured (Total Bilirubin, Direct Bilirubin, Total protein, Albumin, blood urea, Creatinine, Glucose, Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline phosphatase, and LDL) were not statistically ($P>0.05$) different among the rams on treatment groups and all the parameters measured were within the reference values with the exception of Total Bilirubin, TC andTG which is slightly lower than reference values.

The Total Cholesterol value of the fattened Uda rams supplemented with diet containing different levels of groundnut oil ranged from 1.41 to 1.74 mmol/l. lowest values of Total Cholesterol was obtained in treatment 4 groundnut oil supplementation while treatment T1 control diet had the highest values.

The Triglyceride value of the fattened Uda rams supplemented with diet containing different levels of groundnut oil ranged from 0.06 to 0.14 mmol/l. The Triglyceride values was higher for those in treatment 4 diet with 45ml/kg, followed by those in treatment 3 diet with 30ml/kg, then by those in treatment 2 diet with 15ml/kg and least in rams on treatment 1 diet without oil supplementation. The HDL value of the fattening Uda rams supplemented with diet containing different levels of groundnut oil ranged from 0.60 to 1.25 mmol/l. HDL values of sheep was lower for those in treatment 1 (control) without oil supplementation while treatment 4 diet with 45ml/kg diets with highest oil supplementation record highest value among the treatment groups.

Table 19: Influence of graded levels of groundnut oil supplemention on Serum biochemical parameters of fattened Uda rams

Parameters	**T1 (Control)**	**T2 (15ml)**	**T3 (30ml)**	**T4 (45ml)**	**SEM**	Reference values**
Total Bilirubin (mg/100ml)	0.69	0.71	0.60	0.71	0.04	1.71-8.55
Conjugated Bilirubin (mg/100ml)	0.07	0.06	0.05	0.06	0.01	0 - 4.61
Total protein (g/dl)	5.86	5.90	6.23	6.93	0.23	6 - 7.9
Albumin (g/dl)	2.63	2.70	2.86	3.13	0.12	2.4-3
Glucose (mmol/l)	2.86	3.06	3.36	3.50	0.12	1.7-3.6
UREA (mmol/l)	8.23	7.50	7.33	6.10	0.34	3-10
Creatinine (mg/dl)	1.16	1.03	0.96	0.86	0.05	0.79-1.19
AST (iu/l)	145.66	145.66	169.00	184.33	7.44	60-280
ALT (iu/l)	41.00	43.00	43.00	44.66	2.97	22-38
ALP (iu/l)	64.33	70.33	75.66	79.33	3.14	70-390
TC mmol/l	1.74^{a}	1.72^{a}	1.68^{a}	1.41^{b}	0.06	1.05-1.5
TG mmol/l	0.06^{b}	0.11^{ab}	0.13^{a}	0.14^{a}	0.01	0.5-2.8
HDL mmol/l	0.60^{b}	0.85^{ab}	0.98^{ab}	1.25^{a}	0.13	0.8-2.6
LDL mmol/l	0.69	0.62	0.61	0.57	0.03	0.5-4.3

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); GLO=garlic oil SBO= Soybean oil GNO= Groundnut oil, AST – Aspartate Aminotransferase, ALT – Alanine Aminotransferase. ALP- Alkaline phosphatase, TC= Total cholesterol TG= Trigleceride HDL-high density lipoprotein, LDL- low density lipoprotein **source = (Elmhurst *et al*., 2002)

4.3.5 Nutrient digestibility of fattened uda rams fed diet containing graded levels of groundnut oil

The result fattened Uda rams supplemented with diet containing graded levels of groundnut oil is presented in Table 19. Result shows that there was no significant (P>0.05) difference in dry matter (DM), crude protein |(CP), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), hemicellulose (HCL) and cellulose (CELL) digestibilities while ether extract and ash digestibilities were influenced significantly (P<0.05) by diet with 0, 15, 30 and 45ml/kg graded levels of groundnut oil.

The DM digestibility value were ranged from 58.03 to70.13 %. The DM digestibility tended to increase in sequential order from T1 diet with 0ml/kg to T4 diet with 45ml/kg reflecting an increase with oil supplementation. The highest value DM digestibility was record in diet with highest level groundnut oil inclusion.

The CP digestibility of T4 diet with 45ml/kg recorded the highest value of 74.68 %, while T1 diet with 0ml/kg had the lowest value of 65.06 %. The CP digestibility values obtained in this study followed the same pattern with DM digestibility. The CF digestibility increased progressively from T1 diet with 0ml/kg to T4 diet with 45ml/kg which ranged from 43.78 to 62.80 %. The CF digestibilities of diets with oils supplementation were comparably the same ($P > 0.05$) among treatment groups.

The ADF digestibility, NDF digestibility and ADL digestibility ranged from 43.7 to 66.82%, 49.76 to 69.39 % and 44.40 to 71.85 % respectively. Treatment T4 diet with 45ml/kg had the highest values were observed in ADF digestibility, NDF digestibility and ADL digestibility which increased with increasing levels of the oil supplemented in the diet. The hemicellulose digestibility in T4 diet with 45ml/kg recorded the highest value of 76.55% with 45ml/kg of oil supplementation, while T1 diet with 0ml/kg had

the lowest value of 52.81%. The hemicellulose digestibility increase with increasing level oil supplementation. The cellulose digestibility in T4 diet with 45ml/kg recorded the highest value of 72.73 % with groundnut oil supplementation, while T1 diet with 0ml/kg had the lowest value of 55.78 %.

Table 20: Nutrient Digestibility of fattened Uda rams supplemented with diet containing different levels of groundnut oil

Parameters	T1 (Control)	T2 (15ml)	T3 (30ml)	T4 (45ml)	SEM
DM	58.03^{b}	65.06^{ab}	67.94^{ab}	70.13^{a}	1.87
CP	65.06^{b}	70.18^{ab}	74.39^{a}	74.68^{a}	1.53
CF	43.78^{b}	54.61^{ab}	62.43^{a}	62.80^{a}	2.83
EE	97.16	97.52	97.30	97.18	0.09
ASH	78.65	81.31	83.06	81.21	0.78
ADF	43.74^{b}	59.01^{a}	64.24^{a}	66.82^{a}	3.12
NDF	49.76^{b}	62.54^{a}	67.84^{a}	69.39^{a}	2.71
ADL	44.40^{b}	59.46^{a}	62.99^{a}	71.85^{a}	3.37
Hemicellulose	52.81^{c}	63.13^{b}	68.62^{ab}	76.55^{a}	2.92
Cellulose	55.78^{d}	61.15^{c}	68.02^{b}	72.73^{a}	1.99

SEM = Standard error of means; a, b = Means in the same row bearing different superscripts differ significantly (P<0.05); DM= Dry matter, CP= Crude protein, CF= Crude fibre, EE= Ether extract, ADF = Acid detergent fibre and NDF = Neutral detergent fibre, ADL= Acid detergent lignin.

4.3.6 Micro-organisms isolated from rumen liquor of fattened Uda rams fed diet with containing graded levels of groundnut oil

The Morphological identification of isolated Micro-organisms of fattened Uda rams supplemented with the diet containing graded levels of groundnut oil is showed in Table 20. Morphological identification of isolated Micro-organisms in rumen liquer supplemented with different levels of groundnut oil. The *Staphycoccus aureus Escherichia coli, Bacillus subtilis, Listerin spp, Proteus vulgaris,* Micro-organism present in all treatments while these species *Butyrivibrio fibrisolvens, Aspergillus niger Staphycoccus aureus Enterobacter spp*. *Bacillus anthracis, Staphycoccus aureus Enterobacter spp* were only occurred in diets containing oils. The addition of oil in the diet supplements had a variable effect of the numbers of fungi relative to the control.

Table 21 Micro-organism isolated from rumen liquor of fattened Uda rams fed diet with containing graded levels of groundnut oil

Treatments	Micro-organism isolation in rumen liquid
T1 (Control)	*Proteus vulgaris, Aspergillus fumigates, penicillium spp*
T2 (15mL)	*Bacillus anthracis, Staphycoccus aureus Enterobacter spp Escherichia coli Butyrivibrio fibrisolvens, Aspergillus niger, fusarium spp, Absidia spp*
T3 (30mL)	*Bacillus anthracis, Staphycoccus aureus Enterobacter spp, Butyrivibrio fibrisolvens, trychophytan rubrum Aspergillus fumigatus and Aspergillus favus*
T4 (45mL)	*Staphycoccus saprophyticus, Bacillus anthracis, Enterobacter spp, Butyrivibrio fibrisolvens penicillium spp, Aspergillus niger, fusarium spp*

4.3.7 Micro-organisms isolated from rumen liquor of fattened Uda rams offered diet containing graded levels groundnut oil

The Effect of different level groundnut oil supplementation on microbial population is presented in Table 21. The results indicated no significant (P>0.05) effect among treatment groups. The bacteria counts ranged between 7.20 and 7.59 in T_4 and T_2 respectively. The Fungi counts ranged between 0.76 and 1.18 in T_3 and T_4 respectively.

Table 22: Effect of graded levels groundnut oil Supplementation on Microbial Population

Parameters	Treatments				
	T1 (Control)	T2 (15ml)	T3 (30ml)	T4 (45ml)	SEM
Total bacteria (ll CFU 10^6)	7.30	7.59	7.45	7.20	0.70
Fungi (log Cell ml^{-1})	3.28^b	3.66^a	3.70^a	3.77^a	0.26

SEM = Standard error of means, a, b = Means in the same row bearing different superscripts differ significantly (P<0.05);

4.3.8 Fatty acid (FA) composition of the diets supplemented with graded level of groundnut oil.

The effect of fatty acid (FA) composition of the diets supplemented with different levels of groundnut oil is given in Table 22. The effects of groundnut oils on the concentrations of total FA, 18:00, 18:1 cis11, trans18 and 18:3 were not significantly ($P > 0.05$) different between the treatments while 18:2 and Total c18 differed

significantly (P<0.05) among the treatments. The different levels of the Oil had effects on total FA, 18:00, 18:1 cis11, trans18, 18:2, 18:3 and total c18 concentrations in the diets for T1, T2, T3 and T4 for respectively.

Table 23: Fatty Acid Composition of the diet supplemented with different level of groundnut oil.

0	T1 (Control)	T2 (15ml)	T3 (30ml)	T4 (45ml)	SEM
Total FA, % of DM	2.94^{b}	3.38^{ab}	3.73^{a}	3.78^{a}	0.14
Individual FA, g/100 g FA					
Stearic acid (18:00)	8.39	8.45	8.45	9.00	0.15
Vaccenic acid 18:1 cis11	1.03	1.10	1.13	1.20	0.06
Oleic acid 18:1 cis9	14.26	14.57	14.60	14.86	0.23
trans18	0.57	0.61	0.70	0.72	0.05
linoleic (C18:2 *n*-6) acid	26.73^{b}	29.63^{a}	30.23^{a}	31.30^{a}	0.63
linolenic (C18:3 *n*-3) acids	6.30	6.90	7.70	8.07	0.32
Total C18	33.03^{b}	36.53^{a}	37.93^{a}	39.37^{a}	0.80

SEM = Standard error of means; a, b = Means in the same row bearing different superscripts differ significantly (P<0.05);

4.3.9 Metabolism of fattened Uda rams fed diet containing graded levels groundnut oil

The effect of Flow fatty acid and biohydrogenation of long-chain fatty acids (FA) in diets supplemented with different levels of groundnut oil is given in Table 23. The incubation cuture were based on the same diets and differed level groundnut oils added. The result showed that the diets supplemented with groundnut oil with 0, 15, 30 and 45ml/kg had effects on the concentrations of 18:00, bh18:1cis and bh18:3 were no differed significanty ($P > 0.05$) in experiment diets while 18:1 cis11, trans18, 18:1 cis9, 18:2, 18:3, total C18:0, CLA, bh18:2, and bh total C18:0 parameter were differed significantly ($P<0.05$) among the treatments group. The Biohydrogenation (18:2, 18:1cis, 18:3 and total C18:0) concentration was decrease with increasing level groundut oil in the diets while highest in diet without oils supplemented

Table 24: Flow and Biohydrogenation of Long-Chain Fatty Acids in fattened Uda rams fed diet containing graded levels groundnut oil

Time(mL hours)	T1 (Control)	T2 (15ml)	T3 (30ml)	T4 (45ml)	SEM
Flow, mg/d					
Stearic acid (18:00)	497.66	525.33	512.00	556.33	15.88
Vaccenic acid (18:1 cis11)	114.66^{c}	127.33b^{c}	135.33^{ab}	151.00^{a}	4.54
Oleic acid 18:1 cis9	111b	133.33ab	134.33ab	149.00a	5.09
trans18	13.00	14.20	14.33	15.00	0.36
linoleic (C18:2) acid	133.66^{c}	148.66^{b}	166.66^{a}	180.00^{a}	5.58
linolenic (C18:3) acids	64.26^{b}	77.63^{a}	76.90^{a}	87.96^{a}	3.01
Total c18	197.93^{d}	226.30^{c}	243.56^{b}	267.96^{a}	7.93
Conjugate linoleic acid	12.53^{b}	15.20^{a}	16.86^{a}	17.53^{a}	0.66
Biohydrogenation					
Vaccenic acid (18:1cis)	66.33	63.25	64.84	62.18	0.84
linoleic (C18:2) acid	59.47^{a}	59.25^{a}	56.08^{b}	54.28^{b}	0.76
linolenic (C18:3) acids	80.47	78.72	79.67	77.645	0.62
Total C18	39.95^{a}	37.98^{a}	35.76^{ab}	31.92^{b}	1.13

SEM = Standard error of means; a, b = Means in the same row bearing different superscripts differ significantly ($P<0.05$); BH = Biohydrogenation, calculated as percentage of C18 FA that disappeared between intake and effluent flow (Tice *et al*., 1994); total C18 FA were weighted for the number of unsaturated bonds.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Experiment I

5.1.1 Proximate composition of the experimental diets

The dry matter contents of this experimental diet are adequate for storage. Okoruwa *et al*. (2015) reportedthat dry matter content above 70 % can be an advantage during storage as the feed can be stored for a longer period of time without spoilage. Diets with high moisture content are vulnerable to microbial attack which can cause deterioration easily. The crude protein content of diet used in this trial is above the reported value by Minson (1990) who recommended critical CP level of 7 %, below which feed intake of ruminants will be depressed, thus the diets appears to be adequate in meeting the CP requirement of ruminants. The crude protein (CP) content was above the 6 – 9.5% CP recommended by ARC (1980) and NRC (1985) for optimum microbial activities in the rumen. The results of this study are within the normal range of crude protein requirement of 15-18% for growing lambs with weight range of 10-30kg (Church, 1978; ARC, 1990). However, lower than values reported by Okoruwa and Igene (2014) who used concentrate supplement with 20% crude protein to provide adequate nitrogen requirement for rumen microbes to maximally digest the components of dietary fibre. The crude fibre level in this study is within the requirement reference scale of growing sheep as reported by Ganovsk and Ivanov (1982). The authors reported crude fibre requirement of ruminants to be 22% to 25%. The crude fibre could may lock up nutrients in the fibre matrix and makes it not accessible and utilizable by animals for improved performance (Dairo and Egbeyemi, 2012). Crude fibre fermentation ability has been identified as one of the factors in utilization of high fibre content in feeds. The decrease in CF content of diets could be attributed to the possible rumen microbe

secretion of some extra cellular enzymes such as cellulase into the diets and degraded the fibre (you are reporting proximate composition of diet before feeding, reports on extracellular secretion does not arise) (Songsak and Sirilak, 2009). The reduction might be attributed to the ability of fermenting microflora to hydrolyse and metabolise carbohydrates as carbon sources in order to synthesis cell biomass (Madigan *et al*., 2002).

The ether extract values obtained in this study agreed with the finding of Abbator*et al*. (2017) who reported value above 5% fat in the diet of growing animal can supply the essential fatty acids and reduce dustiness of the feed. The ADF and NDF contents of all the diets were all below the reported value of 55-60 %(Meissner *et al*., 1991) which cannot limit feed intake. Thus low proportion of ADF and NDF in these all diets showed that they are of better feeding quality than feeds with higher ADF and NDF contents.

5.1.2 *In-vitro* gas production at different period of time in diets containing different plant oils

The gas produced by feeds is directly proportional to the rate at which substrates are degraded (Dhanoa *et al*., 2000). The highest gas was produced in T1 (control) and the lowest in T4 (groundnut oil) at 24 hours of incubation. Gas production increased progressively throughout the incubation period (Table 3). This observation suggests improvement in digestibility of the feed ingredients, which could be ascribed to the increasing contact between the feed ingredients and microorganisms in the rumen liquor with time. The results obtained in the present study are in line with the findings of Sobhy and Samir (2010) who stated that addition of oil or limonene decreased gas production. However there are many factors that may regulate the amount of gas to be produced during fermentation, depending on the nature and types of diet (Babayemi *et al.*, 2004). Generally, gas production is a reflection of degradable carbohydrate and

therefore, the amount depends on the nature of the carbohydrates (Demeyer and Van Nevel, 1975).Therefore, recent research has been greatly focusedon how to exploit plant bioactive substances as natural feed additives to improve rumen fermentation such as enhancing protein metabolism and decreasing gas production (Wallace *et al*., 2002). This study observed that addition of (garlic, soybean and groundnut oils) had reduced the amount of gas over period of time.

5.1.3 Total *in vitro* gas production and dry matter digestibility of diet containing different plant oils

Akinfemi *et al*. (2009) stated that the fastest and the highest levels of gas production were influenced by the soluble carbohydrate fraction readily available in the feed.The result on Total gas production is in accordance with the study of Sobhy and Samir (2010) who reported that addition of oil decreased total gas production. Kamalak *et al*. (2011) also reported that lipids have different mechanisms to manipulate rumen fermentation and reduce gas production. However this study showed that Groundnut oil in the diet reduced total gas production than garlic and soybean oils. Gas production is associated with production of volatile fatty acid (VFA), methane and other products following fermentation of substrate, so the more the fermentation of a substrate the greater the gas production. The finding in this study is not in line with the studies of Bateman and Jenkins (1998); Ueda *et al*. (2003) who reported no significant difference in gas production due to the addition of oils. The result *in vitro* dry matter digestibility is in line with the report of Benchaar *et al*. (2007) who found that supplementation of essential oil at 200 mg/L did not affect the *in vitro* dry matter digestibility. The result of the present study contradicted the findings of Sobhy and Samir (2010) who showed that essential orange oil supplementation significantly reduced the dry matter digestibility. The differences among results can be attributed to the techniques and type

ofoilsused. In this study, *in vitro* gas production technique was used. Fraser *et al*. (2007) stated that differences in techniques used may affect or cause discrepancies in the results. This study indicated that pH is significantly affected by inclusion of plant oils and fell within the normal level that can support the fermentation process. The normal pH condition is very important to supporting the growth and microbeial activity in decomposing the ration. The normal fermentation process in the rumen is between pH 6.0-7.0 (Franzolin and Dehority, 2010), and play an important role in preserving rumen stability and microbial growth. McDonald *et al*. (2010) found similar outcomes where the normal pH of rumen are presented. The normal pH level indicated no adverse effects on rumen ecosystem condition. The addition of oils might not harm the rumen activities.

5.1.4 *In Vitro* fermentation parameters of diets containing different plant oils

The fementation parameters such as VFA and methane were measured. The result obtained in the present study is in accordance with the reports of Benchaar *et al*. (2007) who found that supplementation of essential oils did not significantly affect the VFA productions. However the result of this finding is not in conformity with Sobhy and Samir (2010) who stated that essential oil supplementation significantly reduced the VFA productions. As mentioned before, the differences among two studies can be attributed to the techniques and diet used. Patra (2014) reported that the addition of oils to ruminant diets reduces the acetic:propionic acid ratio as a result of a lower ruminal degradation of fiber. The VFAs which absorbed across the rumen wall are used by the animal as the primary source of energy (Kamra *et al*., 2012). Subacute acidosis occurs when VFAs production from fermented diets exceeds the ability of the rumen environment to neutralize or absorb it. Absorption of VFAs occurs passively across the ruminal wall and is enhanced by papillae, which provides a large surface area for VFAs absorption. Passive VFAs absorption through the rumen papillae is also increased when

rumen pH drop to threshold value (pH 5.5) because ruminal VFAs are rapidly shifted towards the dissociated form and VFAs are passively absorbed only in the dissociated form.The fermentation of the feed is used by methanogens as their important source of energy to reduce CO2 to CH4 (Hungate, 1967), and may be used by biohydrogenating bacteria to hydrogenate FAs (Chesworth *et al*., 1998).

The finding is in line with Beauchemin *et al*. (2008) who reported that CH4 production is reduced significantly with lipid supplemention. Patra (2013) and Patra (2014), reported linear decreases close to 4.3% of CH4 in cattle and sheep diet supplemented with lipid. Oils have different mechanisms to reduce CH4 production (Machmuller *et al*., 2000; Beauchemin *et al*., 2008). When methane production is reduced, a decrease in acetate which is associated with increase in propionate production is expected (van Nevel and Demeyer, 1996) in diet supplement with lipid. The methane synthesis is usually associated with increased propionate and reduced acetate to propionate ratio (Kamalak, 2011).

5.1.5 Morphological identification of isolated micro-organism in *in vitro* techniques of diets containing different plant oils

Bacteria are principal agents of fermentation in the rumen (Preston and leng, 1987). The bacteria found in this study are involved in the degradation process that occurs within rumen environment (Isah *et al.,* 2015). The most important cellulolytic bacteria species include *Ruminococcus flave faciens, Ruminococcus albus, Bacteriode succinogenes (*or *Fibrobacter succinogenes)* and *Butyrivibrio fibrisolvens* (Cheng *et al.*, 1990) which are all present in all treatments except *Butyrivibrio fibrisolvens* which are only present in diet supplemented with oils. Hespell and O'Bryan-Shah (1988) examined the lipolytic activities of 30 strains of *Butyrivibrio fibrisolvens* and observed that there is wide variation among strains of *Butyrivibrio fibrisolvens. Anaerovibrio*

lipolytica hydrolysed triglycerides less rapidly than diglycerides; however, they did not hydrolyse galactolipids and phospholipids (Henderson, 1971). A*Anaerovibrio lipolytica* documented ruminal lipolytic bacterium (Hungate, 1996) yields an extracellular lipase that has the potential for hydrolysing diglycerides more readily than triglycerides. Galactolipids and phospholipids are not however, affected by these lipases (Kim *et al*., 2009). Bacteria *Butyrivibrio genus* having the capacity to hydrolyse triglycerides (Latham *et al*. 1972). A *Butyrivibrio* strain known to convey out phospholipase action (Hazlewood and Dawson, 1975).

This study indicates that normal Rumen protozoa that support fermention are present. Protozoa are not essential for rumen functioning if number is high. Franzolin and Dehority (2010) described that protozoa had an important role in preserving the rumen pH due to its faster capability in digesting starch, slowing down the fermentation process by the rumen bacteria and producing organic acids (volatile fatty acids). This capability can be linked with protozoa's capability, which is able to grow in the substrate, when ruminal pH decreases to a certain level, the digestion of food ingested could be affected. This study specified that the minimum recorded pH is 6.07 for diet supplement with groundnut oil. De Veth and Kolver (1999); Beauchemin (2007) reported a large reduction in digestion when ruminal pH was less than 5.8. This study indicated that pH significantly affected by inclusion of Plant oils.

The fungi that support fermentation are also present in this study. The fungi may allow fibre that is physically protected by lignin to be fermented by the rumen bacteria (Orpin, 1982). It has been reported that ruminal fungi have enzymes which can hydrolyze the majority of the structural polysaccharides found in plant cell wall (Dehority and Tirabasso, 2001).

In this study, the plant oils (garlic oil, soybean oil and groundnut oil diets) resulted in decrease in the biohydrogenation concentration. Anaerobic fungi have been found to play a role in the bio hydrogenation of linoleic and linolenic acids (Nam and Garnsworthy, 2007). The addition of oil and diet supplements had a variable non-significant effect of the copy numbers of fungi relative to the control. Therefore, it is possible that other microorganisms had a more relevant contribution to bio-hydrogenation of fatty acids (Wallace *et al*., 2006).

5.1.6 Effect of microbial population on the diets containing different plant oils

Microorganisms is very vital in ruminant animal nutrition because it is a directory of the amount of microbial protein made available to the ruminant (Okoruwa 2015). This study has shown differences in microbial polpulation which could primarily be influenced by the plant oils supplementation of the feed. The bacteria counts range between (9.56 and10.09 log CFU 10^9) in T_4 andT_1 (Control) respectively.This is in agreement with the report of Bain *et al*. (2017) who reported no significant (P>0.05) effect in microbial population of ration Supplemented with Soybean Oil, Calcium Soap and Cashew Fruit Flour. The highest number of rumen bacteria counts observed in control diet might be responsible for high fibre and total volatile fatty acids which enhance microbial activities. Bacteria count of rumen fluid depends on the type of diet (Yusuf *et al*., 2013). This study is in accordance with the reportof by Ørskov and Ryle (1990) who reported that species of bacteria have been found in the rumen and about 10^9- 10^{10} bacteria per ml of rumen fluid have been estimated. Okoruwa *et al*. (2013) also reported that the major – fibre degrading bacterial species which are Fibrobacter succinogenes, Ruminoccus albus and Ruminoccus flavfaciens are particularly present under high rumen pH but sensitive to low rumen pH. However, the low bacteria count

in control diet, might indicate ammonia as limiting for bacteria growth (Okoruwa *et al*., 2013).

This study agrees with the reports of Bain *et al*. (2017) who stated that addition oil to concentrate diet did not significantly affect the protozoan population which is in disagreement with Sultana *et al*. (2008) whom reported change in protozoan population of cattle fed with a ration containing soybean and linseed oils. This reduction in rumen protozoa counts with oil supplement, undoubtedly result in a decreased outflow of volatile fatty acids in the rumen. Franzolin and Dehority (2010) reported that protozoa had vital role in conserving pH due to its faster capability in digesting starch, slowing down the fermentation process by the rumen bacteria and producing organic acids (volatile fatty acids). Anaerobia fungi are reported to be the first to reduce the tensile strength of feed particles and increase the particles breakdown in rumination, thus they are important initiators of fermentative breakdown of insoluble plant cell wall materials (Okoruwa *et al*., 2013). This is in agreement with the report of Bain *et al*. (2017) who obtained no significant effect in microbial population of ration supplemented with soybean oil, calcium soap and cashew fruit flour. Bacteria count of rumen fluid is dependent on the type of diet (Yusuf *et al*., 2013).

5.1.7 Metabolism of fatty acid on the diets containing different plant oils

In this study, the dietry oils (garlic oil, soybean oil and groundnut oil diets) resulted in an increase in the CLA and a decrease in the biohydrogenation end product C18:0 concentrations. The higher concentration of 18:2, 18:3 along with the formation of C18:0 with the addition of groundnut oil in the diets to cultures suggest a lower biohydrogenation activity in these cultures. The lower 18:2, 18:3 BH may suggest that plant oilsreduced hydrolysis in culture. This study is in accordance with the finding of Lourenco *et al*. (2008) who reported that 500 mg/l of cinnamaldehyde resulted in an

increased accumulation of CLA. Duckett *et al*. (2002) reported 91% BH for 18:3, 80% for 18:2, and 70% for 18:1 BH when steers were supplemented with typical corn, high-oil corn, and corn oil respectively. These differences in percentages might be due to the differences in type of plant oils, method *in vitro* incubation and diet used. The BH for control was the highest among the treatments while treatment 4 had the lowest BH compared to the other treatments. Wu and Palmquist (1991) reported BH of FA *in vitro* to be an average of 47% in diets containing calcium soap and 71% with animal-vegetable blend. This intermediate of biohydrogenation (C18:2 *cis*-9 *trans*11 CLA) is formed in the first step during the transformation of *cis*-9, *cis*-12 18:2 by the linoleic acid isomerase (LA-I) (Buccioni *et al*., 2012). The finding of the present study suggested that plant oil facilitate the activities of the linoleic acid isomerase which controls the formation of *cis*-9 *trans*11 CLA from *cis*-9, *cis*-12 18:2. The content of C18:2 *n*-6 and C18:3 *n*-3 rapidly decreased with the biohydrogenation of C18:2 *n*-6 and C18:3 *n*-3 was similar to the previous report (Sinclair *et al*., 2005).

5.2 EXPERIMENT II

5.2.1 Growth performance of Uda rams fed diets containing different plant oils

The growth performance of growing lambs showed the highest mean daily live weight gain records in lambs fed diets containing oils and also animal with Groundnut oils supplemented diets performed better than all the Treatments. In this research, oils increased growth performance of growing lambs. The study is in conformity with Benchaar *et al*. (2008) who reported that inclusion of essential oil improved nitrogen and energy utilization in the ruminant nutrition. However,Yang *et al*. (2007) reported that garlic oil numerically increased NH_3-N concentration in the rumen of ruminant animals. The final live weight of lamb fed diet containing groundnut oil was highest probably due to higher metabolizable energy in the diet. Plant oils hold promise as feed

additives in ruminant nutrition to improve feed efficiency and control the spread of pathogens in livestock (Benchaar *et al*., 2008).

The low mean Total feed intake in this study was obtained on diet with groundnut oil supplementeddiet. The result, is not agreement with McDonald (1995) and Schneider *et al*. (1995) who reported that high crude protein influence higher intake. However the finding is in line with the reported of Calsamiglia *et al*. (2007) who offered higher doses: 500 mg/day of cinnamaldehyde and a mixture of eugenol and observed negative effect on feed intake. This results were in accordance with the findings of Reddy *et al*. (2003) and Kumar *et al*. (2006) who found no difference in DMI in sheep due to supplementation of oil in the diet. Similarly, Palmquist, (1984); Schroeder *et al*., (2004) reported a decrease in voluntary feed intakeeven with the use of protected fats. Benchaar *et al*. (2012) found no effect on dry matter intake with the addition of different sources and levels of lipids in TMR systems.Theresultis not in line with Beauchemin *et al*. (2008) who reported that CH4 production is reduced expressively with lipid supplemention

5.2.2 Heamatological parameters of Uda ram fed diets containing different plant oils

The Packed Cell Volume (PCV) values (31.66 to 35.33%) observed in this study for the growing lamb is higher than the values reported by Baneejee (2007); Bianca (1955) as normal range (28.47 to 30.25%) for sheep. But lower than the finding of Swenson (1990) and Dacie and Lewis (1991) who reported range of 38-45% PCV. The result of this study is in conformity with the report of Coles (1986) who stated the normal for PCVto be from 24-45% for growing lamb. The difference in PCV values might be attributed to oils inclusion in the diet, the difference in PCV values may be due to

differences in the diets. The values obtained for PCV show that the experimental diets were adequate for the nutritional requirements.

The values of Haemoglobin (Hb) for the treatments were within the range of 8-16g/dl for growing lambs as reported by Coles (1986). The Hb values were good and could to be capable of supporting high oxygen carrying capacity of blood in the animals.

The Red Blood Cell (RBC) values for the treatments were comparable with values for lambs (4.44 – 8.69 g/dl) as reported by Njidda *et al*. (2014); Campbell *et al*., (2003). The high RBC counts may be associated with Polycythemia conditions that cause the body to make too many red blood cells or impaired pulmonary function, while low RBC counts may be associated with iron deficiency, internal bleeding, some types of anaemia or some vitamin deficiency (Njidda *et al*. 2014). The high RBC counts obtained in T1 control diet, T2 diet with garlic oil and T4diet with groundnut oil may be associated with in normal conditions. Red blood cell indices provide information about the haemoglobin content and size of red blood cells. Abnormal values indicate anaemia and type of anaemia (Gernsten, 2009).

The White Blood Cell (WBC) counts in this study were within the normal range of 6.93 – 12.66 x 10^9/L) observed by Fadiyimu *et al.* (2010). A white blood cell count higher than normal range might be caused by infection, immune system disorders or stress while a low number of WBCs than normal range may be due to bone marrow deficiency or failure, disease of the liver or spleen, radiation therapy or exposure (Bagby, 2007). The higher WBCs count recorded in the control diet may be due to the response of the animals to protect themselves against invading pathogens. The WBCs or leucocytes are the mobile unit of the body protecting system (Aiello, 2000).

The lymphocytes recorded by growing lambs in the present study are within the normal range of 45 – 76 and 40-75% for sheep as observed by Njidda *et al*. (2014); Coles (1986) respectively. The lymphocytes values recorded in this study compared well with the report of Bush (1991). The lymphocytes play an important role of imparting immunity (Sembulingam and Sembulingam, 2002). The Neutrophils value were within normal range (10-50 %) for sheep reported by Coles (1986) which is within the values observed in this study. The eosinophil, basophil and monocyte were all within the normal range of 1-5% monocytes, 1-8% eosinophil and 0-3% basophil as reported by Coles (1986).The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases (Soetan *et al*., 2013).

The MCV, MCH and MCHC values reported for the sheep in this study were within the normal range 38.6 – 103.5 fl, 12-20 pg and 12.8 – 33.11% respectively as reported by Njidda *et al*. (2014). These parameters were used to measure the size and hemoglobin content of erythrocytes and the values are useful in diagnosing various forms of anemia (Kolo *et al*., 2017). The values of MCV and MCH are very important in the diagnosis of anemia and also serve a useful index for the capacity of the bone marrow to produce red blood cells (Awodi *et al*., 2005).Therefore, the differential counts value obtained in this study show that the Lambs were in good health. This indicates that plant oils supplementation is not harmful for growing lambs.

5.2.3 Blood biochemistry parameters of Uda lambs fed diets containing different plant oils

The total and conjugated bilirubin values in this study were in conformity with the account of Coles (1986) and Boyd (1984). Okonkwo (2010) reported mean total bilirubin of 0.65mg/dl in blood of the sheep, which is slightly above the finding from this research. Bilirubin tests measure the amount of the bilirubin in the blood sample and it is considered the true test for the liver function as it reflects the ability of the liver to take up, process and secrete bilirubin into the bile (Frandson, 1981). The results indicate that the experimental diets did not have any debilitating effect on the liver function as seen from the bilirubin.

Total protein values obtained in this study were compared well with the report of total protein of 5.81gm/dl by Coles (1986) and Maigandi (2001). The results were slightly lower than the physiological range of total protein (6 – 9.3g/dl) values for sheep (Borjesson *et al.*, 2000). The levels recorded in this study are slightly lower than the range values (6.0 – 83 g/dl) reported by Njidda (2014) for growing Lambs. Kolo*et al*. (2017) reported that serum total proteins of animals are indirect indices for measuring nutritional protein adequacy in farm animals.

The established albumin values were close to the average value (2.96 g/dl) as reported by by Coles (1986) and and Maigandi (2001) for sheep. Dairo (2005) reported that albumin is an important blood clot factor due to its ability to prevent haemorrhage, therefore the higher the value the better it is to the animals. This could be the reason why the sheep have comparable total protein content among the different groups. The observation is agreement with Allison (1955) and Anon (1980) who found changes in protein reserve in animal as indicated by serum total protein to be associated with alteration in the albumin fraction.

The urea values obtained in this study were within the range (6.1 – 7.5 mmol/l) reported by Njidda *et al*. (2014) for growing lamb, which is not in line with the values reported by Baneejee (2007) who stated urea range of 8 to 20 (mg/dl) value for sheep. The urea values obtained in this studyis good indication of efficiency of utilization of nitrogen and urea recycling and could affect the amino acid balance. The blood urea values in the experiment were within the recommended limits and suggested that the kidneys and liver in the body of the sheep were functioning normal.

The creatinine levels obtained in the study (0.90 to 1.43 mg/dl) are within the normal range reported by Boyd (1984). The urea and creatinine concentration in the blood were used for kidney function (Davis and Burndt, 1994). But highest values of creatinine 1.43 was obtained in control diet, so the muscle mass and kidney function of the animals were normal (Prvulovic *et al*., 2012).

The values on serum aspartate amino transferase (AST) and serum alanine amino transferase (ALT) activity observed in this study were within the normal range (40.0-123 IU/l) and (25.0– 70 IU/l) respectively reported for healthy ewes (Mitruka amd Rawnsley, 1977). The serum alanine amino transferase (ALT) and serum alkaline phosphatase (ALP) activity did not show significant differences among treatments, and values were not within the normal range (IU/l) as reported for clinically healthy ewes (Mitruka amd Rawnsley, 1977). Both ALP and ALT are present in many tissue cells, especially liver and heart cells therefore increased values could indicate necrosis of cells (Kaneko, 1980).Alanine aminotransferase (ALT) is a liver-specific hepatocellular enzyme in some animals measuring hepatic necrosis (Cornnelius, 1986). A decrease in serum ALP and ALT below the normal range has been reported to signify necrosis and mycocardial infarction or response to the presence of a number of toxin factors (Merck, 1998). Normal ALT levels indicate that the activities of osteoblast (a cell from which

bone develops) were not affected because the blood levels of ALT is usually a good indicator of the rate of bone formation (Guyton, 1991). Alkaline phosphatase (ALP) is a sensitive indicator of chondroblast, odteoblast, the hapatobiliary system, renal tubules, gastrointestinal mucusa and placenta (Duncan *et al.*, 1994; Merck, 1998).

The increased serum cholesterol in this trial might be due to oils supplementation in the diets. However the values of cholesterol obtained are in line with the normal range (1.33 – 1.95mmol/*l*) reported for healthy sheep (Cox-Anser *et al*., 1994). But lower than the normal ranges of 1.9–3.5 mmol/l (Sirois, 1995) this may be the differences as a result of feeding material. The high level of blood cholesterol may result in its deposition on the walls of the blood vessels and these deposits may eventually harden to atheroschlerotic plaque, this may block important blood vessels and result in a myocardial infarction.

The TG levels obtained in the present study (0.06 to 0.14 mmol/l) are within the normal range reported by Kaneko *et al*. (1997) but not in line with the report of Daramola *et al*. (2005) whose values range from 0.16 – 1.6 (mmol/l) which is slighy above levels obtained in this study.

The values of high-density lipoprotein (HDL) and low density lipoprotein obtained are within the normal ranges of 0.98– 1.09mmol/l and 0.40– 0.73mmol/l, respectively (Antunovic*et al*., 2011). The two major blood cholesterol carriers are low density lipoprotein (LDL) and high density lipoprotein (HDL). The LDL carried cholesterol which known as “bad” cholesterol, because it deliver the blood cholesterol throughout the body, depositing it as tile in the arterial walls resulting to a condition known as atherosclerosis. On the other hand, HDL cholesterol is known as the ‘good’ cholesterol because it transports cholesterol from the body tissues back to the liver which turns it to bile and is excreted via the gastrointestinal tract (Adeniyi *et al*., 2016). It is needed

for good health, hence, a moderate intake is not harmful but problem arises when the LDL cholesterol levels become elevated and the HDL cholesterol becomes too low. When cholesterol is consumed in excess it elevates total cholesterol (TC) to a high level which may result in atherosclerosis, hence, many people always desire to consume less cholesterol in their diets (Adeniyi *et al*., 2016). It is of interest to note that the body is capable of producing the cholesterol it needs, hence, the extra consumed from foods may predispose the body to some ill health states or conditions. The higher the level of LDL cholesterol in the body the greater the chances of developing heart disease (Varbo *et al*., 2013), while the higher the level of HDL cholesterol in the blood the lower the chance of developing heart disease (Burillo *et al*., 2012).

5.2.4 Nutrient digestibility of Uda rams fed diets containing plant oils

Generally, the nutrient digestibilities were high in all the treatments. The DM digestibility tends to increase as the level of CP increased in the diets. Furthermore, improved fermentation also has a direct effect on DMI by goats (Huhtanen *et al.*, 2002). This contradicted the finding of Sobhy and Samir (2010) who showed that oil supplementation significantly reduced the dry matter digestibility. Comparable result was reported by Thakur and Shelke (2010), who stated no significant difference ($P>0.05$) in digestibility of DM, CP, CF, NDF and ADF with exclusion of EE. Similarly, Sirohi *et al*. (2010) observed similar digestibility of nutrients except that of EE which was higher in bypass fat (300 g/d) supplemented lactating cow.Kumar and Thakur (2007) found higher ($P<0.05$) digestibility of EE with bypass fat supplementation in calves while no adverse effect was recorded on the digestibility of other nutrients. Fraser *et al*. (2007) observed that dry matter digestibility increases significantly as nitrogen content increased in the diet of sheep. The rumen digestibility measured in this study were in line with DM and ADF was not different but is not

inconformity with other parameter measured (Bateman and Jenkins, 1998; Ueda *et al*., 2003).The differences among result can be attributed to the techniques and type of oils used in the study

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5.3 EXPERIMENT III

5.3.1 Proximate Composition of the Experimental Diets for fattened rams

The dry matter content of diet fed during this study was range from (89.12 to 89.53) supported longer period of storage for a diets as reported by Okoruwa (2015) which the feeds can be stored for a longer period of time without spoilage if dry matter above

70%. High moisture content is connected with increase in microbial activities in diets during storage which reduces the nutritional value of the diets (Singh *et al*., 2010). Sharma *et al*. (1995) stated that relatively high levels of unsaturated oil making the product prone to rancidity during storage. The crude protein values recorded for this study were above the critical range of 8 to 10% reported (Isah*et al.*, 2015). The crude protein (CP) content was above the 6 – 9.5%CP as recommended by ARC (1980) and NRC (1985) for optimum microbial activities. This result fells within normal range the crude protein requirement of 15-18% for sheep with weight range of 10-30kg (Church, 1978; ARC, 1990). This study showed that CP are good and accessible and utilizable by animals for improved performance (Dairo and Egbeyemi, 2012). The crude fibre level in this study ranged from 19.50% to 22%, also reported by Ganovsk and Ivanov (1982) when they estimated the crude fibre requirement of ruminants to be 22% to 25%. The decrease in CF content across the diets could be attributed to the possible rumen microbe secretion of some extra cellular enzymes such as cellulase into the diets and degraded the fibre (Songsak and Sirilak, 2009). The low CF content recorded in T_4 might be due to high level of the oil inclusion. Also the reduction might be due to easily fermenting microflora to hydrolyse and metabolise carbohydrates as carbon sources in order to synthesis cell biomass (Madigan *et al*., 2002). The variation in the percentage EE obtained in this study could be attributed to the increasing levels of the oils inclusion in the diets. Higher ether extract has the tendency to reduce dry matter feed intake and may decrease effective digestibility. The ADF and NDF contents of all the diets were below the reported value of 55-60 % which can limit feed intake (Meissner *et al*., 1991). Thus low proportion of ADF and NDF in these by-products showed that they are of better feeding quality than feeds with higher ADF and NDF contents. It has been reported that NDF content of feed can be used to predict the feed intake since it

measures the total fibre component of feed. The variation in nutrient percentage among diets may be due to the levels of oil inclusions in the rations.

5.3.2 Fattened performance of Uda rams fed diets graded levels of groundnut oil

The groundnut oils improve fattening performance since can stimulate the secretion of digestive enzymes most important to improved nutrient digestion and feed intake as reported by Salam *et al*. (2002); Lee *et al*. (2003); Jamroz *et al*. (2005). The highest live weight gain was recorded in sheep fed on the diets containing 45ml/kg groundnut oil which is the highest level of inclusion.Benchaar *et al*.(2008) reported that essential oil improved energy consumption in the animal. In this study it can be seen that increasing level oils in diet with improved growth performance of fattening rams.

The final live weight of sheep fed diet with 45m/kg DM groundnut oil was highest and could probably be due to the increasing levels of energy content in the diet. Groundnut oils hold promise as feed additives in ruminant nutrition to improve feed efficiency and control the spread of pathogens in livestock (Benchaar *et al*., 2008). The Supplementation of oils to animal diet has also resulted in increased feed efficiency (Kung *et al*., 2008, Tassoul and Shaver, 2009).

This study showed no effect on dry matter intake with increasing levels of groundnut oilin the diet, which is in line with the finding of Benchaar *et al*. (2012) who found no effect on dry matter intake with the addition of lipids in TMR systems. Kumar *et al*. (2017) reported that the dry matter intake in Buffalo steers was not affected by gradual increase in dietary supplementation of bypass fat in diet. Reddy *et al*. (2003) and Kumar *et al*. (2006) found no difference in DMI in sheep due to supplementation of protected fat. Purushothaman *et al*. (2008) also noted no difference in DMI of cows with gradual increase in bypass fat supplementation in the diet. Our results were in contrast with the

finding of Kumar and Thakur (2007) who observed higher DMI in ruminant by addition of 2.5% by pass fat to the basal diet. Similarly, Shelke *et al*. (2012) reported that, DMI ($P<0.05$) was improved in Buffaloes with the inclusion of bypass protein and bypass fat (2.5%) to the basal diet. The observed difference in feed intake between the present study and some previous study could be due to the different type of oils or diet ingredient used.

5.3.3 Heamatological parameters of Uda ram fed diets graded levels of groundnut oil

The heamatological performance of fattening sheep Administered varying levels of groundnut oil. The PCV value of sheep Administered varying levels of groundnut oil ranged from 31.16 to 35.50 %. The PCV values obtained in this study were lower than the finding of Egbe-Nwiyi *et al*, (2000); Swenson (1990) whose reported value 43.80.6% and 38-45% respectively, Jain (1993) reported that PCV value fell within the range value of 27 – 45%. The result generally showed growing and fattening sheep to have comparable the same PCV values which is in conformity with the finding of Njidda *et al*. (2014) stated the adults sheep to have higher values in PCV than in lambs, In the sheep, age exhibited remarkable influence on the PCV values.

The haemoglobin values in this study decreasing by increasing levels of groundnut oil supplementation. The haemoglobin values were within the normal range (8 – 16g/d*l*) of haemoglobin for healthy sheep (Greenwood, 1977). Normally, increase in the Hb concentration is connected with better ability to stand firm with disease infection and low level is an indication of easily infection with disease and poor nutrition (Tambuwal *et al*., 2002). The values obtained for PCV and Hb in all the treatment groups; this indicates nutritional adequacy of all diets since values did not indicate mal-or-under nutrition (Church *et al*., 1984).

The RBC values in this trial were slightly above Njidda *et al*. (2014) Observed the RBC values for adult Ouda breed of sheep (6.49– 9.25 g/dl). The values of RBC in this finding were analogous to the reported Frandson (1981); Heath and Olusanya (1988) for sheep. The main function of the RBC is to carry oxygen from lung to the body tissue and transfer carbon dioxide from tissue to the lungs. The high RBC values may be related with conditions that cause the body to build too numerous red blood cells or impaired pulmonary function, while low RBC counts may be associated with iron deficiency, internal bleeding, some types of anemia or some vitamin deficiency (Njidda *et al*., 2014). The Administered varying levels of groundnut oil did not indicate mal-or-under nutrition of the diets.

The white blood cell values ranged from 9.00 to 9.66 x 10^9/L. The white blood cells (WBC) were within the normal range values of sheep 5.2 to 27.70 x 10^9/L and 6.93 – 12.66 x 10^9/L) as reported by Njidda *et al*., (2014) and Fadiyimu *et al.* (2010) respectively. This trial showed that the animals were healthy because decrease in number of WBC below the normal range is an indication of allergic conditions, while elevated values (leucocytosis) indicate the existence of a recent infection, usually with bacteria (Ahamefule *et al.,* 2008).

The lymphocytes, Neutrophils, Monocytes, Eosinophils and Basophils recorded by fatterning sheep in the study are comparable similar with the normal range as observed by Njidda *et al*. (2014) (45 – 76, 19.00 – 48, 0- 3.0, 0 - 4, o %) respectively and Coles (1986) 40-75%, 10-50, 1-5, 1-8 and 0-3% respectively. The variations in the WBC Differentials values recorded in this study compared well with the report of Bush (1991) and may be due to differences in diets. Therefore, the differential counts value obtained in this study show that the animals were in good health. This indicates that groundnut

oil has not toxicity effects in animal. The result was also supported concerns that arose over potential toxicity to the anima and final products

The MCV, MCH and MCHC values reported for the fatterning sheep in this study were within the normal range of 33.12 – 54.09fl, 10.46-17.89 pg and 15.40 – 33.90% as reported by Njidda *et al*. (2014) respectively. These parameters were used to measure the size and hemoglobin content of erythrocytes and the values are useful in diagnosing various forms of anemia. The higher MCH and MCV values may be due to age (Egbe-Nwiyi, 2000). The values of MCV and MCH are very important in the diagnosis of anemia and also serve a useful index of the capacity of the bone marrow to produce red blood cells (Awodi *et al*., 2005).

Mean corpuscular Volume MCV values obtained for sheep were slightly lower than (35.3 – 43.7f*l*) reported by Borjesson *et al.* (2000) with except of control treatment.The values of mean corpuscular haemoglobin obtained in the study were within normal range values (10.46 -17.89 pg) for fattening sheep as reported by Njidda *et al*. (2014). The lower MCV values might be due to oils supplement to the animals. These parameters were used to measure the size and hemoglobin content of erythrocytes and the values are useful in diagnosing various forms of anaemia (Kolo *et al.,* 2017).

5.3.4 Blood biochemistry parameters of Uda sheep fed diets graded levels of groundnut oil

The serum biochemistry of fattening sheep administered varying levels of groundnut oil. The total and conjugated bilirubin values in the study were inconformity with the finding of Coles (1986) who reported the total and conjugated bilirubin of sheep 0.082 to 0.185 and 0.066 to 1.28 mg/dl respectively. Kolo (2017) reported bilirubin (mg/100ml) was between 0.2750 and 1.7900 in blood of West Africa dwarf goat, which the values was fell within normal range of this research. Bilirubin tests measure the

amount of the bilirubin in the blood sample and it is considered the true test for the liver function (Frandson, 1981; Singh, 2004).

The values for total protein and albumin in this study were within the normal range reported for normal healthy rams (Njidda *et al.,* 2014) which are 5.5 to 9.4 and 2.3 to 3.3g/dl respectively. The total protein values and albumin were similar to the range value (6.0 – 9.3g/*dl*) and 30 – 38g/d*l* for sheep by reported of Borjesson *et al.* (2000); Milne and Scott (2006) respectively. Kolo *et al*. (2017) reported that serum total protein and albumin of animals are indirect indices to assess the nutritional protein adequacy in farm animals. Dairo (2005) reported that total protein and albumin as an important blood clotting factor due to its ability to prevent haemorrhage, therefore the higher the value the better for the animals. This study agreed with Allison (1955) who reported changes in protein reserve in animal and indicated that serum total protein is associated with alterations in albumin fraction.

The values of glucose were within the normal range (2.1-3.34 mmol/L) as reported by Njidda *et al*. (2014) for adult sheep and Abbator *et al*. (2017) reported the values of the blood glucose from 2.5 -3.87mmol/L The higher value of glucose found in study of sheep is supplemented with 45ml/kg groundnut oil. The glucose in study is indicator of good metabolism in high energy diets (Coles, 1986). The lower glucose values than the normal range is a sign of hypoglycemia while higher levels are indication of hyperglycemia (Olorunnisomo, 2012).

The urea values obtained in this study were close to the average values (5.28 mg/dl) reported by Baneejee (2007) in matured sheep. The urea values obtained in this study were within reference range (8-26 mg/dl) as reported by Babeker and Elmansoury (2013) but lower than Antunovic (2011) reported the range of urea concentration was 4.30–5.60 mg/dl in sheep. The result of this study was comparable to the report of

Maigandi (2001). Kolo *et al*. (2017) reported that the blood urea levels in the study were fall within the recommended limits and this suggests that the kidneys and liver of the animals were normal. The higher value obtained in serum urea for animals on treatment without oil inclusion diet was a suggestion of poor of efficiency of use of nitrogen and urea recycling and might affect the amino acid balance.

The creatinine levels obtained in the study are fell within the normal range reported by Boyd (1984) which were 1.21 to 1.44 (mg/dl). The highest values were recorded in the control that is treatment without oil supplementation. High creatinine is pointing of poor protein and amino acid metabolism that can cause to impaired renal function and cardiac infarction (Gray and Howarth, 1980). The urea levels in conjunction with creatinine levels indicate normal liver functions.

The serum aspartate amino transferase (AST) serum alanine amino transferase (ALT) and serum alkaline phosphatase (ALP) activity values observed in this study were within the normal range reported for healthy animals (Mitruka amd Rawnsley, 1977). AST level is helpful for the diagnosis and following of cases of myocardial infarction, hepatocellular disease and skeletal muscle disorders in trauma or in diseases affecting skeletal muscle, after a renal infarct and in various haemolytic conditions (Alex and Laverne, 1983). ALT is a liver-specific hepatocellular enzyme that is used to assess liver damage (Mahgoub *et al*., 2008). The results obtained for the all the aminotransferases (AST, ALT and ALP) were normal for sheep which showed the animal are in good condition.

Serum cholesterol values obtained in this study fell within the normal ranged reported for healthy ewes (Mitruka amd Rawnsley, 1977). The cholesterol value of 0.78 to 1.30 were reported by Amin *et al* (2014) which is in conforminity with this study. The values of cholesterol obtained in the study are also in line with the normal range (1.33 –

1.95mmol/*l*) reported for healthy sheep (Cox-Anser *et al*., 1994). The increased in serum cholesterol in this study is indication of oils supplementation in the diets, but is lower than the normal ranges of 1.9–3.5 mmol/l (Sirois, 1995). Consumption of saturated fatty acids has been associated with increased serum cholesterol concentrations which is a risk factor for coronary heart disease (Keys, 1970). The level of plasma lipids and lipoprotein determines the extent of adipose tissue deposition which affects fat composition of meat (Lewington *et al.,* 2007). The high level of blood cholesterol than normal range may result in its deposition on the walls of the blood vessels and these deposits may eventually harden to atheroschlerotic plaque, this may block important blood vessels and result in a myocardial infarction. The TG levels obtained in the study fell within the normal range as reported by Kaneko *et al*. (1997) but not in line with the reports of Daramola *et al*. (2005) who stated values of 0.16 – 1.6mmol/l which is slightly above levels obtained in this study. The HDL and LDL concentration in the current study fell within normal range as Khan*et al*. (2016) reported from 1.47–2.80 mmol/l and 0.10–0.56 mmol/l respectively. The HDL mean value was higher while LDL mean value was lower than those published earlier by Antunovic *et al*. (2011).

5.3.5 Nutrient digestibility of Uda rams fed diets containing plant oils

The nutrient digestibility of fattening rams fed graded levels of groundnut oil. Feed Intake is usually related to the digestibility; Faichney (1993) report that the increase in feed intake normally increases the rate of passage of the digesta through the gastrointestinal tract and reduces its digestibility. The highest average value of Dry matter digestibility, Crude protein digestibility, Crude fibre digestibility, Acid detergent fibre digestibility, Neutral detergent fibre digestibility, Acid detergent lignin digestibility, Hemicellulose digestibility and Cellulose digestibility was recorded in T_4,

that is treatment with highest groundnut oil supplementation, which not in line with what Sirohi *et al*. (2010) reported that increasing levels of palm oil residue did not significantly alter the digestibility coefficients of DM, OM and CP. However, EE, NDF digestibility showed linear effect. Comparable result were reported by Thakur and Shelke (2010), who stated that no difference (P>0.05) in digestibility of DM, CP, CF, NDF and ADF with exclusion of EE. Similarly, Sirohi *et al*. (2010) observed similar digestibility of nutrients except that of EE which was higher in bypass fat (300 g/d) supplemented lactating animal. This finding is in line with reports of Fraser *et al*. (2007) who observed that dry matter digestibility increases significantly as nitrogen content was increased in the diet of sheep. It has been suggested that a lower ruminal digestibility as a result of the use of oils in ruminant diets can be compensated by a higher digestibility in the lower tract (Sutton *et al*., 1983; Faichney*et al*., 2002).Tyagi *et al*. (2009) noted better utilization of DM and CP in lactating crossbred cows with addition of 2.5% bypass fat to the basal diet. Bateman and Jenkins, (1998; Ueda *et al*., (2003) reported DM and ADF digestibility measured were not different significant.The differences among results can be attributed to the techniques and type of oils used in study. Fraser *et al*. (2007) stated that differences in techniques used in different trials may result in inconsistencies among studies.

5.3.6 Morphological identification of isolated micro-organism in Uda rams fed diets containing graded levels groundnut oil.

The addition of oil had a variable effect on the copy numbers of micro-orgsanism relative to the control rumen environment. Therefore, it is possible that bacteria had a more relevant contribution to bio-hydrogenation of fatty acids (Wallace *et al*., 2006). Hespell and O'Bryan-Shah (1988) examined the strains of *Butyrivibrio fibrisolvens* and observed that there is wide variation among strains of *Butyrivibrio fibrisolvens.*

Anaerovibrio lipolytica hydrolysed triglycerides less rapidly than diglycerides; however, they did not hydrolyse galactolipids and phospholipids (Henderson, 1971).

The main group A bacteria have been recognized as *Butyrivibriofibrisolvens* (Harfoot and Hazlewood, 1997). However most bacteria found in this study are commonly documented as cellulolytic bacteria which include *Ruminococcus flave faciens, Ruminococcus albus, Bacteriode succinogenes (*or *Fibrobacter succinogenes)* and *Butyrivibrio fibrisolvens* (Cheng *et al.*, 1990) and other few principally, the *Butyrivibrio* group which are the most active group for fatty acid biohydrogenate (Durmic *et al*., 2008). Latham *et al*. (1972) identified bacteria with the same morphology like those of the genus *Butyrivibrio* having the capacity to hydrolyse triglycerides. A *Butyrivibrio* strain known as LM8/1B and *Butyrivibrio fibrisolvens* have also been observed to carry out phospholipase activity (Hazlewood and Dawson, 1975). Hudson *et al*. (1998), stated *Butyrivibrio proteoclasticus* is the only bacteria capable of converting VA to C18:0, all bacteria in the *Butyrivibrio* group are capable of producing CLA from linoleic acid.The ruminal fungi have not been linked with the hydrolysis of esterified lipids in the rumen; however, there is evidence that they are involved in biohydrogenation (Nam and Garnsworthy, 2007). Biohydrogenation of FFA has been exclusively attributed to rumen bacteria (Lourenco *et al*., 2010).

Fungi appear to be the first organism to attack and begin digesting the structural plant components. The addition of oils in diet had a mutable non-significant effect of the figures of fungi. Therefore, it is possible that other microorganisms had a more relevant influence to bio-hydrogenation of fatty acids (Wallace *et al*., 2006). Fungi possess the exclusive capability to enter to the plant surface and walls of lignified tissues (Akin, 1989). The particles occurrence by fungi allows bacteria to colonize the cell

constituents. Anaerobic fungi have been originate to show a part in the biohydrogenation of linoleic and linolenic acids (Nam and Garnsworthy, 2007).

5.3.7 Effect of microbial population on the diets containing graded levels of groundnut oil

This variation in study could mainly due to the different level groundnut oil inclusionin the feed.This is in line with the report of Bain *et al*. (2017) who reported no significant (P>0.05) effect in microbial population of diet added oil. The highest number of rumen bacteria counts detected in treatment might be responsible for individual difference in animal. Bacteria count of rumen fluid is reliant upon the type of diet and individual difference (Yusuf *et al*., 2013). This study is not in agreement with the report of Ørskov and Ryle (1990) who state species of bacteria have been found in the rumen and about 10^9- 10^{10} bacteria per ml of rumen fluid have been estimated. However, the low bacteria counts in the diets might indicate inclusion of plant extract (Okoruwa *et al*., 2013).

The result is in line with the statement of Okoruwa *et al*. (2015) who reported that fungi population was significantly different when oil was added in diet. The addition of graded levels of groundnut oil in supplement diet had a variable effect in fungi species. Fungi are described to be the principal to decrease the stretchable strength of feed particles and increase the particles breakdown in rumination, thus they are important initiators of fermentative breakdown of insoluble plant cell wall materials (Okoruwa *et al*., 2013). Microbes yield is very important in ruminant animal because it is an index or a function of the amount of microbial protein made available to the ruminant daily (Okoruwa 2015).

5.3.8 Metabolism of fatty acid on the diets containing different level groundnut oil

The saturated FA and Polyunsaturated FA also increased by the addition of groundnut oil. The proportion of fatty acid in diet was increased by level increasing groundnut oil

addition. These FA has been connected with beneficial effects on human health (Rosberg-Cody *et al*., 2011; Yang *et al*., 2015). This study is in line with the finding of Dhiman *et al*. (2000) whoreported that addition of vegetable oils rich in polyunsaturated FA (18:2c9 c12 and 18:2c9 c12 c15) increases the proportions of unsaturated FA and 18:2c9 t11CLA.

In this study, the graded levels of groundnut oil resulted in an increase in the CLA and a decrease in the biohydrogenation end product. The higher concentration of 18:2, 18:3 along with the formation of C18:0 with the addition of groundnut oil diets to cultures suggest a lower biohydrogenation activity in these cultures. This study is in accordance with finding of Lourenco *et al*. (2008) whoreported that 500 mg/l of cinnamaldehyde resulted in an increase of the accumulation of CLA. Addionally, feeding cinnamaldehyde to rams at 200 mg/kg of DM had also no effects on their back fat or liver *trans*FA composition (Chaves *et al*. 2008). The BH which indicated higher of 18:3 than 18:2. Accordingly, Duckett *et al*. (2002) reported BH were 91% for 18:3, 80% for 18:2, and 70% for 18:1 when steers were supplemented with typical corn, high-oil corn, and corn oil. This difference in percentage might be as result of difference in type of of plant oils and method incubation *in vitro* used. The BH for cntrl was the highest among the treatments while treament4 had lower BH compared with other treatments. Wu and Palmquist (1991) reported BH of FA *in vitro* averaged 47% in diets containing calcium soap and 71% with animal-vegetable blend. This intermediate of biohydrogenation (C18:2 *cis*-9 *trans*11 CLA) is formed in the first step during the transformation of *cis*-9, *cis*-12 18:2 by the linoleic acid isomerase (LA-I) (Jenkins *et al*., 2008; Kim *et al*., 2009; Buccioni *et al*., 2012). The finding of the present study recommend that plant oil was facilitated the activities of the linoleic acid isomerase which controls the formation of *cis*-9 *trans*11 CLA from *cis*-9, *cis*-12 18:2. The content of C18:2 *n*-6 and C18:3 *n*-3

rapidly decreased with the biohydrogenation of C18:2 *n*-6 and C18:3 *n*-3 was similar to previous *in* reported (Beam *et al*., 2000; Sinclair *et al*., 2005).

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION AND RECOMMENDATION

6.1 Summary

Plant oils will continue to be important feed additive in developing countries where feed resources are in scarce. Improved ruminant production can be accomplished through improved utilisation of the diets. The studies are conducted in three phases: The first phase was the Influence of plant oils (Garlic, Soybean and Groundnut Oils) on *in vitro* Fermentation Characteristics. Four (4) treatment groups T1 control diet, T2 diet 30ml/kg garlic oil, T3 diet with 30 ml/kg Soybean oil and T4diet with 30 ml/kg groundnut oil. The T4 diet with 30 ml/kg groundnut oil supplemented diets showed better performance in vitro gas production,methane,*in vitro* dry matter digestibility and fatty acid profile 18:1 cis9, 18:2, 18:3, total C18:0, bh18:2, bh18:3 and BH total C18:0.

The second phase was to determine effects of plant oils on the growth performance and blood profiles of growing lamb. Sixteen (16) uda lambs were used for this study. Four (4) treatment groups were formulated as same with first phase were evaluated via in vivo techniques. The results of on growing lamb, showed T4 diet with 30 ml/kg groundnut oil having better performance characteristics in Final weight and Basal feed intake.The addition of groundnut oil at 30ml/kg dry matter, groundnut oil appeared to be the best plant oils in invitro gas production, methane production, fatty acid, growth performance, blood parameter and nutrient digestibilityon growing of lambsbest plant oils identified was selected.

The third phase was to determine the effects of graded levels of groundnut oil on the performance of fattened rams. The experimental animals were from the previous Experimental phase. Graded levels of the best groundnut oil are used at 0, 15, 30 and

45ml/kg for T1, T2, T3, and T4 respectively. The results of fattened characteristics showed that diet containing 45ml/kg groundnut oil had better Final body weight and Body weight gain. However inclusion groundnut oil at level 45ml/kg is the best supplementation in improve performance of fattened uda rams.

6.2 Conclusion

The study conclude the following

On the effect of *in-vitro* fermentation characteristics, the result showed that a better performance were recorded T4 diet with groundnut oil supplementation:

there is decrease of total gas in invitro gas production,

there is decrease in percentage methane (14.29 %) which resulted reduce energy losses and emitted green house gas to the atmosphere and may therefore reduce net energy gain for the respective animals.

3. In term of fatty acid profile there are increase linoleic (C18:2 *n*-6) and linolenic (C18:3 *n*-3) acid and their biohydrogenation which improved potential health benefits to consumers.

The research on the effect on the performance of growing Uda ram lambs supplemented with with different types of plant oils T4 diet with groundnut oil supplementation showed

The highest mean daily live weight gain (77.38) and Feed convention ratio (feed: gain) (6.95) in lambs were better than control and other treatments.

The better performance estimate percentage of CH_4 reduction (66.11) compared control was recorded. In this research,

Groundnut oils increased growth performance of growing lambs was identified.

Based on the above result groundnut was selected as better plant oils to be experiment

The study on effect on hematological and serum biochemical indices of Uda rams of supplemented with different types of plant oils and graded levels groundnut oil which showed

The highest total cholesterol value 1.76mmol/L was recorded T4 diet with groundnut oil in growing lambs.

The HDL value of the growing lambs 1.21mmol/L was obtained, HDL values of sheep

The highes HDL value of the fattening Uda rams supplemented with 45ml/kg diet containing of groundnut oil (1.25) mmol/l, and HDL cholesterol is known as the 'good' cholesterol because it transports cholesterol from the body tissues back to the liver which turns it to bile and is excreted via the gastrointestinal tract

The experiment on the effect on the performance of fattened Uda rams fed graded levels of the groundnut oil showed that a better was recorded in diets supplemented 45ml/kg groundnut oils.

The highest mean daily live weight gain recorded (101.19) and better Feed convention ratio (feed: gain) (10.51) in rams fed diets highest Groundnut oil were better than control and other treatments

There is decrease in percentage methane (79.74) compared control was recorded. In this research, increased groundnut oils increasing growth performance of rams.

T4 diet fatty acid profile there are increase linoleic (C18:2 *n*-6) and linolenic (C18:3 *n*-3) acid and their biohydrogenation which improved potential health benefits to consumers.

The research on the economic performance of growing and fattened Uda rams supplemented with different types of plant oils and graded levels groundnut oil which are economically cheaper and viable than control treatment.

The inclusion of plant oils decrease the cost of feed per Kg. it was more expensive to compound diet control diet (667.00 N/Kg) followed by diet 2 (645.00 N/Kg), diet 3 (589.13N/Kg), and diet 4 (391.45N/Kg).

The increase in inclusion of groundnut oil decrease the cost of feed per Kg. it was more expensive to compound diet control diet (644.33 N/Kg) followed by diet 2 (614.00 N/Kg), diet 3 (569.38 N/Kg), and diet 4 (485.10N/Kg).

6.3 Recommendation

The positive effects of plant oil on sheep performance could be due to the presence of an organo-sulphur compound. Based on the findings of this study the following recommendations were made;

Research and development efforts are required to establish a feed library for feed additive particulatly plant oils.

Plant oils on feeding trials are needed to use to improve the animal response on possible ingredient combination which alternative to synthetic growth promoter.

Plant oils to identify to be good for incubation time that points to maximumin microbial activity in both the *in vitro* and *in vivo* system, so that finishing animals can be targeted.

The smallholder farmer, personnel and institutions engaged in agri-business can try the plant oils pariticualrly groundnut in sheep.

Groundnut oils could be recommended as a better plant oils among garlic and soybean oils as supplement in growing sheep

Groundnut oils supplement above 45ml/kg should be used in diet in fattened sheep

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