KWARA STATE UNIVERSITY, MALETE, NIGERIA

SCHOOL OF POSTGRADUATE STUDIES (SPGS)

EFFECTS OF COMPOUND TANNINFEROUS PLANT FODDERS ON MICROBIAL ECOLOGY AND GAS PRODUCTION BY GOATS

By

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BSc (KWASU)

(19/57MMB/00007)

APRIL, 2022

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SCHOOL OF POSTGRADUATE STUDIES (SPGS)

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A MSC. THESIS SUBMITTED AND PRESENTED

BY

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In Partial Fulfillment For The Award Of Master Of Science Degree

DEPARTMENT OF MICROBIOLOGY

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DECLARATION PAGE

I hereby declare that this thesis titled “effect of compound tanninferous plant fodders on microbial ecology and gas production by ruminant animal” is a record of my research. It has neither been presented nor accepted in any previous application for higher degree.

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APPROVAL PAGE

This is to certify that this thesis by Folashade Gift NTAGBU has been read and approved as meeting the requirements of the Department of Microbiology for the award of the degree of Masters M.Sc. in Environmental Microbiology.

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Dedication

This work is dedicated to the Almighty God who has been my Father and my all . To Him alone be the glory and to the love of my life Chima J Ntagbu, my princes and princesses Goodluck, Elvis, Gideon and Goodness Ezinne-Carol, who have been the source of my inspiration.

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I wish to sincerely register the appreciation of love and care from my loving, and caring husband and lovely children.

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Abstract

Livestock farming had been reported to contribute large amount of greenhouse gases in the environment due to their feed composition with respect to their intestinal microbiota. This study investigated the effects of compounded tanninferous plant fodders on microbial ecology and gas production by ruminant over 64 days period. Twelve growing red Sokoto goats were placed on four differently formulated experimental dietary treatments, Control, *Detarium microcarpum, Daniella oliveri* and *Afzelia Africana* (Tc, TDm, TDo and TAa) of tanniferrous plants in a completely randomized design. Rumen fluid was collected from the goats at the end of the feeding trial and analyzed for pH, methylene blue reduction time (MBRT), nitrate reduction, cellulose digestion, glucose fermentation sedimentation activity rate, colour, odour, consistency and microbial population. Results showed that the tanniferrous plants significantly reduce ($p < 0.05$) the pH and MBRT values as compared to control. There was significant reduction in the period for Nitrate reduction, glucose fermentation and cellulose digestion in all the treatments. Consequently, significant increases ($p < 0.05$) in the period for cellulose digestion was observed in all the treatments as compared to control. For microbial counts, the highest numbers of protozoa and bacterial population were recorded in Tc. However, significant increases ($p < 0.05$) in fungal counts value was only observed in TAa treatment. The highest significant amount of carbon (iv) oxide (8.67 ml / mg) and methane gases (17.80 ml / mg) were recorded in the control compared to other treatments. In general, rapid production of gases were observed between 6 to 24h, with gradual decline in gas production at 27h to 48h in the treatments except in control, This has shown that 2-5% inclusion of tanniferrous foliage in the experimental feed

fed to goats had drastically reduced the emission of pollutant gases into the environment thereby could had significant impact in depletion of ozone layer.

1.0 INTRODUCTION

Background of the study

The gut microbiota had enormous contribution to the nutrient availability and nourishment of the ruminant animals. The microbial communities (archaea, viruses, protozoa, bacteria and fungi) inhabiting the rumen have the capability to convert low-quality, fibrous plant contents into available nutrients for the ruminants. Likewise, report have shown that rumen microflora could contribute to immune status of their host (Danielsson 2016).

The first reported domesticated animals were ruminants with about 4 billion domesticated worldwide (Malmuthuge *et al*., 2012). Ruminants had contributed to the sustainability of the agriculture systems due to their unique ability to convert low-quality forages into high-quality food products. They can utilize crop residues and by-products as feed sources thereby adding tremendous sustainable value to food animal production (Myer *et al*, 2015). Ruminants animals are highly valuable because of the demand for their meat and fiber products.

Currently, increases in human population had led to decrease in arable land resulting from urbanization, industrialization and soil degradation, in association with increase in the demand for livestock products. All these had brought about dramatic changes in the global ruminant livestock sector. Among these changes were shift in the size of regional livestock populations and in the management and feeding systems. It was noted that there will be increased demand of

a wider range of quality attributes from animal agriculture, not just of the products themselves but also of the methods used in their production. The livestock sector will therefore need to respond to new challenges of increasing livestock productivity while protecting the environment and human health and conserving biodiversity and natural resources. The microorganisms in the digestive tracts of ruminant livestock have a profound influence on the conversion of feed into end-products which can impact on the animal and the environment. As the livestock sector grows in numbers and productivity particularly in developing countries there will be an increasing need to understand these processes for better management and use of both feed and other natural resources that underpin the development of sustainable feeding systems. (Ellison *et al*., 2017)

Reports have shown that ruminant husbandry contributed about 25 % of the global greenhouse gases (Malmuthuge *et al*., 2012). Though, fermentation activities by ruminant gut flora are an important process of feed digestion and acquisition. However, large amount of methane is generated through the process of methanogenesis by rumen methane producing microbes. All these gases had been reported to end up been released into the environment as a waste products from ruminants (Wallace *et al.*, 2015)

Rumen gut flora comprises of both anaerobic and facultative anaerobes communities that have the capability to degrade lignin and cellulose containing feeds. Report have shown that in developing countries ruminants are majorly maintained on low grade fodder and usually grazing on degraded range of land which could result in their poor nutrient acquisition and productivity. Therefore, selective manipulation of rumen fermentation had been a major area of research and evaluation over the last two decades for potential optimization of ruminant fermentative capacity

thereby improving nutrient acquisition and productivity in animals as well as reducing the environmental pollution (Wallace *et al.*, 2015).

Under standard condition, the co-evolution of animal and its gut microbiota could resulted in stable and the most favoured stable microbial communities that will perform the require fermentation process. Therefore, these (manipulation and process) had led to the quest for the possible optimization of rumen gut flora. Among these are the technology that introduce genetic manipulation of plant fibres for more nutrients acquisition and increase productivity. Likewise, there exist considerable scope for selection and improvement of rumen microbial strains for improved feed utilization, better feed conversion efficiency and production performance of the animals. The biotechnology could also be used for the selection of rumen gut microbial for effective digestion forage plants and reduction in the amount of gas produced. (Ellison *et al*., 2017)

Rumen gut microbial communities could also be modified through chemical additives that will support and stimulate the microbes of interest. Likewise, the naturally occurring beneficial microbes or genetically modified microbes could be introduced into the rumen gut. Report have shown that defaunation processes has successfully eliminated rumen protozoa and brought about increases in protein contents thereby improve growth and productivity of the experimental animals (Ellison *et al*., 2017). Further studies on interspecies trans-inoculation processes of rumen microbes had been reported to be successfully for elimination of dietry toxic products. Others successful methods include introduction of probiotics microbes in animal feeding in other to stabilize rumen fermentation, prevent incidence of diarrhoea and thereby improving feed conversion and growth in livestock. (Myer *et al*, 2015).

It is envisaged that the digestion of most of the constituents of ruminants feed (cellulose and lignin) that form the basis for their nutrients source could not be possible without the fermentative capability of their gut microbes (Myer *et al*, 2015). Also, report had shown that the nature and composition of the ruminants feed could have possible effect on the ecology of the microbes inhabiting their gut and consequently the amount of gases been produced (Malmuthuge *et al*, 2012). Therefore, this research assesses the effect of compound tanniferous plant fodders on microbial ecology and gas production by ruminant animal. Genetic manipulation of rumen microorganisms has enormous research potential in developing countries. In view of feed resource availability more emphasis has to be given for manipulating rumen fermentation to increase cellulolytic activity for efficient utilization of low-grade roughage.

1.2 Statement of Problem

The effect of accumulated greenhouse gases on the environment and associated loss of produced energy by the animals is of great concern to the global world. Microbial rumen fermentation processes has been implicated to increase energy losses such as methane which contribute to the release of greenhouse gas pollutants in the environment and protein losses such as Nitrogen that limit the production performance of the animal. The manipulation of rumen microbial population using antibiotic feed additives has led to risk of residues in the food of animal origin and emergence of multi-drug resistant bacteria that may threaten human health. (Olafadehan and Okunade 2016).

1.3 Justification of the Study

The manipulation of the rumen microbial population to reduce the loss of methane production by use of plant phytochemicals will go a long way to reduce the global challenge and improve

animal yield. Therefore, researchers are increasingly looking into plant extracts and plant secondary metabolites that generally are recognized as safe for human consumption, reduce methane production and consequently improve animal performance. (Olafadehan and Okunade 2016). The antimicrobial activity of plant extracts is attributed to a number of secondary plant metabolites, which include saponins, tannins and essential plant oils. (Myer *et al*, 2015). Many of these beneficial compound in tanniferrous plant foliages have not been fully investigated for their ability to manipulate rumen microbial population and environment for effective ruminant animal production.

1, 3 Aim of the study

Aim:

- The aim of this research is to assess the effect of compound in tanninferous plant fodders on microbial ecology and gas production by ruminant animal.

Objectivesi

1.3.1 The objectives of this research are to:

1 Determine chemical (proximate and phytochemical) composition of the tanniferous foliage plants.

2 Enumerate microbial load in rumen fluid of goat fed with the tanniferous foliage.

3 Characterize the bacterial isolates by Gram staining and biochemical tests for proper identification.

4 Estimate the amount of gases produced by the experimental animals.

5 To determine the total *in vitro* gas production of the tanniferous browse foliage supplements on goat.

Significant of the Study

Strategies to reduce enteric methane production by the ruminant are often limited by the diet , physiological state and the management conditions of the animal, as well as government regulations; resulting in difficulties of approach to the problem of enteric methane mitigation. To this end, the study is therefore significant to determine the effect of three selected browse foliage fed as supplement on the microbial population and methane reduction in goat.

2.0 LITERATURE REVIEWS

2.1 Rumen ecology

The ruminant digestive system is composed of reticulum, rumen, omasum, and abomasum. The rumen is a large fermentation chamber providing an anaerobic environment, constant temperature and pH, and good mixing.

The rumen is the main site for the major fermentation processes (Tharwat *et al.,* 2012). Enzymes present in the rumen are produced by microorganisms. These enzymes are used to digest and ferment food eaten by ruminants, thus, the rumen is considered as a fermentation vat (Aschenbach *et al.,* 2011).

2.2 Rumen microbial biomass and environment

Ruminants are herbivorous mammals considered as latecomers in evolution. The rumen microbial population contains billions of complex microbiota which anaerobically break down and ferment the ingested plant materials Their fore stomach is a very complex environment, which allows them to convert plant tissues into nutritious and useful products. The digestive tract of ruminants is formed by various compartments such as reticulum, rumen, omasum, abomasum, small intestine, cecum, colon and rectum (Appuhamy *et al.,* 2014) The ruminant stomach is

composed by three pregastric fermentation chambers (rumen, reticulum and omasum) (Attwood *et al.,* 2011) Environmental conditions such as temperature (38–42°C), redox potential (250 to 450 mV), pH (5.5–7) controlled by buffer in saliva and osmolarity (260–340 mOsm) (Castilo *et al.,* 2014.) provide the ideal conditions for the digestion of plant material by microorganisms. Fibrous components are hydrolyzed and fermented by the interactions among different microbial communities inhabiting the rumen, producing mainly acetate, propionate and butyrate, CO_2, H_2 and CH_4. Volatile fatty acids (VFAs) are the most important source of energy for the animal (75% of the total amount of the digested energy) (Costa *et al.,* 2013, Belanche *et al.,* 2015) Moreover, microbial cell biomass is the major source of protein and amino acids. Microbial population also synthetizes vitamins B and K and employs detoxification mechanisms for phytotoxins and mycotoxins .

Microbial ruminant ecosystem is composed by a high microbial population density, predominantly obligate anaerobic microorganisms (Castilo *et al.,* 2014). Bacteria are the most abundant microorganisms and more than 50% of the cell mass in the rumen are comprised of at least 50 bacterial genera (10^{10} –10^{11} ml^{-1}), followed by 25 genera of ciliate protozoa (10^4 –10^6 ml^{-1}), six genera of fungi (10^3–10^6 ml^1), methanogenic archaea (10^7–10^{10} ml^1) and bacteriophages (10^8–10^9 ml^1) (Cieslak *et al.,* 2014, Belanche *et al.,* 2014), Danielsson 2016). Nevertheless only 10% of these microbiomes have been identified and described (Danielsson 2016). The interactions of these microorganisms are widely different, namely mutualism, commensalism, syntrophy, competition and depredation (Hook *et al.,* 2012, Gunun *et al.,* 2013, Gunun *et al.,* 2016).

Hydrolysis of plant polysaccharide material is the first step in the enteric fermentation process, and 80% of plant cell material degradation is carried out by bacteria and fungi, and the rest 20% is by protozoa (Gunun *et al.,* 2013, Isah *et al.,* 2014).

In the second stage, monomers are fermented to Volatile fatty acids (VFAs), branched chain Volatile fatty acids (VFAs), organic acids (lactate), alcohols, CO_2 and H_2. VFAs are absorbed by the rumen and omasal walls of the host animal for its nutrition (Danielsson *et al.,* 2016), though several parameters such as rumen fluid, volume, pH and VFAs, concentration can disturb this absorption (Denman, 2015). Free acids can be oxidized by obligate hydrogen producing bacteria to acetate, albeit this reaction is thermodynamically non-favorable, and hence are carried out only in synthropic association with hydrogen consuming bacteria or archaea, which diminish the partial pressure of H_2. When the conditions are not favorable, Volatile fatty acids (VFAs) are accumulated, decreasing the pH and inhibiting rumen microbiome (Freitag *et al.,* 2010, Henderson *et al.,* 2015). NH_3 is produced due to proteolysis and can be used by microorganisms to build their own proteins. The excess of NH_3 is absorbed by the rumen wall and transported by the animal blood (Haas, 2011, Henderson *et al.,* 2015). The digested proteins, lipids and the carbohydrate constituents of microbial cells are exploited in the small intestine for the maintenance of the animal and the production of meat and milk. During enteric fermentation, a large quantity of CO_2 is produced due to diverse biochemical processes. A part of this CO_2 produced is released through eructation or normal respiration, and other part is reduced with H_2 to CH_4 by hydrogenotrophic methanogens. Methane produced is primarily released through eructation and approximately 10–15% is emitted by normal respiration and via flatus (Hristov *et al.,* 2015_a). CH_4 production can be accomplished by the reduction of acetate and methyl-containing C1 compounds, nonetheless these pathways are not common in the rumen

(Kittelmann *et al.,* 2016). About 2–12% of gross energy intake (GEI) produced in the rumen by fermentation is converted to methane, which apart from leading to the loss of the feed energy, results in the emission and consequently, global warming (Leahy *et al.,* 2010, Kittelmann *et al.,* 2016).

2.3 Ruminant microbial diversity and functions

Bacteria are among the important and functional populations in rumen gut and represented about 60 % of the total gut flora (Castilo *et al.,* 2014). Rumen bacteria communities are mostly facultative anaerobes and / or strictly anaerobic non-sporulating genera. Thirty-nine genera and 63 species have been fully described, and more than 300 bacterial species have been isolated. (Kittelmann *et al.,* 2016). Bacterial species are classified based on their morphology and motility, growth factor requirements, substrates degradation and products produced in axenic cultures or according to their substrate preference. Alternatively, they can be classified based on their environmental niche to include free-living bacteria genera colonizing the rumen liquid phase, bacteria loosely associated with feed particles, bacteria firmly adherent on feed particles, bacteria colonizing the rumen epithelium and bacteria attached to the surface of protozoa or fungal. Rumen bacteria comprises of cellulotic bacteria (e.g; *Fibrobacter succinogenes, Butyrivibrio fibrisolvens, Ruminococci albus, Clostridium lochheadii*). The reported bacterial genera with proteolytic efficiency are *Bacteroides rutminicola*, *Bacteroides amylophilus*, *Butyrivibrio* fibrisolvens, *Prevotella albensis* and *Streptococcus bovis* (Kumar *et al.,* 2014). While the amylolytic bacteria (e.g *Bacteriodes ruminicola Ruminobacter amylophilus, Selenomonas ruminantium, Succinomonas amylolítica, Streptococci* bovis), lipolytic bacteria (*Anaerovibrio* lipolytica), lactate-degrading bacteria (*Selenomonas lactilytica, Megasphaera* elsdenii) and pectin-degrading bacteria which includes *Lachnospira multiparus* (Mc-Alister *et*

al., 2015). Although it has been known for some times that the rumen was a complex microbial ecosystem, more recent molecular studies have revealed more complex diversity. (Mc-Alister *et al*., 2015).

Protozoa also constitute part of the large proportion of the rumen biomass, 20 to 40 %. More than 100 protozoal species have been identified but no more than 15 to 20 species are found in an individual ruminant (Chaucheyras-Durand and Ossa, 2014). The most common genera are *Dasytricha*, *Entodinium*, *Polyplastron*, *Eudiplodinium*, *Epidinium* and *Ophryoscolex* (Kenters *et al*., 2011). Overall estimates based on numbers generic composition may not give a clear insight due to differences in cell size to volume ratio. For example, the genus *Entodinium* had been reported to account for about 80 % of the total protozoa. However, the volume ratio of *Entodinium* was estimated to be about 40 % of the total protozoa population in the rumen. The possibility exists that the voluminous ciliary protozoa (*Isotricha*, *Metadinium*, *Polyplastron*, *Ophryoscolex*, and *Epidinium*) might contribute to a greater extent to the metabolic action of the total protozoa in the gut. (Kim *et al.*, (2011); Hook *et al.*, 2012). Among the gut protozoan, Holotrichid metabolic function include soluble sugars utilization, meanwhile *Entodiniomorphs* species was reported to have a diverse capability for different substrates utilization. *Entodiniomorphs* species, with the exception of small *entodinia*, were capable of ingesting small plant particles and metabolize the cell wall components. Reports have emphasized the capability of *Entodiniomorphid* ciliates genera on amylase production and the engulfment of starch granules (Hook *et al.*, 2012). The phagocytized starch granules are usually stored as granules or in incorporated in the skeletal plate as amylopectin. (Kim *et al.*, 2011). Likewise, assimilated proteins get digested within the cells. Comparatively, ciliated protozoa have been reported to have lower capacity to transport amino acids into the cell as compared to bacteria. (Kenters *et*

al., 2011).Though, some researches had reported that their function is not yet entirely clear. (Kenters *et al*., 2011). However, major reports have shown that they are known to digest plant materials and may help slow the rate of fermentation with high concentrate diets by engulfing starch granules which may moderate rumen acidosis (Hook *et al.*, 2012). Rumen protozoa exhibit relatively low activities against soluble proteins, but rapidly engulf and degrade rumen bacteria and other particulate protein sources (Hook *et al* 2012). It has been shown that predation of protozoa on bacteria in rumen greatly increased intra ruminal N recycling and that the removal of protozoa from the rumen (defaunation) can markedly increase the flow of bacterial protein leaving the rumen (Cieslak *et al*., 2012; Castillo *et al.*, 2014).

Anaerobic fungi account for approximately 8 % of the microbial biomass in the rumen and have a slower generation time (24 to 32 h) than bacteria. They are believed to play a role in the degradation of recalcitrant fibre. Six genera, *Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyces* and *Piromyces*, and twenty species have been described (Sebata, 2011; Gunun *et al*., 2014; Elghandour *et al*., 2015).

Methane producing organisms in the rumen belong to the domain archaea. (Hook *et al., (*2012) Pilajim and Wanapat 2013). They are characterized by a unique cellular membrane that differs from that of the bacteria. Most archaea identified in the rumen belong to known methanogen clades with a predominance of methanogen Brevibacter spp. In the rumen, the major substrates used by methanogens are CO_2 as the carbon source and H_2 as the main electron donor. Formate is also an important electron donor used by many rumen hydrogenotrophic methanogens and may account for up to 18% of the methane produced in the rumen. (Sebata, 2011). Methanogens in rumen microbial community are estimated to comprise, 0.3% to 3% of the total rumen microbial biomass. (Salah *et al*. 2014) The diversity of the rumen methanogens is much smaller,

and their diversity is much lower than that of rumen bacteria. Surveys of archaeal 16S ribosomal RNA gene sequences from ruminants around the world fed a variety of diets conducted by Salah *et al*., (2014) show that three methanogen groups dominate. These are *Methanobrevibacter spp*., *Methanomicrobium spp*. and Rumen Cluster C (RCC), also known as Thermoplasmatales Aafiliated lineage C but more recently proposed as a seventh order of methanogenic archaea, the 'Methanoplasmatales. Salem *et al*. (2014) reported that only eight species of ruminal methanogens have been isolated into pure cultures: *Methanosarcina barkeri , Methanobacterium bryanti, Methanobacterium formicicum, Methanomicrobium mobile, Methanobrevibacter millerae, Methanobrevibacter olleyae, , Methanoculleus olentangyi, and Metanobrevibacter ruminantium*.

2.4 Manipulation of the rumen microbiome

Adequate knowledge about ruminant digestion and manipulation of the rumen micro biome has been seen as an efficient way ruminant can effectively utilize feed. (Salah *et al*. 2014) This is necessary for better ruminant production in a cost effective manner. In order to effectively manage the production costs in animal agriculture, it is highly important to improve feed efficiency, most especially during elevated feed costs or reduced livestock values period. Also, enhancements in feed efficiency can be one of the effective ways of reducing feed usage as well as maintaining animal performance. (Salah *et al*. 2014) Likewise the synergy in feed efficiency and rumen gut flora could facilitate selection of more efficient breeding stock with little stress on individual feed intake data collection and other intensive task. Despite how important this area is to scientific scope of environmental ecology as related to feed efficiency with rumen gut flora in animal, limited research interest has been published to date. (Ellison *et al*., 2017). However, the research in this area has become more interesting with improved and affordable sequencing

technologies (Mbomi *et al*., 2011; Ellison *et al*., 2017). Biotechnology has been used to modify ruminal fermentation by promoting or diminishing certain fermentation processes, leading to a higher efficiency in animal productivity (Mosoni *et al*., 2011; Aguiar *et al*., 2014). This biotechnological management can be categorized into the following five groups: 1) modification of diet and fermentation profile, 2) transformation of food before consumption, 3) manipulation of ruminal microorganisms, 4) use of microorganism fermentation activators, and 5) use of substances with ruminal activity (Singh *et al*., 2011; Castillo *et al*., 2014).

Defaunation can be used to change rumen community to favour rumen digestion which lead to boom in bacteria biomass, and consequential improvement in starch metabolism, and decreasing methane and propionate concentrations as well as efficiency in fiber digestion (Morgavi *et al* 2010). Protozoa are known to be effective in Hydrogen ion (H+) production, which are then used by methanogens to reduce carbonIVoxide (CO_2) to methane (CH_4) gases. Therefore, the removal of protozoa could disrupt the process of methanogenesis due to the reduction of available Hydrogen ion (H+) for methane production (Mosoni *et al*., 2011). Additionally, defaunation can be used to manipulate proteolysis process and thereby resulted in changes in the ammonia production (Ozutsumi *et al.*, 2005; Bouazza *et al*., 2011). The positive correlation between the amount of ammonia in defaunated animals and proteolysic activities had been reported (Olafedahan and Okunade, 2016). Therefore, the important function of protozoa in ruminant growth has been shown through their contribution to the degradation of the main feed components, which is an important part in the fermentation process. Also, decreases in post prandial pH was reported to be regulated by protozoa because of their modulating effect on amylolytic bacterial populations that uses starch as a substrate for its fermentation. (Belanche *et al.*, 2019)

2.5 Effect of wild legume seed based diets on rumen ecology

Most of the wild legume seeds contain phenolic compounds such as tannins, saponins etc which have been stragetically used to manipulate rumen environment for omptimal feed utilisation. Recently, the use of plant secondary compounds, including tannins and saponins that are potent modifiers of ruminal fermentation and intra-ruminal recycling of microbial protein aims at improving the efficiency of dietary energy and nitrogen utilization while mitigating nitrogen losses and rumen methane production in ruminants (Hristov and Jouany, 2005; Kreuzer *et al*., 2009;; Mao *et al*., 2010, Patra *et al*., 2017).

2.6 Effect of wild legume seed based diets on rumen fermentation parameters

Animal nutritionists have intensified their search to exploit or develop new natural products as feed additives to manipulate ruminal fermentation for better feed efficiency utilization, essentially to replace antibiotics in the feed. The use of phenolic containing feed ingredients as one of the common natural products (probiotics or directfed microbials (DFM), prebiotic oligosaccharides and exogenous enzymes) to manipulate rumen fermentation without compromising the growth, the production and health of the ruminants had been identified. Such compounds like saponins, tannins, lignins, flavanoids, and essential oils are particularly prevalent in many tropical plants. (Patra *et al*., 2017). At low doses, they have the potential to improve ruminal fermentation, but at high doses, they have adverse effects on ruminal fermentation and animal health and immunity. The trend is particularly evident in Europe, Australia and New Zealand (Wallace *et al*., 2002; Calsmiglia *et al*., 2007). The major phytochemicals that have been tested in *in vitro* ruminal fermentation and in *in vivo* studies include essential oils, tannins, and saponins. There are many wild legume seeds such as Daniella seeds which naturally possess plant secondary compounds such as tannins and saponins which

can drastically manipulate the ruminal protein fermentation to the benefit of ruminant animals. These compounds, including tannins, saponins and flavonoids, are known to have an impact on rumen microbial metabolism by inducing changes in the fermentation conditions (pH, propionate proportion, and protein degradation) (Balcells *et al*., 2012). Rumen acidity (pH) normally remains within the normal range when moderate tannin containing based diets are fed to ruminants. For instances, Cieslak *et al*. (2014) observed that pH was not affected by tannins addition, which is consistent with what was observed by Balcells *et al*., (2012). where condensed tannins from *Leucaena* sp. Affected IVDMD only by 7% and did not change ruminal pH. Similar trends were observed when Olafadehan and Okunade (2016) fed tannin-containig diet to Sheep and goats respectively. Legumes is important as animal feed due the presence of large amount of nutrients and protein quality which could be expressed as nitrogen. (Olafadehan and Okunade 2016).

Protein from legumes are mostly degraded and metabolized rapidly into amino acids and ammonium in the rumen. These metabolic products contribute to the growth of ruminant gut microorganisms, but they can also be dissipated as ammonium when energy in the diet is not sufficient. Report had shown that excessive degradation of proteinous compound in the rumen resulting from consumption of leguminous fodder could decrease the nutrient efficiency of the ruminant animals (Valderrama and Anrique, 2011). Feed is highly important to animal health when it contains large amount of proteins that could be metabolize into amino acids and absorbed into the blood stream (Kononoff *et al*., 2007). The average rumen hydrogen ion concentration and ammonia-nitrogen (NH_3-N) concentration from several works on supplementation of legume seeds were about 6 and > 15 mg/dL for pH and NH_3-N respectively.

which signifies the optimal levels for microbial growth in tropical conditions (Hristov and Jouany, 2005; Gunun *et al*., 2013; Gunun *et al*, 2016).

In studies involving ruminant metabolism and nutrition, Daniellia and propolis seeds reduced ammonia (NH_3) production (Aguiar *et al*., 2014; Olafadehan and Okunade 2013). These reports Salso agreed with the observations of Gunun *et al*., (2013 and Gunun *et al*, (2016).

The reduction in ruminal NH_3-N may be as a result of displacement of strong antimicrobial activity against hyper-ammonia producing rumen bacteria by phenolic compounds. Bhatta *et al*., (2013) attributed reduction in rumen ammonia nitrogen to the formation of complexes between hydrolizable tannins (HT) and condensed tannins (CT) with proteins that are minimally degraded by ruminal microbes and this resulted in reducing the concentration of ammonia in rumen fluid. Similarly, Olafadehan and Okunade (2016), reported that ruminal ammonia concentration was lower in the rumen of dairy cows supplemented with *Acacia mearnsii* tannins, which may lead to i) reduction of the protein degradation rate in the rumen (Patra and Saxena, 2011); ii) improved efficiency of microbial protein synthesis (Bhatta *et al.,* 2013); and iii) reduced urea N excretion in urine (Grainger *et al.,* 2009). Phenolic compounds significantly changed ruminal fermentation, a quadratic response was observed in acetate molar proportion and total Volatile fatty acid (VFA) concentration (Paula *et al*., 2016). Costa Jr. *et al.,* (2012) and Morsey *et al., (*2015) also observed greater total VFA concentration and greater molar proportion of acetate when propolis extract was added in the diets. Most of the wild legume seeds based diets offered to ruminant animals in moderate level furnish them with adequate Volatile fatty acid (VFAs) (majorly propionate and acetate) for efficient energy metabolism (Olafadehan 2013: Morsey *et al*., (2015). Moreover, acetate formation may be an indication of healthy ruminal fermentation which is important for the animal's production and wellbeing (Paula *et al*., 2016). However, Phenolic

compounds such as tannins and saponins may not have any significant difference on fermentation parameters. Wei *et al.,* (2012) reported that the addition of three plant extracts of tea saponin, mulberry i.e *Afzelia* extract and ecdysterone did not show any significant difference on fermentation parameters such as pH, NH_3 N, total VFA, individual VFA and the ratio of acetic/propionic acid compared to concentrate and with inclusion of no extracts at 28 h of incubation. According to McSweeney *et al.,* (2001) hydrolizable tannins can be degraded by some microbial species in the rumen, the toxicity appears to be due to absorption of degraded products of hydrolizable tannins and high amount of phenols in the bloodstream, which increase the capability of liver to detoxify phenols. In summary, feeding ruminants with low to moderate doses of some of the phenolic compound such as tannins which is present in legume seeds have the potential to improve ruminal fermentation, (Patra *et al*., 2017). but at high doses, have adverse effects on ruminal fermentation and animal health and immunity. (Wei *et al.,* 2012) The major phytochemicals that have been tested in *in vitro* ruminal fermentation and in *in vivo* studies include essential oils, tannins, and saponins (Patra *et al*., 2017). The effectiveness of plant compounds in modifying ruminal fermentation has not been consistent or conclusive. A better understanding of the chemical structure and activity relationship is required to fully exploit the application of plant compounds in ruminant animal production (Patra *et al*., 2017).

2.7 Effect of wild legume seed-based diets on rumen microbial count and Isolates

Many previous studies reported that tannins-and saponins-containing plants and their extracts such as wild legume seeds at an appropriate dose, appeared to be useful in suppressing the proliferation of rumen ciliate protozoa and methanogens (Chanthakhoun *et al*., 2011; Anantasook and Wanapat, (2012). For instance, supplementation with Mao seed has the potential to manipulate rumen fermentation, by decreasing protozoa (Gunun *et al*., 2014, Gunun *et al*.,

2016). Several researchers (Singh *et al*., 2011; Isah *et al*., 2014; Morsey *et al*., 2015; Nguyen *et al*., 2017) had reported reduction in rumen protozoa population with increasing phenolic compounds, especially tannins and saponnins in the diets fed to ruminant. Anantasook and Wanapat, (2012) found that supplementation with rain pod meal has the potential to manipulate rumen fermentation, by depressing protozoa and methanogens and by reducing methane production in in vitro. Similar results were obtained when (Gunun *et al*., 2014) supplemented rain tree pod meal containing tannins and saponins in the diets of dairy steers. Protozoa are sensitive to saponins because their membrane sterols bind with saponins (Wei *et al*., 2012) while they have an indirect action on methanogens by reducing the activity of methanogen for a lower supplying of hydrogen (Mao *et al*., 2010). This process resulted in increases in bacteria and fungi population, propionate production, partitioning factor, and protein yield and efficiency of microbial protein synthesis, hence improving performance in ruminants thus Wanapat *et al.* (2012) ; Nguyen *et al*., 2017) also reported increase or flow in bacteria and zoospores with increasing levels of Leucaena silage, but not at the highest level of condensed tannin (CT) uptake with 100% Leucaena silage**.** This could be due to the interaction between protozoa and fungal zoospores. Also, the cellulolytic and proteolytic bacterial population is a response to the types and level of phenolic compounds in the diets. Isah *et al*., (2014) reported protozoa to be phagocytic (they engulf ruminal bacteria), therefore, decrease in their population will resulted to increase in bacteria and fungi populations. Rumen microbes have different tolerance capacity to tannins and differed among the groups also (Kumar *et al*., 2014). They further explained: rumen fungi, proteolytic bacteria and protozoa are more resistant to tannin as compared to other microbes. There was decrease in protozoal population in goats offered pakar leaves (tanniferous leaves) by (Singh *et al*., 2011) showed that the growth of cellulolytic and proteolytic bacteria

was inhibited by tannins in an artificial rumen. This corroborates the submission of Vliwisky *et al*., (2002) on significant decrease in bacterial count by adding hydrolysable tannin in the diet of lamb at 1 and 2 g/kg DM, whereas, protozoal population remained unaffected. Inhibitory activity of tannins against bacteria may be due to the ability of tannins to form complexes with the cell wall and membrane of bacteria causing morphological changes of the cell and the secreted extracellular enzymes (Smith *et al*., 2005; Cieslak *et al*., (2012).

Literature data reporting the effects of phenolic compounds on ruminal protozoa strains is scarce. However, Paula *et al*., (2016) found that there was a significant linear reduction of the *Entodinium* protozoa population as with increasing level of phenolic compound. This is further confirmed by Cieslak *et al*., (2014). They found a significant reduction of *Entodinium* in the rumen of water buffaloes fed propolis extract compared to a control diet. *Entodiniomorphids* protozoa, such as, *Entodinium* and *Epidinium* are capable of ingesting and digesting insoluble proteins, as well as bacterial proteins therefore suppressing these protozoa may reduce NH_3–N formation in the rumen. Furthermore, Cieslak *et al*., (2014) demonstrated inbibitory effects of tannins on proteolytic rumen bacteria: *Streptococcus bovis, Eubacterium* sp., *Prevotella bryantii, Butyrivibrio fibrisolvens* and *Clostridium proteoclasticu* Bodas *et al., (*2012), reported reduction in some of the rumen bacteria strains: *Ruminococcus albus*, *Fibrobacter succinogenes*, *Streptococcus bovis, Prevotella bryantii*, *Butyrivibrio fibrisolvens* when various concentration levels (0.05-0.30) of tammic acid .Min *et al*., (2005) found that when the diet of sheep changed from perennial rye grass/white clover pasture (which does not contain condensed tannin (CT) to *L. corniculatus* (32 g CT/kg DM), the population of proteolytic rumen bacteria *Clostridium proteoclasticum, Eubacterium sp., Streptococcus bovis, and Butyrivibrio fibrisolvenes* were decreased. They reported that some of these strains of rumen microorganisms, such as

Clostridium proteoclasticum and *Ruminococcus albus*, were resistant to condensed tannin in leaves at a level of 200 μg /mL. Addition of phlorotannins to rumen bacterial cultures inhibited growth of *Fibrobacter succinogenes*, but stimulated growth of *Streptococcus bovis* and *Prevotella bryantii* (Wang *et al.*, 2009). Not all rumen microbiota are affected by phenolic compounds, reports on resistance of rumen microbes had been documented. The inhibitory effect of some bacterial species is due to the long period of exposure to tannins, rumen bacteria could acquire resistance. Patra and Saxena, (2011) suggested mechanisms of bacterial tolerance to dietary tannins such as synthesis of tannin-complexing polymers, formation of extracellular glycocalyx from tannins and cell wall/membrane, tannin degradation and synthesis of siderophores which chelate tannins and cations. Microorganisms have different tolerance capacity to tannins and differed among the groups also. Rumen fungi, proteolytic bacteria and protozoa are more resistant to tannin as compared to other microbes (Kumar *et al*., 2014).

2.8 Effect of wild legume seed based diets on methanogens and enteric methane mitigation

Rumen methanogens are the major culprits of rumen methanogenesis by utilizing the enteric fermentation end products (i.e, H_2, CO_2, formic acid, or methylamines) to form methane (CH_4). In the rumen ecosystem, rumen protozoa are also involved in methanogenesis because of their ecto and endo symbiotic association with methanogenic archaea which utilize H_2 produced by the protozoa to produce CH_4 Research on enteric methane production in the rumen has attracted great interest in the last decade, not only because it is a feed energy loss to the ruminat animals (Patra *et al*., 2017), but also its major contribution to devastating effect of global warming In a report, about 19 % of global methane emissions were attributed to enteric fermentation by ruminant gut microbes and manure composting (Knapp *et al*., 2014). Domesticated animals had been reported to contribute more than 13% of global methane emissions. The ruminants can

release about 10.0 % of their ingested feed through methane production by way of eructated gases from the rumen resulting from feed fermentation by gut flora (Nicholson *et al*., 2007). Therefore, reduced CH_4 production by ruminants has been recognized as an important goal because it reduces the GHG emission and improves feed efficiency. (Nicholson *et al*., 2007).

Research has confirmed that many wild legume seeds naturally possess plant secondary compounds such as tannins and saponins which can drastically manipulate the ruminal protein fermentation to the benefit of ruminant animals if strategically used in ruminant nutrition. For instance, supplementation with Mao seed has the potential to manipulate rumen fermentation, by decreasing protozoa (Gunun *et al*., 2014; Gunun *et al*., 2016). At an appropriate dose, Condensed tannin and saponin-containing plants have been shown to suppress protozoal and methanogenesis population and increase bacteria and fungi population, propionate production, partitioning factor, and yield and efficiency of microbial protein synthesis, hence improving performance in ruminants (Wanapat *et al*. 2012). Mangosteen (*Garcinia mangostana*) peel is a fruit by-product containing high level of condensed tannins (CTs) and saponnins (SPs), and it has been reported that supplementation of mangosteen peel powder (MSP) at 100–300 g/head/day exhibited no negative effect on feed intake,nutrient digestibility, ruminal fermentation characteristics, nutrient utilization, and microbial protein synthesis while influenced on rumen methanogen population, hence possibly mitigating CH_4 production based on the previous findings in in vitro (Wanapat *et al*. 2014;), dairy cows (Trinh *et al*. 2012; Buccioni *et al*. 2015), beef cattle (Wanapat *et al*. 2014), and buffaloes (Wanapat *et al*. 2014; Buccioni *et al*. 2015), respectively. In many studies (*in vitro* and *in vivo*) it has been demonstrated that with temperate legumes (*Hedysarium coronarium, Lespedeza cuneata*, *Lotus corniculatus* and *L. uliginosus*) and tropical legumes (*Calliandra calothyrsus, Flemingia macrophylla*) that contain secondary compounds such as condensed

tannins (CT) have possibility to reduce methanogenesis. Tannins and phenolic monomers have been found to be toxic for some of the rumen microbes, especially ciliate protozoa, fiber degrading bacteria and methanogenic archaea, and as a result methanogenesis in the rumen can also be reduced. However. Wanapat *et al*. (2014) indicated that with some tropical legumes with tannins (i.e.*Calliandra calothyrsus* and *Fleminigia macrophylla*) there low fiber digestibility also contributes to the reduced in vitro CH_4 production measured with these legumes. Recently, Babayemi and Bankole (2006) and Isah *et al*., 2015) had reported the methane mitigation effects of slected tropical tanniferous legume leaves and seeds both in vitro and in vivo repectively without compromising the animals' performance. However, few contrary reports abound on lack of effect of plant secondary metabolites on CH_4 (Isah *et al*., 2015). Non-effect of CT-containing forages on protozoa and bacteria populations together with a decrease in CH_4 production had been reported. The mitigating effect of tannins on gut microbes is often considered to be due to a decrease in methanogens, as several experiments reported a decrease in methanogens in tanniferous forages feeding ruminants. Report have indicated that TRP shown no effect on methanogens (Pilajun and Wanapat, 2013). An absence of toxic effect on methanogens when feeding TRP could be related to tannin-degrading enzymes and microbial extracellular secretions which could reduce the effect of tannin (McDonald *et al*., 2012, Anantasook *et al*., 2012, Weiner 2015).

More studies on tolerance, or adaptation and methanogens resistance of the gut flora to tannins are needed to characterize the causes of this effect (Carberry *et al*., 2012). Newbold *et al*. (2015) reported initial decreases in methanogen populations in the presence of TRP with sharp recovery after 24h of observation. Studies had also demonstrated that microorganisms develop ways to survive the prolonged tannin exposure, (Kittleman 2014, Myer *et al*., 2015).

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The study was carried out in Animal production Technology Department Farm, Federal College of wildlife Management, New Bussa, Borgu Local Government area of Niger State. Borgu is located in the savannah zone between latitude 10° 75' N and 10° 15' N as well longitude 40° 33' E. Its elevation was 125.5m above sea Level (Abu, 2003).

3.2 Chemical analysis of Tanninferous Foliages

Some selected tanniferrous plant fodder were (**Fm** = ***Flemingo*** *macropum* **Dm** = *Detarium microcarpus*; **Aa** = *Afzelia africana***;** **As** = *Acacia saligma*; **Do** = *Daniellia oliver**i;*** **Gs** = *Gliricida sapium*) pre - screened and analyzed for its proximate composition (crude protein, crude fibre, ether extract and ash) according to AOAC (1995). The fibre fractions; neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were

determined according to Van Soest et al. (1991). Cellulose and hemicellulose were calculated as the differences between ADF and ADL, NDF and ADF respectively in order to know and select the suitable fodder based on concentration or amount of condensed tannins for the experimental diet after which the basal diet comprised of (Maize, offal 25kg, cowpea husk 45,50kg, Groundnut cake (GNC) 20kg, Brewers dried grain 5kg, Vitamim – mineral Premix 1kg, Dicalcium phosphate 2kg,sulphur powder 1kg and Table Salt 1kg = 100kg) supplemented with suitable selected Samples of browse foliage were equally analyzed according to the standard methods of Association of Official Analytical Chemists (AOAC,2002). Dry matter (DM) crude protein (CP), ether extract (EE), ash and fibre fractions (NDF, ADF, ADL) were determined as described by Non-fiber carbohydrates which were estimated as 100−CP−NDF−EE−ash (AOAC, 1995)

3.2 Experimental animals and management

Twelve growing Red Sokoto bucks of 7 - 9 months old with average initial weight of 9.00±0.25 kg were used for the study. Each goat was housed in individual pen (1.20 m × 0.80 m × 0.70 m) furnished with drinking and feeding facilities. The goats were treated against endo parasites and ecto parasites prior to the commencement of the experiment. They were randomly allocated to three dietary treatments in a completely randomized design. Each treatment was replicated with three animals. The experimental diets were formulated to meet the nutritional requirements of growing goats. The animals in first treatment (control) were served with basal diet of threshed sorghum top only, while the remaining three treatments were supplemented with *Detarium microcarpum, Daniellia oliveri, and Afzelia Aaricana.* The experimental diets were offered as complete ration mix (forage and concentrate) in two equal meals. The experiment lasted for 64 days. The leaves including petioles were plucked, wilted overnight and served to the animals.

Known quantity of each browse leaf and the basal feed were chopped manually into 10cm pieces and fed to the goats at 09:00 hour and 16:00 hour, respectively. Browse foliage were served after the basal feed. Provision was made for daily feed allowance of 10% above that of previous day`s intake. Clean water was provided ad libitum daily.

3.3 Sample Collection.

About 100 mL of representative rumen content were collected before morning feeding on the 64th day of the experiment from each buck with the aid of suction tube i.e the last day of the experiment as described by (Babayemi and Bamikole, 2006). The rumen liquor was collected into the thermo flask that had been pre warmed to a temperature of 39˚C.

Before commencing the length of the tube required to be passed was measured, to ensure that the tube was rested in the rumen. Tubes with internal diameters of 1.5 cm were used because tubes less than 1.5cm may become obstructed with ingesta A speculum was fixed in the mouth to allow the tube to be passed without damage from the teeth. The tube was passed over the base of the tongue (or through the gag) into the oesophagus. As the animal swallows, the tube is advanced down the esophagus until it reaches the rumen. Placement of the tube within the rumen is confirmed by auscultation of the abdomen as air was simultaneously blown into the tube. The distinctive odor of fermented gas was detected coming from the end of the tube .A stirrup pump was attached to the tube and was used to withdraw a sample. The initial fluid portion was discarded because it often contains an excessive amount of saliva and always elevates the pH of the fluid. When the sample was collected, the tube was kinked to prevent loss of ruminal fluid from the tube, and removed with a downward motion to prevent rumen contents from leaking out of the tube and entering the trachea

3.4 Physical characteristics were carried out by observation.

Colour : The colour of rumen fluid was determined by visual inspection or macroscopical examination in a transparent plastic and glass tube of a smaller diameter

Odour : The odour was determined by smell, the sample was closed in an air-tight container for a minimum of five minutes and then opened to perceived the odour

Consistency : Rumen fluid consistency was assessed by slowly turning a glass tube half-filled with rumen fluid 45-60° left and right from an upright position in a fast motion for foam or bubbles to form on the top of the sample and the result from viscous to watery was observed. (Wanapat *et al*. 2012).

3.5 Chemical characteristics

3.5.1 The pH was measured using pH meters immediately after sampling.

3.5.2 Sedimentation activity test

10ml of rumen fluid was put in a test tube and allow to stand. The time needed for completion of sedimentation of fine particles and floatation of coarse solid particles was measured.

3.5.3 Methylene blue reduction test

20 ml of rumen fluid was mixed with 1 ml of 0.03 % methylene blue in a test tube and allow to stand at room temperature. The time needed for the color of the mixture to be changed was measured.

3.5.4 Cellulose digestion test.

10 ml of rumen fluid was mixed with 0.3 ml of 16 % glucose. A thread of pure cellulose was immersed and the lower end is weighted by a glass bead. The tube were Incubated at 39ºc and the time for the bead to be dropped free at the bottom of the tube was recorded..

3.5.5 Nitrate Reduction test

10 ml of sieved rumen fluid was placed into each of three test tubes and 0.2, 0.5, 0.7 ml of 0.025% potassium nitrate solution were added to the three tubes. The three tubes were put in a water bath at 39 ºc. Every five minutes one drop from each tube were placed in a small ceramic plate. 2 drops of reagent I (2 ml of sulphanilic acid in 30 % acetic acid to make 200 ml) and 2 drops of reagent II (0.6 ml alpha-naphthylamine+16 ml conc. acetic acid were added to each drop and 140 ml distilled water was also added. The change of color was observed

5.6 Glucose Fermentation test

0.5 ml of 16 % glucose solution was added to 10 ml of rumen fluid. The mixture was place in a fermentation saccharometer and keep at 39 ºc. The result was read after 30 and 60 min.

3.6. Examination for protozoa

3.6.1. Qualitative examination:

A thin film of freshly collected rumen sample was prepared on free grease microscopic slide and the motility of protozoa was examined under low power magnifying microscope.

3.6.2. Quantitative examination

1 ml of strained sample was diluted with 15 ml saline solution and 5ml logo's iodine solution and shake gently. 0.1ml of the mixture was spread on glass slide in an area under cover glass of 22 X 50 mm. 30 fields in the slide was counted using low power (X10).

3.6.3. Microscopic examination of Bacteria

Rumen fluid were Centrifuge at 100 per revolution, the supernatant was discarded and a smear was made by spreading a drop of the sediment on a clean grease free microscope glass slide allow to air dried and stained using gram staining techniques .

3.6.4. Microscopic examination of Fungi

A drop of sample from supernatant was drop on a clean grease free glass slide and a drop of lugol's iodine was added and slide was examined using low power magnification

3.6.5 Medium Preparation

The following media were prepared according to manufacturer instruction. Thioglycollate broth, Anaerobic blood agar, Egg-yolk agar (EYA), Bacteroides bile esculin agar (BBE), Laked Kanamycin-vancomycin blood agar (LKV), Anaerobic phenylethyl alcohol agar (PEA), Cycloserine cefoxitin fructose agar (CCFA) and Medium 10.

The growth medium 10 contained: 15 ml Mineral Solution I (KH_2PO_4 3.0 g; $(NH_4)_2SO_4$ 6.0 g; NaCl 6.0 g; $MgSO_4$ 0.6 g; $CaCl_2 \cdot 2H_2O$ 0.795 g per Litre), 15 ml Mineral Solution II (K_2HPO_4 3 g/litre), 0.25 g Yeast Extract, 1 g Tryptone, 0.1 ml Resazurine (0.1%), 0.2 ml Hemin (0.05%), 0.5 g Microcrystaline Cellulose, 0.1 g Cellobiose, 0.4 g Sodium Carbonate, 20 ml Clear Rumen Fluid, 100 ml Distilled Water and 50 mg Cysteine Hydrochloride were prepared and used . Slight modifications were made and the final medium were composed of additionally, 22.85 µL Griseovulvin (2.5µ g mL^{-1}) and Augmentin to prevent rumen fungi and rumen bacteria from growing on the plates After autoclaving.. For the defined medium, 310 µL volatile fatty acid was added. The media was autoclaved in the anaerobic bottles for 20 min at 121°C.

3.6.6 Culturing of Rumen Microorganisms

Serial dilution of rumen sample were prepared up to 10^{-10} using conventional techniques Strict anaerobic conditions were maintained during the culture procedure. 100 µL aliquot of serially diluted rumen sample were plated in duplicate on each agar medium. The plates were incubated at 39 °C inside the anaerobic chamber for 3 days, 52 plates were used overall. For control I, 2 plates from each media type were not inoculated but were incubated along with the other plates. Additionally, all buffers that were used for the dilutions were plated without the rumen samples for control II. Total viable bacteria and fungi were enumerated using colony counter, afterward each colonies observed were subjected to sub-culturing which depend on the colour, smell and shape observed, another sub-culture was done after 48h which led to pure culture of this organism in another sterile Petri dishes for each bacterial colony using the same medium (selective, differential and indicator) , after obtaining a pure culture of this bacterial, they were store in nutrient agar medium.. Biochemical tests were performed for the identification of bacterial.

3.6.7 Isolation and identification of Rumen Microorganisms

Gram staining test was performed on each of the isolated bacteria. The bacteria slide was heat fixed by passing it through the flame of a bunsen burner, this slide sample was kept for some seconds before a dropper was used to apply the primary stain (crystal violet) to the slide and allowed to stay for 1 minute. The slide was gently rinsed with water to remove excess stain The slide was covered with alcohol for 10 seconds, after which was immediately rinsed with water. The secondary stain, safranin, was applied and allow it to stay for 1 minute. It was gently rinse

with water for 5 seconds. The slide was viewed using a compound microscope. The gram-negative cells stained red while the gram-positive cells appeared purple and blue.

Bacterial isolates were identified using their morphological and biochemical characteristics with the guidance from Bergey's Manual of Determinative Bacteriology . (Hook *et al., (*2012).

Catalase test: This test was used to check the production of enzyme catalase. For this test a clean microscopic slide was used. A drop of 3% H2O2 was put on the microscopic slide aseptically. A loopful of bacterial culture was taken and mixed with 3% H2O2 solution on the slide and the presence of the bubble production observed.

Sugar fermentation test: Approximate 100 ml of the nutrient broth solution was prepared in conical flask and 1 ml phenol red was added to it. This medium was autoclaved at 121°C for 15 min and cooled at room temperature. A syringe filter sterilized solution of 1% glucose was prepared under aseptic conditions. In all sterilized test tube, 5 ml of the broth and 100 µl of the glucose solution was taken and labelled. Then these test tubes were kept at room temperature for 24 h to check the contamination. After 24h, all the test tubes were inoculated with freshly grown bacterial culture and incubated at 37°C for 24h - 48h. for homofermentation, there were production of acid along with the change in colour of the medium from red to yellow, and in heterofermentation there was gas production in Durham tube alongside the change in the colour.

Coagulase test: A suspension of bacterial cells was prepared and mixed into a drop of rabbit plasma on a microscope slide. The presence of bound coagulase resulted in visible clumping of bacterial cells on the microscope slide.

Indole Test (IND) was performed by culturing the isolated bacteria organisms in peptone water medium containing tryptophan in a screw capped tube, incubated for 24 h at 37°C.Kovac's 0.5mL was added for each of the bacteria isolated, observed and result recorded accordingly.

Oxidase Test (OXI) test was used to assess the bacteria which produce the enzyme cytochrome Oxidase. Filter paper was moistened with a few drops of 1% tetramethyl-p-phenylene diamine di-hydrochloride. With a wooden applicator, growth from pure culture plate was smeared on the paper then the experiment was observed and result recorded accordingly.

Urease test: Christensen's Urea Agar was used, a heavy inoculum from an 18- to 24-hour pure culture was used to streak the entire slant surface. The tube was incubated with loosened caps at 37^{o}C. The color change from bright pink (fuchsia) was observed at 6 hours, 24 hours, and every day for up to 6 days.

3.6.8 In Vitro Gas Production

All laboratory handling of rumen fluid were carried out under a continuous flow of carbon IV oxide. Two hundred milligram (200 mg) of the oven dry and milled leaves of each experimental diet were accurately weighed into a calibrated transparent 100 ml glass syringes fitted with plungers. In vitro incubation of the samples was conducted in triplicates. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution (g/liter of 1.985 (Na_2) HPO_4 + 1.302 KH_2PO_4 + 0.105 $MgCl_2.6H_2O$ + 1.407 NH_2HCO_3 + 5.418 $NaHCO_3$ + 0.390 Cystene HCl + 0.100 NaOH) and three blank samples containing 30 ml of medium (inoculums and buffer) only were incubated at the same time. The syringes were placed in a rotor inside the incubator (39°C) with about one rotation per min. The gas production was recorded at 3, 6, 9, 12, 18, 24, 36 and 48 h. At post incubation period, 4 ml of (10M) Sodium hydroxide

(NaOH) were dispensed into the each incubated sample. Sodium hydroxide was added to absorb carbon dioxide that was produced during the process of fermentation and the remaining volumes of gas were recorded as methane according to the report of (Isah *et al*., 2015). The averages of the volume of gas produced from the blanks were deducted from the volume of gas produced from samples.

3.6.9 Statistical analysis

Data obtained were subjected to Analysis of Variance (ANOVA) and the significance mean value were identified using Dunnet posthoc analysis at $P < 0.05$ on SPSS version 21 software**.**

4.0 CHAPTER FOUR

4.1 RESULTS

The results for chemical composition of tanniferrous plant samples prescreened for selection are presented in Table 4.1.The dry matter content values obtained were 87.08, 90.00, 92.00, 92.00, 92.88, 95.86 for Fm, Dm, Aa, As, Do and Gs samples respectively. Statistically the significant ($P < 0.05$) highest value were obtained in, Aa, As and Dm respectively. Organic matter values obtained were 87.78, 86.22, 85.83, 85.30, 84 .00 and 81.35 for As, Aa, Do, Dm, Fm and Gs respectively. Therefore the significantly higher organic matter ($P < 0.05$) were observed in As, Aa and Do respectively. For crude protein the values obtained were 20.26, 16.80, 16.68, 16.50, 16.85 and 11.46 for Gs, Fm, Aa, Do ,Dm and As respectively. Therefore the significantly higher crude protein ($P < 0.05$) was observed in Gs, Fm and Aa respectively. For Non-fiber carbohydrate values obtained were 39.17, 33.80, 28.30, 28.00, 26.04 and 26.00 for Fm, As, Dm, Gs, Aa and Do respectively. The significantly higher value for Non-fiber carbohydrate ($P < 0.05$) were observed

in Fm,As and Dm respectively. In Neutral detergent fibre (NDF), the values obtained were 61.66, 46.00, 44.76 42.00, 36.00 and 32.99 for Fm, Do, As, Dm, Aa and Gs respectively, however the significantly higher value Neutral detergent fibre (NDF) ($P < 0.05$) were observed in Fm, Do and As respectively. 9.36, 6.02, 4.19, 3,60, 3,00 and 0.90 were the values obtained in condensed tannins for Fm, As, Do, Dm, Aa and Gs respectively, in which the significantly higher value for condensed tannins ($P < 0.05$) were observed in Fm, As and Do respectively. Also for saponnin concentrations P^H the values obtained were 10.20, 9.00, 8.80, 6.60, 5, 71 and 1.58 for Do, As, Dm, Aa and Gs respectively, Statistically, the significantly higher value for saponins ($P < 0.05$) were observed in Do, As and Do respectively,

The chemical composition of the feed supplements was presented in Table 4.2. The dry matter content values obtained were 92.28, 92.00, 90.02 and 88.95 for Tc, TDm, TDo and TAa respectively. Therefore the statistically significant lowest values were obtained from TAa. However there is no significant difference among the value obtained in TDo and TDm respectively, Organic matter values obtained were 88.05, 86.22, 85.85 and 85.30 for Tc, TDm, TAa and TDo respectively. Therefore the statistically significant highest values were obtained from Tc as compared to TDm, TAa and TDo respectively. Crude protein values obtained were 28.28, 16.85,16.50 and 15.85 respectively. The statistically significant highest values were obtained in Tc however there is no significant difference among the value obtained in TDo and TDm respectively, Non-fiber carbohydrate values obtained were 28.00, 26.00, 25.08 and 22.00 for TDo, TAa, TDm and Tc respectively. The statistically significant lowest values were obtained in Tc however there is no significant difference among the value obtained in TDo and TAa respectively, In Neutral detergent fibre, the values obtained were 46.00, 42.00, 36.00 and 30.00 for TAa, TDo, TDm and Tc respectively. The statistically significant lowest values were obtained in

Tc however there is no significant difference among the value obtained in and TAa and TDo respectively, The condensed tannins values were obtained 3.60, 3.30. 2.90 for TDm, TDo, TAa, and below determined level for Tc (the concentration is too low to be determined). respectively. Statistically no significant difference values were obtained in Tc however there is no significant difference among the value obtained in TDo and TDm respectively, saponnin values obtained were 10.20, 8.80, 6.60 and and below determined level for Tc respectively. Statistically there is no significant values were obtained in Tc however there is no significant difference among the value obtained in TAa and TDo and respectively,

The biochemical characteristics of rumen fluid After 60 days of experimental feeding and observation were presented in Table 4.3. The PH ranges from 6.10 (TDm) to 6.43 (Tc). But, no significant differences were observed in the values obtained for the PH. Methylene blue reduction test values obtained were 33.30, 9.00, 8.66 and 7.66 for Tc, TDo, TDm and TAa respectively. Therefore, highest values were obtained in Tc however there is no significant difference among the value obtained in TDo and TDm respectively. Glucose fermentation test of rumen fluid values obtained were 357.87, 254.33, 216.66 and 172.33 for Tc, TDo, TDm and TAa respectively. The statistically significant highest values were obtained in Tc however there is no significant difference among the value obtained in TDo and TDm respectively. Cellulose digestion test values obtained were 68.33, 63.33, 60.67 and 48.00 for TDo, TDm, TAa and Tc respectively. The statistically significant lowest values were obtained in Tc however there is no significant difference among the values obtained in TDo and TDm respectively. Nitrate reduction test values obtained were not significantly difference in the values obtained for TDo, TDm, TAa respectively except Tc where significant value were recorded.

The microbial population of rumen fluid After 60 days of experimental feeding and observation were presented in Table 4.4. The total protozoa values obtained were (39.00, 29.60.18.50) ml/l. and 13.70 for Tc, TDm, TDo and TAa respectively. Therefore, the statistically significant highest values were obtained in Tc however there is no significant difference among the value obtained in TDm and TDo respectively. The total bacterial values obtained were 7.00, 5.06, 4.07 and 4.40 for Tc, TDo, TDm and TAa respectively. The statistically significant highest values were obtained in Tc however, there is no significant difference among the value obtained in TAa, TDm respectively. The total fungi values obtained were 4.20, 3.63, 3.43 and 3.33 for TAa, TDo, TDm and Tc, respectively. The statistically significant highest values were obtained in TAa however, there is no significant difference among the value obtained in TDo and TDm respectively.

The morphological and biochemical characteristics of screened bacteria isolates obtained from rumen fluid were presented in Table 4.5. Isolates I, III – IV, VI-XI were gram positive rod shape while isolates IV was gram positive cocci and II V&VI were gram negative rods. Isolates I , III - V&VI were motile organism except isolates IV which were not motile. Isolates I, IV &VI were citrate positive while isolates III &V were citrate negative. Isolates I, II &V were oxidase positive while isolates II, IV &VI were oxidase negative. Isolates I, II, V &VI were catalase positive while isolates III &IV were catalase negative. Isolates II were indole positive among the six isolates while isoltes I & III were indole negative respectively. Only isolates III were urease positive while isolates I, II, IV,V&VI were urease negative respectively. Isolates II & III were positive for methyl red while isolates I, IV, V &VI were methyl red negative. Isolates III, IV & VI were positive for voges proskauer test while isolates I, III &V were voges proskauer negative.

Average gas production of experimental animals fed with Tanninferous foliage samples over the period of 48 hours were presented in Table 4.6. Production of carbon (iv) oxide (Co_2) values obtained were 8.67, 6.00, 5.00 and 3.67 for Tc, TDm, TDo and TAa respectively. The statistically significant highest values were obtained in Tc, however there is no significant difference among the value obtained in TDm and TDo respectively. Productions of methane (CH4) values obtained were 17.80, 8.67,7,67 and 5.33 for Tc, TDm, TDo and TAa respectively. The statistically significant highest values were obtained in Tc, however there is no significant difference among the value obtained in TDm and TDo respectively. The overall gas production values obtained were 19.96, 13.54, 11.46 and 10.23 for Tc, TDm, TDo and TAa respectively. The statistically significant highest values were obtained in Tc however there is no significant difference among the value obtained in TDm and TDo respectively.

Overall physical parameters were analysed in Table 4.7. The colour obtained ranges from brown to milky grey and grey for Tc, TDm, TDo and TAa respectively. The odour obtained ranges from rotten, putrefaction to pungent for Tc, TDm, TDo and TAa respectively. The consistency were thick and viscous, purulent and viscoust for Tc, TDm, TDo and TAa respectively. Rotten odour was observed in control as compared to others. Thick and viscous consistency was observed in the control as compared to others. Based on the sedimentation activity per minutes of the rumen contents, the average sedimentation period observed for the rumen content are 4.66,7.00,6.33 and 7.00 respectively for Tc, TDm, TDo and TAa. The fastest sedimentation period was observed in control as compared to others.

Figure 1 clearly presents the *in vitro* gas production pattern of the dietary treatments (control and tanniniferous supplemented diets). The cumulative volume of the gas production by the dietary treatments with it directly correlated to CO_2 and methane production increased with

increasing hour of the *in vitro* incubation. The gas produced significantly ($p<0.05$) differed at all stages of incubation. In general, the gas volume of treatments fast produced from 6 to 24h, then it did slowly from 27 to 48 h and was significantly different ($P<0.05$) among the treatments. Particularly at 42 h it was significantly lower ($P<0.05$) for the *D. oliveri* supplemented diet (Do) compared with other dietary treatments (Tc, Dm, and Aa) respectively. The total in vitro gas production obtained in this study was between 16.0-39.0 ml/200g DM with *D. oliveri* supplemented diet (Aa) producing the lowest ($p<0.05$) and control diet (T1) yielding the highest ($p<0.05$) total gas as shown in Figure 1. The control diet (Tc), lead ($p<0.05$) in gas production in all the four dietary treatments (Figure 1). In general, the largest volume of gas was produced within the first nine to eighteen hour, and the least between 3 to 12 hours (Fig. 1). All the browse species produced less than 100 ml/g DM between 24- 39 hours (Fig. 1).

Table 4.1: Chemical composition of tanniferous browseplant sample

Plant Samples	Dry matter (%)	Organic matter (%)	Crude Protein (%)	Non fibre carbohydrate (%)	Neutral Detergent fibre (%)	Condensed Tannins (%)	Saponnins (%)
Fm	87.08 ^{cd}± 0.21	84.00 ^{ab} ± 0.44	16.80 ^{b}± 0.33	39.17^{a}± 0.49	61.66^{a}± 0.22	9.36^{a}± 0.32	5.71^{d}± 0.28
Dm	90.0 ^{ab} ± 0.52	85.30 ^{ab} ± 0.32	15.85^{b}± 0.12	28.30 ^{c}± 0.38	42.00 ^{d}± 0.38	3.60 ^{d}± 0.43	8.80^{b}± 0.41
Aa	92.00 ^{a}± 0.34	86.22 ^{a}± 0.36	16.68 ^{c}± 0.23	26.04 ^{b}± 0.49	36.00 ^{c} ± 0.31	3.00 ^{b}± 0.44	6.60^{b}± 0.22
As	92.00 ^{a}± 0.29	87.78 ^{a}± 0.41	11.46 ^{c} ± 0.44	33.80 ^{b}± 0.34	44.76 ^{ab}± 0.24	6.02 ^{c} ± 0.15	9.00^{c}± 0.18
Do	88.95 ^{a}± 0.55	85.83^{a}± 0.44	16.50 ^{c} ± 0.51	26.00 ^{c} ± 0.14	46.00 ^{b}± 0.16	4.19 ^{e}± 0.46	10.20^{d}± 0.13
Gs	86.76 ^{a}± 0.45	81.35 ^{a}± 0.50	20.26 ^{c} ± 0.62	28.00 ^{d}± 0.53	32.99 ^{b}± 0.49	0.90 ^{e}± 0.14	1.58^{e}± 0.33
P-value	0.00	0.13	0.00	0.001	0.00	0.001	0.001

KEY; Fm = *Flemingo* mac; **Dm = *Detarium microcarpus***; **Aa = *Afzelia africana*; As = *Acacia saligma***; **Do = *Daniellia oliveri;* Gs = *Gliricida sapium***

a,b,c, means within a row not bearing a common superscript letter significantly differ, ($P < 0.05$)

Table 4.2: Chemical composition of control diet and supplemented browse plants (% DM)

Compounded feed	DM (%)	OM(%)	CP(%)	NFC (%)	NDF(%)	CT (%)	Sap (%)
T_C	92.28 [a]± 0.50	88.05 [a]± 0.23	18.28 [a] ± 0.62	22.00 [c]± 0.23	30.00 [d]± 0.67	BDL	BDL
T_{DM}	92.00 [a] ± 1.52	86.22 [ab]± 0.22	16.85 [b] ± 0.54	25.08[b]± 0.44	36.00[c]± 0.48	3.30 [b]± 0.32	6.60 [c]± 0.29
T_{DO}	90.02 [a] ± 0.9	85.30 [b] ± 0.51	15.85 [c]± 0.35	28.00 [a]± 0.39	42.00 [b]± 0.56	3.60 [a]± 0.28	8.80 [b]± 0.14
T_{Aa}	88.95 [a] ± 1.3	85.85 [b] ± 0.43	16.50 [b]± 0.41	26.00 [b]± 0.50	46.00 [a]± 0.38	2.90 [e]± 0.33	10.20 [a]± 0.36
P-value	0.036	0.013	0.017	0.021	0.029	0.037	0.041

KEY; C = Control **Dm =** ***Detarium microcarpus***; **Aa =** ***Afzelia africana*****; = Do =** ***Daniellia oliveri;***

[abc] means in the same column with different superscripts differ significantly ($P < 0.05$). BDL = below detectable limit

SEM: Standard Error of Mean. DM: Dry matter, OM: Organic matter, CP: Crude protein, NFC: non fibre carbohydrate, NDF: Neutral detergent fibre, CT: Condensed tannins, Sap: Saponnins

Table 4.3: Biochemical Characteristics of Rumen fluid

Treatment	P^H	MethyleneBlue Reduction Test (MBRT) (Minutes)	Glucose Fermentation Test (mg/dl)	Cellulose Digestion Test (Hours)	Nitrate Reduction Test
T_C	6.43± 0.12	3.3± 0.50	357.87± 0.52	48.00± 0.43	4.61± 0.40
T_{DM}	6.10^b± 0.32	8.66± 0.46	216.6^c± 0.38	63.33± 0.12	3.72 ± 0.33
T_{DO}	6.20± 0.54	9.00± 0.37	254.33^b± 0.41	68.33± 0.33	3.55 ± 0.46
T_{Aa}	6.16± 0.42	7.66± 0.49	172.33± 0.58	60.67± 0.36	3.60 ± 0.23
P-value	0.025	0.006	0.000	0.014	0.015

KEY;; **C =** Control, **Dm = *Detarium microcarpus***; **Aa = *Afzelia africana*; As = Do = *Daniellia oliveri;***

Table 4.4: Microbiology Characteristics of Rumen fluid

Treatment	Total Cell (Protozoa) Count per ml)	Total Cell (Bacteria) Count (x10^6 cfu/ml)	Total Cell (Fungi) Count (x10^6 cfu/ml)
T_C	39.00 ^{a}± 0.4	7.00 ^{a} ± 0.4	3.33 ^{b} ± 0.5
T_{DM}	29.60 ^{b}± 0.3	4.76 ^{b} ± 0.5	3.43 ^{b} ± 0.3
T_{DO}	18.50 ^{c} ± 0.3	5.06 ^{b} ± 0.2	3.63 ^{b} ± 0.2
T_{Aa}	13.70 ^{c} ± 0.7	4.40 ^{b} ± 0.4	4.20 ^{a}± 0.3
P-value	0.0001	0.001	0.110

a,b,c, means within a row not bearing a common superscript letter significantly differ, ($P < 0.05$)

KEY;; **C =** Control, **Dm = *Detarium microcarpus***; **Aa = *Afzelia africana*; = Do = *Daniellia oliveri;***

Table 4.5: Biochemical Characteristics of isolates from animal on control diet

Isolates	Gram stain	Motility	Citrate	Oxidase	Coagulase	Catalase	Indole	Urease	Methyl red	Voges Proskauer	Glucose	Maltose	Sucrose	Lactose	Mannitol	Probable Bacteria
I	+ rod	+	+	+		+	-	-	-	-	**+/A**	**-**	**+/A**	**+/A**	**-**	***Bacillus sp***
II	-rod	+	-	+		+	+	-	+	-	**+/A**	**+/A**	**+/A**	**+/A**	**+/A**	***E. coli***
III	+ rod	+	-	-	-	-	-	+	+	+	**+/A**	**+/A**	**+/A**	**+/A**	**+/A**	***Lactobacillus sp***
IV	+ cocci	-	+	-	-	-	-	-	-	+	**+/A**	**+/A**	**+/A**	**+/A**	**+/A**	***Streptococcus sp***
V	- rod	+	+	+	+	+	-	-	-	-	**+/A**	**-**	**+/A**	**-**	**-**	***Pseudomonas sp***
VI	- rod	+	+	-	-	+	-	-	-	+	**+/A**	**+/A**	**+/A**	**-**	**+/A**	***Serratia sp***
VII	- rod	+	+	-	-	+	-	-	-	+	**+/A**	**+/A**	**+/A**	-	**+/A**	***Clostridium sp***
VIII	+cocci	+	+	-	+	-	+	+	_	+	**+/A**	**+/A**	**+/A**	**+/A**	**+/A**	***Staphyloccocus sp***
IX	- rod	+	+	+	-	-	+	+	-	+	**+/A**	**+/A**	**+/A**	**-**	**+/A**	***Bacteriods***
X	- rod	+	+	+	-	-	-	-	-	+	**+/A**	**+/A**	**+/A**	**-**	**-**	***Fusobacterium***
XI	- rod	+	+	+	-	-	-	-	-	-	**+/A**	**+/A**	**+/A**	**-**	**+/A**	***Proteobcterium***
XII	- rod	+	+	+	-	-	-	-	-	-	**+/A**	**+/A**	**+/A**	**+/A**	**-**	***Listeria sp***
XIII	- rod	+	+	+	-	-	-	-	-	-	**+/A**	**+/A**	**+/A**	**+/A**	**-**	***Bifidobacterium***

KEY: + =Positive, - = Negative

Table 4.5a: Biochemical Characteristics of isolates from experimental animal diets

Isolates	Gram stain	Motility	Citrate	Oxidase	Coagulase	Catalase	Indole	Urease	Methyl red	Voges Proskauer	Glucose	Maltose	Sucrose	Lactose	Mannitol	Probable Bacteria
I	-rod	+	-	+		+	+	-	+	-	**+/A**	**+/A**	**+/A**	**+/A**	**+/A**	***E. coli***
II	+ rod	+	-	-	-	-	-	+	+	+	**+/A**	**+/A**	**+/A**	**+/A**	**+/A**	***Lactobacillus sp***
III	+ cocci	-	+	-	-	-	-	-	-	+	**+/A**	**+/A**	**+/A**	**+/A**	**+/A**	***Streptococcus sp***
IV	- rod	+	+	-	-	+	-	-	-	+	**+/A**	**+/A**	**+/A**	-	**+/A**	***Serratia sp***
V	- rod	+	+	-	-	+	-	-	-	+	**+/A**	**+/A**	**+/A**	-	**+/A**	***Clostridium sp***
VI	+cocci	+	+	-	+	-	+	+	_	+	**+/A**	**+/A**	**+/A**	**+/A**	**+/A**	***Staphyloccocus sp***
VII	- rod	+	+	+	-	-	+	+	-	+	**+/A**	**+/A**	**+/A**	-	**+/A**	***Bacteriods***
VIII	- rod	+	+	+	-	-	-	-	-	+	**+/A**	**+/A**	**+/A**	-	-	***Fusobacterium***
IX	- rod	+	+	+	-	-	-	-	-	-	**+/A**	**+/A**	**+/A**	-	**+/A**	***Proteobcterium***
X	- rod	+	+	+	-	-	-	-	-	-	**+/A**	**+/A**	**+/A**	**+/A**	-	***Listeria sp***
XI	- rod	+	+	+	-	-	-	-	-	-	**+/A**	**+/A**	**+/A**	**+/A**	-	***Bifidobacterium***

KEY: + =Positive, - = Negative

Table 4. 6: Average gas production of experimental animals fed with Tanninferous foliage samples over the period of 48 hours

Gas Production (ml / mg)			
Forage Samples	CO_2	CH_4	Overall Gas Production
Tc	8.67 [a] ± 2.6	17.80[a] ± 1.3	26.47 [a] ± 2,5
TDm	6.00 [a] ± 0.6	8.67[b] ± 0.3	14.67 [b]± 1.5
TDo	5.00 [a] ± 1.0	7.67[b] ± 0.3	12.67 [b]± 1,3
TAa	3.67 [a] ± 0.3	5.33[b] ± 0.3	9.00 [c]± 1.0
P-value	0.17	0.001	0.001

[ab] Means in the same row with different superscripts differ significantly (P<0.05)

KEY;;T**c =** Control **Dm = *Detarium microcarpus***; **Af = *Afzelia africana*; = Do = *Daniellia oliveri;***

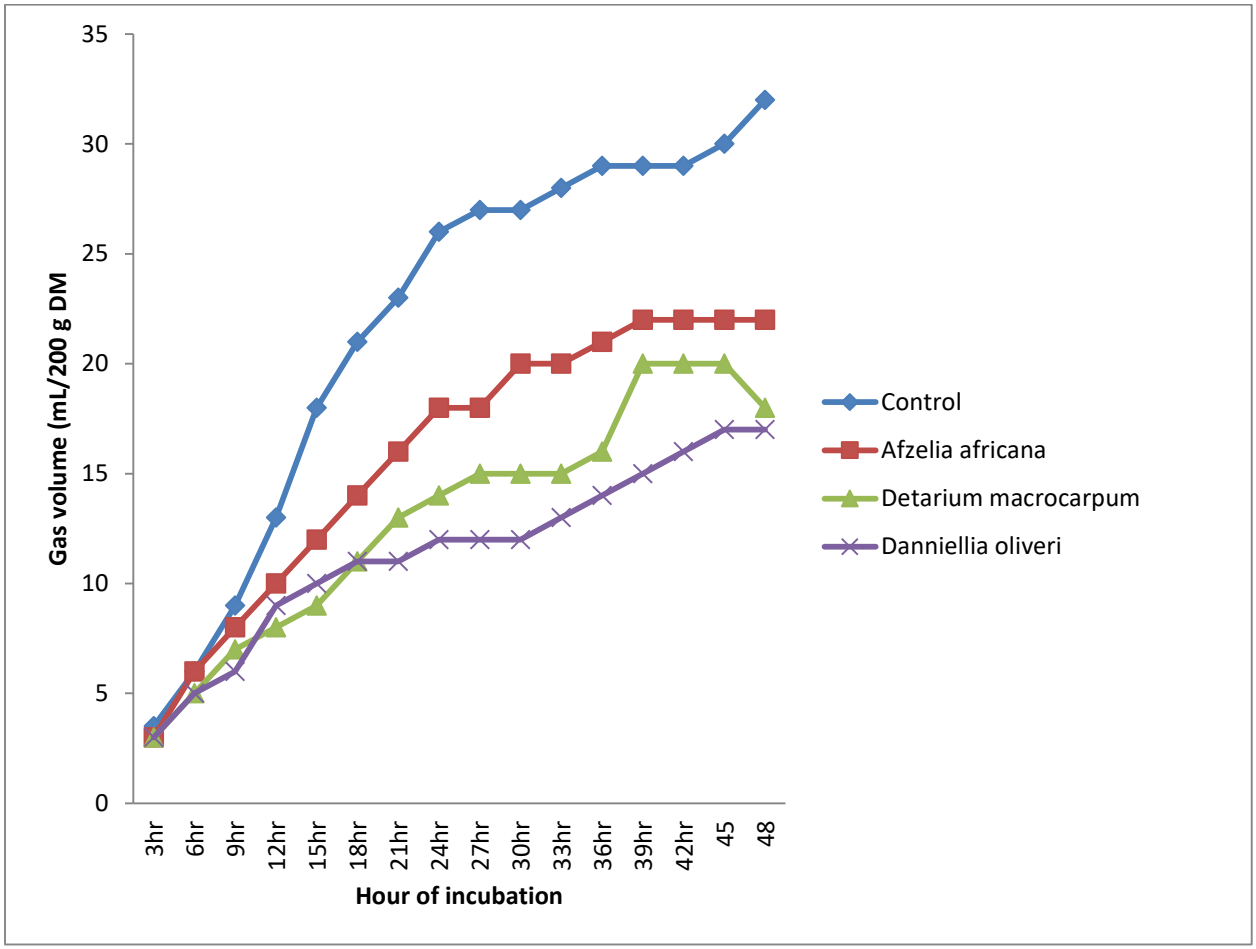

Figure 4.1: In- vitro gas production (ml/200 mg DM) of animals fed selected browse Plants

Table 4.7: Physical Parameters of Rumen fluid

Treatment	Colour	Odour	Consistency	Sedimentation Activity (Minutes)
T_C	Brownish	Rotten	Thick and Viscous	4.66
T_{DM}	Grey	Putrefaction	Purulent	7.00
T_{DO}	Milky grey	Pungent	Viscous	6.33
T_{AA}	Grey	Pungent	Viscous	7.00

KEY;; Tc = Control **Dm** = ***Detarium microcarpus***; **Aa** = ***Afzelia africana*****;** = **Do** = ***Daniellia oliveri;***

5.0 CHAPTER FIVE

5.1 DISCUSSION

5.1 Chemical composition of selected tanniniferous plants

The amount of crude protein obtained in the selected tanniniferous plants foliage use in this experiment was within the range that had been previously reported by McDonald (2012) and Okunade *et al*., (2014) for tropical tanninferous plants foliage. Non fibre carbohydrate and NDF were within the normal level required for growing goats (Isah *et al*., 2014). Condensed tannin and saponins concentrations in all the plant foliage studied in this work are moderate except for *F. macro* and *A. saligma* for the level ruminant animal can tolerate without any detrimental effect. Chemical composition is subjected to wide fluctuations depending on soil and climate characteristics. The nutrient density of these selected tanniferous plant foliage in terms of CP, NFC and NDF are adequate to meet nutrient requirement of growing goats (Salah *et al*., 2014). In addition, moderate CT and saponins contents reported in this study which can increase rumen undegraded protein and help in enteric methane (CH_4) and carbon (iv) oxide (CO_2) mitigation indicate that all the selected tanniniferous plants have potential feeding value as a dietary plant supplement for ruminants in areas where one of the most important factors limiting productivity is limited feed supply.

For chemical composition of control diet and supplemented plants, the lowest value of CP (12.64 g/100 g DM) for *D. microcarpum* is well above the range of 7.0–8.0 g/100 g DM suggested as critical limit below which intake of forages by ruminants and rumen microbial activity would be adversely affected (Van Soest, 1994). The CP content of the browse fodders is an indication of

their nutritional quality since CP content is a very important index of nutritional quality of a feed. This justifies their use as supplements to poor quality natural pastures and crop residues. The NFC contents of the browse species should be adequate to stimulate NH_3–N utilization in the rumen (Tylutki *et al*., 2008). The optimal concentration of NFC is important in ruminant diets to avoid acidosis and other metabolic problems. Diets with excess NFC can cause ruminal upsets and health problems. The fibre fraction contents of the plant species were generally moderate and within the limits established by NRC for ruminant animals for ensuring proper digestion and rumination. The mean NDF values of 48.28 and 27.61 g/100 g DM were low to moderate when compared with low quality roughages, which ruminants can effectively degrade (Salah *et al*., 2014). The low to moderate fibre contents of the browse fodders suggests their high nutritive value since fibre plays a significant role in voluntary intake and digestibility. The range of cellulose concentration shows that the fodders have the potentials to support intestinal movement, proper rumen function and promote dietary efficiency. (Kittleman *et al.,* 2014). opined that the higher the hemicellulose fraction, the higher is the feed value.

The levels of CTs recorded in for TDm, TDo and TAa in this study are much below the range of 60 to 100 g/kg DM, considered to depress feed intake and growth (Mbomi *et al*., 2011). Therefore, the plants species contained CTs at levels beneficial to ruminants because CTs at low level produce mild or low protein binding effect (Olafadehan, 2013). Similarly, CT-containing forage minimizes methane emission by ruminants (methane mitigation), in addition to other benefits, when not included at a high proportion of the diet (Bodas *et al*., 2012; Cieslak *et al*., 2012). Saponin levels in all the samples were lower than the tolerable level of 15-20 g/kg DM reported for goats, which suggests the levels reported herein are not likely to affect nutritional

potentials of the plants to ruminants. Feedstuffs containing saponin have been shown to act as defaunating agents and capable of reducing methane production (Teferedegne, 2000).

Rumen microbial population was not significantly different among supplemented diets. The results of this study shown that tanniferous plants supplementation changed the population of rumen microbes significantly. This is in trend with earlier reports (Manasri *et al*., 2012; Isah *et al*., 2014) who opined that plants containing tannins and saponins have antimicrobial properties. The inhibitory activity of tannins against bacteria has been linked to the ability of tannins to form complexes with the cell wall and membrane of bacteria causing morphological changes of the cell wall and the extracellular enzymes secreted (Jones *et al.*, 1994). However, (Pilajun and Wanapat, 2012) reported that supplementation with tannins and saponins rich-plants did not change the microbial population in swamp buffalo. Different results obtained by these authors could be due to the different sources of diets, dose of tannins, animal type, feeding program and environmental conditions between the previous studies and the present work. In contrast, the number of fungal zoospores were increased ($p<0.05$) with tanniniferous plants supplementation. The results obtained could be due to increase in fiber and a higher pH which would increase fiber digestibility and consequently increased fungi colonization in tanninferous plants supplemented diets (Denman *et al*., 2015). The decrease in protozoa was observed due to tanniniferous plant supplementation (Ningrat *et al*., 2017). Protozoa population in the rumen is directly proportional to methane gas production, because archaea have symbiotic relationship with protozoa in the rumen. Tanniniferous plant supplemented diets were significantly different ($p<0.05$) compared with control diet (Tc). This indicated that tannins and saponnins containing plant foliage decreases protozoa population. That implies that the population of archaea will be decreased which finally lead to reduced methane gas during rumen fermentation of the diets. The higher CP

in the control diet (Tc) coupled with low fibre fraction (NDF) probably made protein available for rapid proliferation of total rumen bacteria than tanniniferous plant supplemented diets (Isah *et al*., 2014). Higher fungi population may be as a result of increase in NDF of supplemented diets compared with control (Tc). Fungi proliferates more on recaltrant fibre in the rumen.

During fermentation process, varied amounts of gases are produced within the rumen, which mainly constitutes H_2, CO_2 and CH_4. Hydrogen in the rumen together with CO_2 is used to synthesize CH_4 by methanogenic microorganisms. The emissions of CH_4, CO_2, and other greenhouse gases into the environment are the major causative agents of global warming (Elghandour *et al*., 2017). Some plant phytochemicals such as tannins found in many tanniferous plant foliages has been studied to reduce enteric CO_2 and methane (Okunade *et al*., 2014; Elghandour *et al*., 2017) when supplemented to ruminant animals. This exertion is confirmed in this present study. The higher level of CO_2, methane and methanogenic properties from the control compared with tanniferous plant supplemented diets (TDm, TDo, and TAa} respectively, obtained in this study may due be to higher gas production from rapidly degradable concentrate by rumen microbes as a result of its higher unprotected CP, adequate NFC and NDF. Ningrat *et al*. (2017) reported rapid digestion of fibre (NDF) in the rumen resulted into higher H_2 which serves as precursor for enteric methane and directly increase the multiplication of methanogens (archaea) in the rumen. Generation of these environmental pollutants (CO_2 and CH_4) were drastically reduced in tannniferous plant supplemented diets (T2, T3 and T4) respectively compared to the control diet (T1) due to the presence of concentration of tannins. Reduction or mitigation of CO_2 and CH_4 from the goats fed tanniniferous supplemented diets appears to be attributed to a concomitant decline in digestibility of nutrients. The latter is the results of complexes formed by tannins with

carbohydrate and proteins and under rumen pH condition. This is in agreement with earlier reports (Salem *et al*., 2014; Kholif *et al*., 2015; Elghandour *et al*., 2017) Moreover, the effectiveness of phytogenic feed additives depends upon the type, source and level of active secondary metabolite (Elghandour *et al*., 2017). Phytogenic feeds, rich in plant secondary metabolites, have been reported to reduce enteric CO_2 and methane.

Feed fermentation and digestibility determine nutritive values and these two are further influenced by chemical and phenolic compositions of the diets. In vitro total gas production is a faster procedure to determine the fermentation and digestibility of feed(s). A high GP indicates greater fermentation to support rapid rumen microbial proliferation and activity. It has been reported that under normal situation the greater the quantity of gas generated during ruminal digestion is accompanying by greater greenhouse gases (CO_2 and CH_4) (Elghandour *et al*., 2017). Therefore, reduction of in vitro total gas production reduces its accompanying enteric pollutants (CO_2 and CH_4). In current study, the main factors affecting total in vitro gas production are tannin and saponins contents since all the diets understudied had adequate CP, NFC and moderate NDF that is below the level that can lower or hinder digestibility. This observation agrees with the report of (Isah *et al*., 2014, Okunade *et a*l., (2014). (Elghandour *et al*., (2017) also opined that the molecular weight of tannins influences the rate and the quantity of gas production in- vitro. In the present study tannin showed depressing effect in fermentability and digestibility of plants. Control diet (Tc) which is without tanniniferous plant suplementation was the most fermentable and digestible dietary treatment that could be associated to the non-detectable level of phenolic compounds (tannins and saponin) which probably allowed the faster degradation of high CP, NCF and NDF this lead to the higher gas production compared to other diets. On the other hand tanniniferous plant supplemented diets (TDm, TDo, and TAa} recorded

lower total in vitro gas production in that chorological order compared to control diets that could be due to the negative influence of high CT in that order.and this is in agreement with earlier works of Guglielmelli *et al*., (2011) ; Jayanegara *et al*., (2011) and Sebata *et al*., (2011). The negative effect of tannins on fermentation and digestion could be related to the formation of tannin–carbohydrate and tannin– protein complexes that are less degradable or toxic to rumen microbes (Elghandour *et al*., 2017; Khejornsart, 2021).

Conclusion

Results obtained showed that the concentration of condensed tannins in the selected plant have inhibitory effect on rumen microbial ecosystem thus suppressing their proliferation by binding to form complexes with the cell wall and membrane which lead to reduction in total gas production and its accompanying enteric pollutants (CO_2 and CH_4) there by reduces the GHG emission, improves feed efficiency and animal productivity.

Contribution to Knowledge

This study has shown that the tanninferrous plants used can reduce the production of enteric methane and improved feed efficiency.

Recommendation

Plant secondary metabolites such as tannins and saponins can be used strategically to manupulate rumen microbes for reduction of CO_2 and enteric methane. This will eventually improve

ruminant performance and minimized green gas emission. Further work can be carried out to determine the different combination of these tanniferrous plants that will give best result.

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APPENDIX

Table 1: Chemical composition of selected tanniferous browse plants

	Dry matter	Organic matter	Crude Protein	Non fibre carbohydrate	Neutral Detergent fibre	Condensed Tannins	Saponnins
Flemingo macrophylia							
r1	86.08	82.00	16.60	38.54	62.00	9.35	5.80
r2	87.18	84.00	16.80	38.98	60.00	9.05	5.48
r3	88.00	86.00	17.00	4.00	63.00	9.70	5.86
Detarium microcarpum							
r1	89.02	85.80	15.75	28.00	42.05	3.80	8.40
r2	91.50	85.50	16.00	29.00	41.85	3.20	9.00
r3	89.48	84.60	15.80	27.90	42.10	3.80	9.00
Afzelia Africana							
r1	92.00	86.20	16.60	25.08	36.00	3.02	6.60
r2	92.00	86.26	16.45	25.04	36.00	3.28	6.58
r3	92.00	86.20	17.00	25.00	36.00	2.70	6.62
Acacia saligma							
r1	92.30	88.10	11.54	33.42	45.28	5.98	9.00
r2	93.05	86.23	11.26	32.99	42.00	6.20	9.00
r3	95.20	89.02	11.60	35.00	47.00	5.88	9.00
Daniellia oliveri							
r1	88.05	83.30	16.25	25.95	45.54	4.00	10.00
r2	88.95	85.85	16.50	26.00	46.00	4.30	10.20
r3	89.85	88.35	16.75	26.05	46.46	4.26	10.40
Gliricida sapium				28.00			
r1	86.80	82.00	28.80		33.00	0.92	1.55
r2	85.95	80.05	29.00	28.00	32.50	0.88	1.61
r3	87.50	83.50	27.00	28.05	33.45	0.91	1.58

Table 2: Ingredient composition (% DM) of formulated concentrate

Ingredient	% DM
Maize offal	25.00
Cowpea husk	45.50
Brewers dried grain	5.00
Groundnut cake	20.00
Dicalcium phosphate	2.00
*Vit-Miniral Premix	1.00
Sulphur powder	1.00
Table salt	1.00
Total	**1000**

*supplied the following per kg of complete diet:
Vitamin A 4,000,000 IU; Vitamin D3 2,000,000 IU; Vitamin E 7,000 IU; Vitamin B2 4,000 mg; Nicotinic acid 15,000 mg; Calcium D-pentothenate 8,000 mg; Biotin 40 mg; Vitamin B12 10 mg; Mn 20,000 mg; Fe 50,000 mg; Zn 100,000 mg; Cu 10,000 mg; Iodine 750 mg; Co 3,000 mg.
KEY ;- DM - Dry Matter.

Table 3: In vitro gas production (ml/200 mg DM) of tested substrates

Substrate	***Hour of incubation***														
	3hr	**6hr**	**9hr**	**12hr**	**15hr**	**18hr**	**21hr**	**24hr**	**27hr**	**30hr**	**33hr**	**36hr**	**39hr**	**42hr**	**45h**
T_C	3.5	6.0	9.0	13^{a}	18^{a}	21^{a}	23^{a}	26^{a}	27^{a}	27^{a}	28^{a}	39^{a}	29^{a}	29^{a}	30^{a}
T_{DM}	3.0	6.0	8.0	10^{ab}	12^{b}	14^{b}	16^{b}	16^{b}	17^{b}	18^{b}	18^{b}	18^{b}	20^{b}	21^{b}	22^{b}
T_{DO}	3.0	5.0	7.0	8^{b}	9^{c}	11^{c}	13^{c}	14^{c}	15^{c}	15^{c}	15^{c}	16^{c}	18^{c}	20^{c}	20^{c}
T_{AA}	3.0	5.0	6.0	9^{c}	10^{c}	11^{c}	11^{d}	11^{c}	12^{d}	12^{d}	13^{d}	14^{d}	16^{d}	16^{d}	17^{c}

^{abcd} means in the same column with different superscripts differ significantly ($P < 0.05$).

NB: Carbon IV oxide (CO_2) and Methane gas (CH_4) were estimated at 24 hrs.

APPENDIX

Table 4. 9: In vitro gas production (ml/200 mg DM) of tested substrates

Substrate	***Hour of incubation***														
	3hr	**6hr**	**9hr**	**12hr**	**15hr**	**18hr**	**21hr**	**24hr**	**27hr**	**30hr**	**33hr**	**36hr**	**39hr**	**42hr**	**45h**
T_C	3.5	6.0	9.0	13^{a}	18^{a}	21^{a}	23^{a}	26^{a}	27^{a}	27^{a}	28^{a}	39^{a}	29^{a}	29^{a}	30^{a}
T_{DM}	3.0	6.0	8.0	10^{ab}	12^{b}	14^{b}	16^{b}	16^{b}	17^{b}	18^{b}	18^{b}	18^{b}	20^{b}	21^{b}	22^{b}
T_{DO}	3.0	5.0	7.0	8^{b}	9^{c}	11^{c}	13^{c}	14^{c}	15^{c}	15^{c}	15^{c}	16^{c}	18^{c}	20^{c}	20^{c}
T_{AA}	3.0	5.0	6.0	9^{c}	10^{c}	11^{c}	11^{d}	11^{c}	12^{d}	12^{d}	13^{d}	14^{d}	16^{d}	16^{d}	17^{c}

^{abcd} means in the same column with different superscripts differ significantly ($P < 0.05$).

NB: Carbon IV oxide (CO_2) and Methane gas (CH_4) were estimated at 24 hrs.