THE EFFECT OF SHEA NUT MEAL INCLUSION IN DIETS OF GROWING SHEEP ON PERFORMANCE, RUMEN PARAMETERS AND METHANE MITIGATION

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DECLARATION AND RECOMMENDATION

Declaration

I declare that this is my own work and it has not been submitted to any institution for any award.

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DEDICATION

I dedicate this thesis to my father Odeke Bernard, who sacrificed everything so I could go to school and have a better life but left so soon before seeing the fruit.

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ABSTRACT

Livestock agriculture provides more than 34% of proteins, other essential nutrients and services, and is an integral part of the livelihood to millions of people in the world. However, due to increasing population, the demand for animal protein is on the rise yet free-range farming barely sustains this demand. Intensive systems can resolve this challenge but are contested with inadequate feed, digestion inefficiencies, food & environmental safety concerns. This study assessed shea nut meals (*Vitalleria paradoxa*) potential as an alternative protein source for sheep diets. Varying levels of shea nut meal (SNM) at: 0, 5, 10, 15 and 20% were supplemented in diets of fifteen (15) female growing sheep aged 5 months and weighing 25±0.8 kg. The sheep were grouped in five, with 3 replicates (n=3) in a completely randomized design (CRD), and were fed basal diets of 70% Rhodes grass hay (RGH) and 30% maize bran (MB). Data was analyzed for proximate, dry matter intake (DMI), apparent nutrient digestibility, rumen fermentation parameters (pH, ammonia nitrogen concentration, volatile fatty acids, acetate: propionate ratio & protozoa count), average daily gain (ADG), feed conversion ratio (FCR), cost of feed/kg gain, *in vitro* gas production, *in vitro* organic matter digestibility, (IVOMD) and % methane reduction using the general linear model of SAS version 9.0 (2002) and means separated using the least significance difference (LSD). Shea nut meal ($p<0.05$) increased NDF, ADF, CP, EE and ME in diets. Dry matter intake was improved at 5% but was not significantly different ($p>0.05$) from 0% diet. Nutrient digestibility was ($p<0.05$) improved for 5% and 10%. Average daily gain and feed conversion ratio were ($p<0.05$) improved in all SNM diets. The cost of feed/kg gain was ($p<0.05$) lowered with increased SNM inclusion in the diets. The sheep fed SNM diets had a higher pH range of (6.4-6.9) compared to 0% (5.5-6.8). Rumen ammonia nitrogen concentration, total volatile fatty acids concentrations and protozoa counts were lower in sheep fed SNM diets compared to 0%. However, the acetate: propionate ratio was improved for sheep that were on SNM diets. The 24-hour *in vitro* gas production and organic matter digestibility were improved for 5% but declined with increasing SNM inclusion. Methane in total gas reduced with SNM increasing inclusion in the diets with the maximum reduction recorded at 20%. It was concluded that at 10% SNM inclusion had the best results and SNM is a moderate nutrient source as it modulated the rumen, improved nutrient digestibility, enhanced growth and reduced methane production.

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LIST OF ABBREVIATIONS AND ACRONYMS

Ace	Acetate
ADF	Acid detergent fibre
AOAC	Association of Official Analytical Chemists
Buty	Butyrate
CBI	Confederation of British Industry
CBR	Cost Benefit Ratio
CF	Crude fibre
Coefvar	Coefficient of Variation
CT	Condensed tannins
DMI	Dry Matter Intake
FADH	Flavin Adenine Dinucleotide Hydrogen
FAO	Food and Agricultural Organization of the United Nations
GHG	Greenhouse Gas
GLM	General Linear Model
Hb	Haemoglobin
IPCC	Intergovernmental Panel for Climate Change
LGW	Live Weight Gain
LSD	Least Significance Difference
ME	Metabolizable Energy
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NDF	Neutral detergent fibre
NRC	National Research Council
IVOMD	*In vitro* Organic Matter Digestibility
ppb	Parts per billion
ppm	Parts per million
Prop	Propionate
RBC	Red Blood Cells
RGH	Rhodes grass hay
RUP	Rumen Un-degradable Protein

TMP	Total microbial population
TPP	Total protozoan population
tVFAs	Total Volatile Fatty Acids
WBC	White blood cells

CHAPTER ONE
INTRODUCTION

1.1 Background Information

The livestock production component of agriculture constitutes very important economic and socio-cultural roles, providing more than 34% of global proteins, other essential nutrients, goods and services and is an important livelihood to millions of populations in the globe (FAO, 2017). The constant growth and transformations in the sector therefore are opportunities for the sectors development, poverty reduction, reduced negative environmental impacts, food security improvements and improved human nutrition (FAO, 2020).

In the horn of Africa, livestock production plays a significant role in off farm employment and income generation. The ruminants form large herds and high per capita contribution in more than ninety developing countries (FAO, 2017). Sheep and goats especially are the most peculiar species with adaptation to survive diverse range of environment and management systems. Sheep are mainly kept for meat, milk and wool and the economic importance of each of the products varies with regions. Corriedale sheep are a dual-purpose cross breed of Merino and Lincoln reared mainly for meat and wool. They are large framed and have a broad body, polled and with good quality carcass (Meggitt, 2017).

Despite the livestock contributions to human wellbeing, livestock production still faces critics among other challenges. The complexity in nutrient utilization from feeds which is affected by both nutritional and animal factors Wilkinson (2011) and Phuong *et al*. (2013), and nutrient deficiencies especially essential nutrients such as Sulphur and nitrogen are indispensable (Migwi *et al.,* 2013). Moreover, the growing environmental concerns from enteric methane emissions are quite crucial. Methane, nitrous oxide and carbon dioxide (CO_2) are three important greenhouse gases GHG) from livestock activities. Methane is emitted from the enteric fermentation, nitrous oxide from manure decomposition and CO_2 from all the process, signifying about 14.5% global GHG emissions from the sector (Hristov *et al*., 2013). This estimates could even be higher, The United Nations (UN) inter-governmental panel for climate change (IPCC), France-Presse (2017) report designated that the revised calculations of methane emission per head of cattle is 11% higher than previously thought and that methane from ruminants alone could account for 11-17% global emission (Beauchemin *et al*., 2009).

Methane emission is also associated with feed nitrogen (N) and energy losses (Gerber *et al*., 2013). Enteric methane is produced when rumen methanogens utilize hydrogen to reduce carbon dioxide, an inevitable physiology that lessens the build-up of reducing equivalents, and maintains a virtuous fermentation vat, but also results in an averagely 7% gross energy loss in the feed taken (Beauchemin *et al*., 2009). It's reported that enteric methane production could decline if digestible nutrients escaping rumen fermentation are improved, but high fibre in diet will result in arise in methane production (Dijkstra *et al*., 2011).

Feeding poor forages alone also favors production of acetate and butyrate and little of propionate (Hristov *et al.*, 2011; Knapp *et al*., 2014). When comparing propionate, acetate and butyrate are not hydrogen sinks and their production results in the build-up of hydrogen through oxidation of reduced co-factors i.e. NADH, NADPH and FADH Newbold *et al*. (2015) which are then used in the formation of methane (Knapp *et al.*, 2014). Supplementation with concentrates at 30 – 40 % is reported to improve rumen efficiency by maintaining high pH, optimum NH_3-N concentration and increasing microbial protein synthesis and VFAs (Sittisak & Wanapat, 2007). This then results in improvements in digestibility and reduced methane production (Gerber *et al.*, 2013; Ungerfeld, 2015).

Fat addition at 5%-8% could even yield better results as unsaturated fatty acids especially act as hydrogen sinks when dehydrated, reducing methane production by about 33% (Beauchemin *et al.*, 2009). Moderate tannin levels of less than 4% and saponins as well are said to inhibit protozoa growth, causing a beneficial ruminal response by increasing availability of bacterial protein flow (Newbold *et al.*, 2015). Surprisingly shea nut meal possess all these very important metabolites (Abdul-Mumeen, 2013). However, the impact of new feed on rumen function must be understood to inform tolerable limits for better performance. This is because the rumen function influences the ruminants' digestive heath, and could also have a general impact on the human wellbeing through its effect on the quality of animal products produced and the enteric methane emitted (Ungerfeld, 2020).

1.2 Statement of the Problem

The demand for animal protein is increasingly on the rise yet free-range farming can barely sustain it. Intensive systems may offer a solution but are accompanied with new contests such as

competition with humans for high energy cereals, digestion inefficiency, and food and environmental safety concerns. Ruminants' ability to efficiently and economically meet the food needs thus remains a challenge. Also, roughage which is the most widely available biomass and the cheapest feed source is constrained by high lignification and inadequate microbial nutrients. This affects its digestibility and favors production of methane, a potent greenhouse gas of environmental concern to the globe. Additionally, currently employed technologies are also perceived either toxic, uneconomical, unsafe for food or environment and could encourage drug resistance.

1.3 Objectives

1.3.1 Broad objective

To contribute towards providing a solution to alternative protein and energy sources in sheep feeds, afforestation and reduction of ruminal methane emission through use of shea nut meal as a supplement in sheep diets.

1.3.2 Specific objectives

i. To determine the effect of different SNM inclusion levels on nutrient content, feed intake, growth performance (ADG & FCR) and cost benefit ratio when fed to growing sheep on Rhodes grass hay (70%) and maize bran (30%) basal diets.

ii. To determine the effect of different SNM inclusion levels on rumen fermentation parameters (pH, NH_3-N concentration, VFAs & protozoa count) of growing sheep fed Rhodes grass hay (70%) and maize bran (30%) basal diets.

iii. To determine the *in vitro* fermentation, methane reduction, metabolizable energy and organic matter digestibility of Rhodes grass hay (70%) and maize bran (30%) basal diets with SNM different inclusion levels.

1.4 Hypotheses

i. Shea nut meal (SNM) inclusion at different levels will have no significant effect on nutrient content, feed intake, performance (ADG & FCR) and cost to benefit ratio in growing sheep fed Rhodes grass hay (70%) and maize bran (30%) basal diets.

ii. Shea nut meal inclusion at different levels will have no significant effect on rumen fermentation parameters (pH, VFAs, NH_3-N concentration & protozoa) of sheep fed Rhodes grass hay (70%) and maize bran (30%) basal diets.

iii. Shea nut meal inclusion at different levels will have no significant effect on the *in-vitro* fermentation, methane reduction, metabolizable energy and organic matter digestibility of Rhodes grass hay (70%) and maize bran (30%) basal diets

1.5 Justification of the Study

Feed scarcity is increasingly evident as even wastes from agricultural harvests, agricultural product processing and manufacturing are being commercialized (Katongole *et al.,* 2012). For ruminant feeds, the situation is even worse as majority farmers are low input ranchers who depend entirely on natural forages. Natural forages have several limitations, they become scarce during the dry season and highly lignified as they age thus negatively impacting on the nutrient contents, intake, digestibility and performance (Migwi *et al.,* 2013). Supplementation of ruminants fed poor forages with concentrates is reported to modulate the rumen, improving digestibility of forages (Gerber *et al*., 2013; Ungerfeld, 2015). However, concentrates are as well scarce and preferably used in the non-ruminants' diets like poultry. Non-conventional concentrates especially from noncompetitive human edible products could bridge the gap and also promote a sustainable food system (Schader *et al.,* 2015).

Shea nuts are kernels from shea fruits of the *Vitellaria paradoxa*, a tree native to Africa. The trees self-plant and are later farmer-selected in a grassland system which is traditionally managed hence boosting afforestation. It is estimated that the trees natural range extends across 21 countries, from the eastern part of Senegal to Gambia, to the high plateaus of East Africa into North Eastern Uganda, forming almost an unbreakable belt of 6, 000 KM long, and an average of 500 KM wide (Boffa, 2015). There are two different tree species; *V. paradoxa* that grows mainly in West Africa and *V. nilotica* which is native to East Africa. These trees are of economic importance as their kernels are processed to obtain butter. Their butter has a nutrient composition that is almost similar to coconut butter Hatskevich *et al.* (2011) and thus the butter is being used as a replacer for coconut butter in the cosmetic, chocolate, soap and food industry (Honfo *et al.,* 2014; Okullo *et al.,* 2010).

Of recent, there has been an increase on the harvest, processing and export of the shea nut butter fat Boffa (2015) and CBI (2019) which has made the waste (shea nut meal) readily available. Numerous studies on the SNM from *V. paradoxa* of West Africa have been done but no research has yet been reported for *V. nilotica* which is SNM of East Africa.

CHAPTER TWO

LITERATURE REVIEW

2.1 Atmospheric Methane and its burden on Earth

Compared to chlorofluorocarbons (CFC), methane is the second atmospheric potent greenhouse gas of importance Etminan *et al.* (2016) with literature showing conflicting atmospheric methane trends. The methane burden was greatly observed in 2007 which rapidly grew in 2014 at a rate of (12.7 ± 0.5 ppb/year), in 2015 at a rate of (10.1 ±0.7 ppb/year), in 2016 at a rate of (7.0 ± 0.7 ppb/year) and in 2017 at a rate of (7.7 ± 0.7 ppb/year), increasing rates that were never observed since 1980s (Nisbet *et al.*, 2019).

Climate change is attributed to accumulation of atmospheric greenhouse gases like methane that have capacity to trap and radiant heat before it's transmitted to space (Darkwah *et al.,* 2018). A recent study by Hmiel *et al.* (2020) reveals that the natural fossil and biological methane emission in the past few centuries had no disparity and also the methane emission was under reported by about 38-58 tera-grams of methane per year. it was also found out that almost all the methane emitted in the atmosphere was biological in nature for the past 200 years until about 1870 when the fossil component begun to rise rapidly and coinciding with anthropogenic time.

2.2 Anthropogenic Methane from livestock

Livestock farming activities (feed production, processing and enteric fermentation) are responsible for the production of the most potent greenhouse gases (Sarkwa *et al.,* 2016). Methane produced from enteric fermentation and manure storage is estimated to account for 44% of total gas emissions. Nitrous oxide from manure storage and use of organic/inorganic fertilizers, accounts for 29% and carbon dioxide which is about 27% generated from both process including transportation of livestock (Grossi *et al.*, 2019).

Livestock farming activities alone are liable for 14.5% of methane produced from the human activities, accounting for more than a third of total emission from agricultural activities (Gerber *et al.,* 2013). Feed production and processing accounts for 45% and enteric fermentation 39%. Most emissions are from dairy and beef contributing about 20 and 41% respectively (Sarkwa *et*

al., 2016). Its estimated that an average ruminant produces 250-500 liters of methane per day and livestock emit methane equivalent to 3.1 gigatonnes of carbon dioxide annually in to the atmosphere (Gerber *et al.,* 2013).

2.3 The rumen

The rumen is a fermentation vat, well developed to provide an excellent anaerobic environment and good mixing of substrate for rumen microbial growth, fermentation, and absorption of fermentation end products, making it one of the most important component of metabolism besides the liver (Faniyi *et al.,* 2019). The rumen does not secrete any enzymes or acids but its stability is maintained by the balance between production of fermentation acids by microbes in the rumen absorption, passage, neutralization and the buffering effects of continuous saliva flow stimulated by rumination and chewing (Maekawa et *al.,* 2002). Saliva also contains urea, and urea from saliva finds its way back to the rumen and is cleaved by bacterial urease to ammonia, becoming again available for microbial use, a process of ammonia balance (Getahun *et al.,* 2019).

2.3.1 The rumen pH

The rumen pH is the function of substrate fermentability and the buffering effect of bicarbonate that regulates the state of acidity or alkalinity of the rumen (Faniyi *et al.,* 2019). The pH range of 6.5-7 is considered almost neutral but when it drops to 6.0-6.2, fiber digestion starts to declines and further drop to 5.2- 5.5, animals succumb to acidosis Jaramillo-López *et al.* (2017) and Kung (2014), which is the most common digestive health in cattle (Al-Husseiny & Zenad, 2018). Rumen pH has a high influence on microbial shift and growth, cellulose digestion, bio-hydrogenation, defaunation, methanogenesis, volatile fatty acid absorption rate and rumen health (Faniyi *et al.,* 2019).

The pH is influenced by nutritional factors and the diet fed to the animals. For instance high concentrate proportions in diet results in rapid fermentation, short lag time of digestion and lowered saliva buffering effect which then results in lactic acidosis (Nagaraja & Titgemeyer, 2007). Consequently, this inhibits the activity of cellulolytic bacteria Kung (2014) favoring the growth of lactic acid bacteria. In addition, high energy feeds such as grains and protein concentrates undergo a rapid microbial fermentation (hydrolysis) resulting in production of large

quantities of sugars and gases to levels that overwhelm the rumen buffering capacity and the animals' abilities to rid the gases (Nagaraja & Titgemeyer, 2007). For this reason, NRC recommends that for a healthy rumen, ruminant rations should contain at least 25% neutral detergent fiber of which 18.7% must be from the forages.

2.3.2 The rumen microbes

The health of the digestive system and the productivity of the ruminant livestock depends on the rumen microbiota, being bacteria, protozoa and anaerobic fungi. The bacteria and protozoa dominate in numbers and metabolic process that happen (Kung, 2014). Specific bacteria type predominate based on diet; amylolytic bacteria (firmicutes) dominate with starch (grain) diets while fibrolytic bacteria (bacteroidetes) with high fiber diets but both could have a relevant abundance of about 80% or more at times (Faniyi *et al*., 2019). The protozoa roles in rumen digestion are still not clear but their presence or absence have effect on rumen pH, concentration of ammonia, VFAs and bacteria population (Patel, 2018). There are two types of protozoa, the ciliate and the flagellates.

Ciliates are classified into two groups; holotrichs that have cilia all over their bodies, they use soluble sugar which is rapidly converted to polysaccharides, used when sugars are unavailable and the entodinomorphs that have cilia at discrete site, they are attached to the fiber and utilize starch plus other plant materials (Patel, 2018). It is reported that elimination of the ciliate protozoa could increase the microbial protein supply by 30% and reduce methane production by 11% (Newbold *et al.,* 2015). The fungi form the least of the microbial population present even in calves (Li *et al.,* 2012). They are usually in three growth stages. The flagellated zoospores that rapidly colonize the stem plant fragments and form hyphae that penetrate the plant tissue, the growing fungus that develop extensive rhizoids for anchorage and nutrient supply and then sporangium that later rapture and release zoospores completing the developmental cycle. Fungi population are greater in the rumen when diets are more fibrous making them major actors in initial fiber digestion and paving for bacterial colonization (St-Pierre, 2018).

2.4 Digestion of fibre

Digestion of fibre is a complex process and is assisted by the complex rumen ecosystem of microorganism that include bacteria, ciliate protozoa, anaerobic fungi, bacteriophages, viruses

and methanogens living in a symbiotic relationship. They ferment fibre to carbohydrates, proteins and lipids Castillo-González *et al.* (2013), and later to acetate, butyrate, propionate, and hydrogen (H), CO_2, ammonia (NH_3) and subsequently methane (CH_4) (Knapp *et al.*, 2014). The rate of ruminal degradability is influenced by several factors such as pH fluctuations, available substrate and the shift in microbial populations that in turn causes an increase in the lipopolysaccharides concentration in both the gut and blood, which also influences rumen stability (Faniyi *et al*., 2019).

During normal ruminal fibre degradation, methane production is estimated to cause 5-15% feed gross energy loss (Wanapat *et al.*, 2015). The ciliate protozoa particularly (methanogen archaea) are believed to be responsible for this process while acetogenic bacteria are hydrogen sinks and are believed to interfere with methanogenesis process (Leahy *et al.*, 2010). It has been therefore established that effective feed utilization and enhancement strategies should target improvements to feed intake, nutrient balance and nutrient metabolism at tissue levels Migwi *et al.* (2011), and improvement of acetogenic bacteria population that provide a substitute for hydrogen sink and reduction of methanogens or members of the rumen microbes that produce substrates of methanogenesis (Leahy *et al.*, 2013).

2.5 Ruminant feeding in East Africa

Ruminant feeding in East Africa largely depends on the rangeland and grassland forages, and crop residues, which are high in fiber and low in nutrients. During the early rainy season, these forages are often lush and present fewer nutritional deficiencies. However, as the rains progress, the grasses rapidly mature, becoming highly lignified. Inclusion of at least 7% protein levels is reported to sustain microbial growth and efficient ruminal degradation of the fibrous and low quality forages (Lazzarini *et al*., 2009). Mature forages and crop residues are also high in lignin content and low in protein and other essential nutrients such as Sulphur (S) and nitrogen (N) required for ideal microbial growth.

2.6 Supplementation of ruminant diets

Supplementation with grains/carbohydrates, protein concentrates and other essential nutrients is important in enhancing nutrient utilization from feeds of ruminants livestock (Migwi *et al.*, 2013). High energy and protein supplements increases intake, fiber digestibility, microbial

protein synthesis and enhances absorption at tissue metabolism (Migwi *et al.,* 2013). Excess supplementation with concentrates beyond optimal levels however, can cause metabolic disorders, uneconomical and can reduce nutrient digestibility and utilization (Quang *et al.*, 2015). Supplementing without precautions also may have considerable consequences to the animal digestive health resulting from sudden ruminal pH fluctuations, microbial shifts, and imbalance in the ruminal metabolism (Jaramillo-López *et al.*, 2017).

2.7 Effect of supplementation with concentrates on intake, nutrient digestibility and methane mitigation

Increasing concentrate ratio in diets of ruminants has potential in improving intake, digestibility and mitigating methane by 15% (Knapp *et al.,* 2014). This is because enteric methane production is likely to decline if digestible nutrients escaping rumen fermentation are improved but if the fiber content is high, it will result in arise in methane production (Dijkstra *et al.,* 2011). Low quality forage digestion also favors production of more acetate and butyrate and little of propionate (Knapp *et al*., 2014).

Comparing propionate, acetate and butyrate are not hydrogen sinks and their production results in the buildup of hydrogen through oxidation of reduced cofactors i.e., NADH, NADPH and FADH Hristov *et al.* (2013) and Dijkstra *et al.* (2011) which are then used in the formation of methane (Knapp *et al.,* 2014). Subsequently, acetate and butyrate which are considered as the two main acetogenic substrates have a low glucogenic potential and therefore low glucose supply. Consequently, there will be low metabolizable glucose which is a limiting factor to low quality roughage intake and metabolism at the tissue level. This results in inadequate supply of reducing equivalents such as NADH that are necessary in the conversion of acetate to long chain fatty acids (LCFAs) for adipose tissue formation and storage in the body tissue (Migwi *et al*., 2011).

2.7.1 Why supplementation with fats

Fats (triglycerides) whether in saturated or unsaturated form, are an excellent source of energy and are normally included in animal diets to improve the feed energy value (Behan *et al.,* 2019). Fats possibly function by modulating the rumen microbial population, which could result in improvements in rumen fermentation, nitrogen metabolism, animal performance and reduction in

methane production (Hristov *et al.,* 2013). Inclusion of fat especially those from unsaturated fatty acids have been reported to induce a negative growth of protozoa and fibrolytic bacteria Enjalbert *et al.* (2017), resulting in subsequent inhibition of methane emission in particular animals species (Rasmussen & Harrison, 2011).

Individual fatty acids have different effect on metabolism and performance of animals (Dai & Faciola, 2019). Fats in ruminant diet are utilized in two ways i.e., high inclusion of saturated fats which have a lesser damaging effect in the rumen. This method is satisfying, but could have negative effects on the degree of milk fat saturation Marín *et al.* (2013) and the use of "protected fat" meant to overcome the negative effects of fats in the rumen by encapsulating it or creating a temporal digestion barrier by treating with aldehyde such as formaldehydes. The method however is limiting because it makes milk fat go rancid so fast and protected fats also have lower animal performance compared to un-protected fat (Bhatt *et al.,* 2013).

2.7.2 Effect of fat supplementation on feed intake, nutrient digestibility and performance of sheep

High fat inclusion in diets of ruminants have been reported to decrease feed intake. This is due to the negative digestibility Marín *et al.* (2013) resulting from disruption of the normal rumen digestion process because fat forms a coat film on the feed, causing a decreased fiber digestion or may decrease rumen cation availability creating a toxic environment for the microbes hence reducing intake and performance (Behan *et al.,* 2019).

In an experiment comparing protected and un protected fats fed to steers, Fiorentini *et al.* (2015) reported a higher intake in dry matter (DM) and organic matter (OM) for animals fed diets containing lactoplus protected fat (PF) and without fat (FW) 4.38 and 4.20, respectively, compared to diets with palm oil (PO) and linseed oil (LO) that recorded 10% decreased digestibility on DM and OM. Pi *et al.* (2019) reported an improvement in the apparent total tract digestibility on DM, NDF and EE when cows were supplemented with 4% rubber seed oil and 4% flax seed oils. Bhatt *et al.* (2011) observed decreased digestibility of organic matter and neutral detergent while ether extract increased linearly with increasing supplementation with coconut oil in lambs. Delgado *et al.* (2013) reported a diminishing ingestion effect with coconut oil on DM (3.03 and 2.39% TP) and OM (0.93 and 0.63% TP) for treatments without coconut oil

respectively. Nogueira *et al.* (2019) reported 22% greater digestibility of EE and 9% lower digestibility of non-fiber- carbohydrates when 30% cottonseed was added in cattle diet which also improved feeding behavior and ruminal parameters.

In an experiment evaluating alternative protein sources i.e. castor bean cake, sunflower cake and sunflower seed to soybean, Alves *et al.* (2016) reported a no difference in nutrient intake of castor bean diet compared with soybean meal. Nutrient intake for sunflower cake and sunflower seed decreased compared to soybean meal. The apparent digestibility of sunflower cake and sunflower seed diets for DM, OM, CP, and NDF were also decreased compared to soybean meal. However, an average daily gain in animals fed castor diet of (0.190 kg) which was not different from animals fed soybean meal (0.217 kg) was observed. The sunflower cake and sunflower seed diets recorded lesser weight gain (0.171 and 0.135 Kg/d respectively) than the soybean meal and castor bean meal diets. Bhatt *et al.* (2011) reported an improved feed conversion and production of carcass with acceptable characteristics at an optimum 50 g/kg supplementation with coconut oil. However, higher levels of coconut supplementation (75%) depressed growth and feed conversion due to suppression of rumen protozoa in the study lambs.

2.7.3 Effect of fat supplementation on ruminal volatile fatty acids proportions

Volatile fatty acids are a result of rumen microbial degradation of nutrients directly absorbed into the portal circulation through the rumen papillae, influenced by the rumen microbiota and diet. Hristove *et al.* (2011) reported that fats caused lowered ruminal acetate concentration and molar acetate: propionate ratio. The low acetate concentration is attributed to the depression in activity and growth of the fibrolytic bacterial species. Pi *et al.* (2019) observed a significant change in rumen VFAs profile by increase in the propionate proportion and decrease in VFAs concentration, the proportion of acetate and the ratio of acetate: propionate when rubber seed oil and flaxseed oil was supplemented to diets of cows. Kim *et al.* (2015) reported tVFA concentration increase by coconut material.

The acetate concentration was significantly lower while propionate was significantly higher with the addition of the coconut materials than the control. Bhatt *et al.* (2013) reported a decrease in total volatile fatty acids and individual volatile fatty acid concentration which later increased 4 hrs post feeding when lambs were fed rice bran oil and Ca-soap. Nogueira *et al.* (2019) reported

36% higher molar proportion of propionate, and butyrate and acetate: propionate ratios which were 27% and 30% lower when cottonseed was added to diets of cows. Vargas *et al.* (2020) reported increased propionate production and butyrate and ammonia out puts and the acetate to propionate ratios were decreased with supplementation of olive, sunflower and linseed oils at 6%. The effect of different vegetable oils on the concentration of VFAs in the rumen from different studies is presented in Table 2.1 below;

Table 2.1 Effect of the different vegetable fats on the concentration of ruminal VFAs

Fat source	**VFAs production (mmol/l) (%)**					
	PROP	ACE	BUTY	ACE: PROP	TVFAs	References
Palm oil	23.1	65.2	11.7	2.82	-	Fiorentini *et al.* (2015)
Linseed oil	25.1	64.3	10.6	2.56	-	Fiorentini *et al.* (2015)
Whole soybean	25.5	63.5	11.0	2.49	-	Fiorentini *et al.* (2015)
Olive oil	19.62	63.70	8.83	3.72	95.58	Atikah *et al.* (2018)
Sunflower oil	20.22	63.72	8.37	3.15	95.79	Atikah *et al.* (2018)
Palm oil	22.15	56.79	8.13	2.56	91.11	Atikah *et al.* (2018)

VFA- volatile fatty acids, PROP- propionate, ACE- acetate, BUTY- butyrate, ACE: PROP- acetate to propionate ratio, TVFA- total volatile fatty acids

2.7.4. Effect of fat supplementation in diets of sheep on the concentration of the different rumen fermentation parameters

Diverse information on oil supplementation in ruminants is available. The type of ration fed to ruminant influences the metabolic process and can even result to acute gastrointestinal disorders if the rumen pH is decreased to a range of 5.5-5.0 and maintained that way for 24 hours (Jaramillo-López *et al.*, 2017). Subsequently, rumen microbial population are influenced by complex ruminal cofactors such as diet Faniyi *et al.* (2019) which in turn influences the rumen pH, and pH subsequently affects feed intake, digestibility and ruminal NH_3-N concentration (Dai & Faciola, 2019).

Supplementation with different dietary fats of different fatty acid profiles have the capacity to improve rumen fermentation through reducing ammonium concentration, increasing total volatile fatty acid concentration, altering fiber degrading bacterial population and improving apparent digestibility of nutrients (Atikah *et al.*, 2018). Rumen protozoa are also important in

methanogenesis and reduction of their numbers below 10^7 cells/ml could potentially reduce methane production (Dai & Faciola, 2019). Phytochemicals of some plants and C-12:0 & C-14:0 medium chain fatty acids have been reported to be toxic and could reduce rumen protozoa (Faniyi *et al.,* 2019). Majewska *et al.* (2017) in an experiment comparing two oil seeds included in diets of sheep at (5% rapeseed) and (5% linseed) observed a low pH range of 5.92-6.55, with the lowest pH observed in the control group before feeding followed by diet with rape seed.

Rumen protozoa density at 2 hour and 4 hour after feeding was lower than before feeding in each experimental groups and rapeseed oil decreased total number of ciliates and entodinium species compared to the control and the linseed groups. Kim *et al.* (2015) reported a 50% reduction in the diversity of ciliate associated methanogens with coconut materials. Bhatt *et al.* (2011) reported a linear decrease in total nitrogen and trichloroacetic acid precipitable nitrogen with increasing coconut supplementation.

The concentration of total nitrogen and trichloroacetic acid precipitable nitrogen decreased at a decreasing rate, whereas ammonia nitrogen in rumen liquor decreased at an increasing rate with increased coconut supplementation. The population of the rumen protozoa had a linear decline with the increased level of coconut fat supplementation and the lowest value (8.6x104) was in the highest coconut oil supplemented group. Fiorentini *et al.* (2015) observed a pH range of 6.21-6.51 and a decrease in rumen protozoa by 50% with palm oil (PO), linseed oil (LO) and whole soybean (WS) while somewhat protected fat diets (PF and WS) decreased the effects of lipids on ruminal fermentation. Vargas *et al.* (2020) reported no effect on ruminal pH for all treatment diets but there were a reduced number of bacteria with 6% level inclusion of olive and linseed oil relative to the control group. The effect of the different vegetable fats on rumen fermentation parameters from different studies is presented in table 2.2 below;

Table 2.2 Effect of the different vegetable fats on the ruminal pH, ammonia nitrogen concentration and protozoa count

Fat source	Inclusion level (%)	pH	NH_3-N (mg/l)	TMP	TPP	REF
Olive oil	-	-	-	10.20	2.63	Atikah *et al.* (2018)
Palm oil	-	-	-	10.01	3.30	Atikah *et al.* (2018)
Sunflower oil	-	6.33	36.9	9.83	3.22	Atikah *et al.* (2018)
Palm oil	-	6.37	7.09	-	7.92	Fiorentini *et al.* (2015)
Linseed oil	-	6.51	7.33	-	2.23	Fiorentini *et al.* (2015)
Whole soybean	-	6.32	9.28	-	2.25	Fiorentini *et al.* (2015)
Coconut oil	2.5	6.62	6.0	-	74.6	Bhatt *et al.* (2011)
Coconut oil	5.0	6.62	5.8	-	57.7	Bhatt *et al.* (2011)
Coconut oil	7.5	6.31	3.4	-	8.6	Bhatt *et al.* (2011)

TMP- total microbial population, TPP- total protozoa population

2.7.5 Effect of fat supplementation in diets of sheep as a sink to hydrogen gas

Methane production is reliant on the VFAs (acetate, butyrate and propionate) produced. This occurs from the microbial fermentation of hydrolyzed dietary carbohydrates in the rumen, in which the H_2 produced during the conversion of hexose into acetate or butyrate is used by the methanogenic bacteria to reduce CO_2 into CH_4 to form energy Rasmussen and Harrison (2011), and prevent the accumulation of reducing equivalents that would otherwise hinder fermentation (Beauchemin *et al*., 2009). Rasmussen and Harrison (2011) reported inhibition of methane production when ruminant diets were supplemented with medium chain fatty acids and polyunsaturated fatty acids. Patra (2013) reported a marked inhibitory effect on methanogenesis with fatty acids C12:0 and C18:3 compared to other fatty acids. Mao *et al*. (2010) reported an inhibitory effect on methane when tea saponins and soybean oil were added to diets of growing sheep.

2.7.6 Effect of fat supplementation in sheep diets on methane gas reduction

Reducing methane emission is one most important challenge faced with the ruminant sector in livestock production (Gerber *et al.*, 2013). Presence of vegetable fat in ruminant diets is said to

decrease methane emission, a virtual contribution that might help alleviate the environmental impact of ruminant production (Vargas *et al.*, 2020). Patra (2014) reported a greater methane suppressive effects expressed as digestible DM intake in sheep compared to cattle in a meta-analysis study. Vargas *et al.* (2020) reported 21-28% methane production reduction with 6% supplementation with olive, sunflower and linseed plant oils. Mao *et al.* (2010) reported 13.9% daily methane decrease with soybean oil in diets of weaned lambs in an *in vivo* study. Wang *et al.* (2018) reported a linear decrease in total gas production with increasing anise oil (ANI) and fixed eucalyptus oil (EUC) in an *in vitro* and *in vivo* study in sheep.

The methane emission was less in sheep fed ANI than EUC. In an experiment evaluating the effect of coconut materials on *in vitro* ruminal methanogenesis and fermentation characteristics on cows by addition of coconut oil and coconut powder, Kim *et al.* (2015) reported gas production inhibition and reduction by15% and 19% in ruminal methane production respectively, compared to the control. Delgado *et al.* (2013) reported methane production reduction when coconut oil was included in diets of sheep at (18.73 and 12.16 kgDM/d). Bhatta *et al*. (2012) reported a higher ratio methane reduction from shea nut meal obtained by expulsion (0.482) followed by shea nut cake (0.301) and lastly in shea nut solvent extracted by products (0.261).

2.8 The shea nut tree

Shea nut tree (*Vitellaria paradoxa*) is a deciduous tree that grows to about 25 M in height. It is a native to Africa and a sole species to *Vitellaria genus* (Boffa, 2015). The trees self-plant and are later farmer-selected in a grassland system and traditionally managed. Trees attain maturity at about 10-15 years but full production starts at 20-30 years after planting. Its fruits are flat and round containing up to four shiny brown seeds. Both tree species produce different shea butter in terms of texture, density, consistency and nutrient content (Moore, 2008).

2.8.1 The shea nut fruits and kernels

Shea fruit is ovoid shaped and resembles small avocado fruits. The pulp cover on the shea kernels is delicious when ripe. The pulp is rich in vitamin C about 196.1 mg/100g and the pulp is consumed mainly by children and pregnant women (Honfo *et al.*, 2014). After the pulp removal are the kernels that contain varyingly high amounts of fat levels of 17.4 -59.1 g/100g dry weight

(Honfo *et al.*, 2014). Traditional methods are the most applied for extraction of the butter from them but also industrial methods are used.

2.8.2 The shea nut butter

Shea butter is the oil that is extracted from the dried kernels. The fat has omega 3 properties almost similar to that of coconut butter (Moore, 2008). In East Africa, Uganda, South Sudan and Kenya are the main producers with their butter having 56-60% and 29-32% oleic and stearic composition respectively, higher than that of West Africa which has composition of 44-50% and 40-44% oleic and stearic acid respectively (CBI, 2019). Shea butter is increasingly gaining use as a replacement for coconut butter in the international cosmetic industry, inclusion in the European and Japanese foods and a replacer for coconut butter in the chocolate manufacturing industries (Bup *et al.,* 2014). There is an increase in shea butter extraction and so are its by-products (shea cake/meal) which are currently being disposed or incinerated and very little utilized as fuel (Honfo *et al.*, 2014). The annual production of shea for high production zones are estimated at 70,000-300,000 tones while for low production zone is estimated at 10,000-70,000 tones (Bup *et al.*, 2014).

2.8.3 Chemical composition of shea nut butter

The concentration of fat and fatty acids in the butter vary in content and chemical composition depending on location, season, age and mode of treatment, genetic differences and provenances. Honfo *et al.* (2014) reported a fat content range of 43.9% -58.4%. Shea nut fat contains 16 fatty acids but five are the most dominant (Okullo *et al.,* 2010). They include two unsaturated fatty acids; oleic, and linoleic acids, and saturated fatty acids; palmitic and stearic fatty acids. Over 87% of the composition of fatty acids is stearic acid which ranged between (25-38%), oleic acid (47-62%), linoleic (6%) and palmitic (4%) (Gwali *et al.,* 2012). The shea nut butter fatty acid profile is presented in Table 2.3 below;

Table 2.3 Profile of shea nut butter fatty acids and fat content: mean, minimum and maximum

Variables	Minimum		Maximum		Mean		SD		Coef Var	
	1	2	1	2	1	2	1	2	1	2
Fat content	43.88	-	58.40	-	53.46		2.22	-	4.14	-
Palmitic acid	3.55	3.3	5.31	7.5	4.44	4.4	0.29	-	6.46	-
Stearic acid	25.31	29.5	38.48	55.7	32.49	40.4	1.99	-	6.13	-
Oleic acid	47.35	37.2	62.04	60.7	54.06	49.3	1.99	-	3.68	-
Vaccenic acid	0.29	-	0.62	-	0.42	-	0.07	-	15.52	-
Linoleic acid	4.72	4.3	8.97	8.0	6.21	6.6	0.54	-	8.65	-
Linolenic	0.13	0.2	0.37	1.7	0.25	0.4	0.05	-	19.08	-
Arachidonic acid	0.76	0.8	1.12	1.8	0.98	1.3	0.07	-	7.26	-
Sat:unsat acid ratio	0.43	-	0.81	-	0.62	-	0.06	-	8.91	-

Source: Honfo *et al.* (2014) for results in columns indicated 2 and Gwali *et al.* (2012) for results in columns indicated1.

2.8.4 Shea nut meal

Shea nut meal is a brown amorphous waste from the shea nut butter processing industry. The by-product is composed of approximate constituents, minerals and phytochemical components that are used to gauge its quality as a feed (Abdul-Mumeen, 2013). Shea nut cake production from dry savanna zones of Ghana extract processing is estimated at 500,000 metric tonnes Boffa (2015) with no clear data for Uganda and Kenya. Due to abundance of shea processing extracts and the good nutrient properties it exhibits, shea nut cake is now used as substitute for proteins in the livestock feeds

2.8.5 Chemical composition of shea nut meal

Literature reports show that the meal contains substantial amount of nutrients such as crude protein, ether extract, crude fiber, ash and nitrogen free extracts. The meal also contains plant compounds like tannins, theobromine and saponins. Other phytochemicals like terpenoids, reducing sugars and alkaloid as well as minerals have been reported Abdul-Mumeen (2013), making it a unique feed supplement that may variably impact rumen fermentation. The chemical composition of the shea nut by-products from different studies conducted in West Africa are presented in Table 2.4 below;

Table 2.4 Chemical composition of shea nut by-products from different studies

Nutrient	**Replicates**					
Composition	1 (gkg^{-1} as fed)	2 (%) DM	3 (g/kgDM)	4 (g/kgDM)	5 (g/kgDM)	6 (%) DM
Dry matter	-		905		915.0	84.9
Crude protein	265.4	13.03 ± 1.70	159	80-250	122.4 -189.0	11.6
Ether extract			320	17-362	123.0 – 175.0	4.90
Crude fibre	73.3	8.71 ± 0.85	93.8	53-138	75.0 – 138.0	
Ash	58.7	4.25 ± 0.79	-	33-76	41.6 – 53.0	5.10
NFE	-	-	-	318-675		
NDF	-	-	570		100.7	52.9
ADF	-	-	450		92.3	35.5
Tannins	-	#	0.447	98-156.4	#	7.37
Saponin	-	#	-	3.0-30.0	#	
Theobromine	-	-	#	4.5	#	
Crude lipid	148.2	23.38 ± 10.15			-	
ME (MJ kg-10	19.70	-			7.12	8.2

Sources: Obioha (2018) :5, Agbo *et al.* (2015) :1, Abdul-Mumeen (2013) :2, Bhatta *et al.* (2012) :6, Oddoye *et al.* (2012) :3 and Dei *et al.* (2007) :4. Note. Numbers 1-6 indicate the citations in the table. # presence - not given.

2.8.6 Phytochemical components in shea nut meal

Phytochemicals are reported to be effective in reduction of methane production especially if their supplementation can successfully reduce ruminal protozoa numbers below 10^7 cells/ml (Dai & Faciola, 2019). The presence of tannins in shea nut cake was first reported in studies done by Morgan and Trinder (1980) and theobromine and saponin reported in later studies by Atuahene *et al.* (1998), presence of alkaloids, terpenoids and reducing sugars was reported in (Abdul-Mumeen, 2013).

2.8.7 Effect of saponins on nutrient utilization

Saponins are secondary plant compounds that foam in aqueous solution (Newbold *et al.*, 2015). They are believed to have defaunating effects, they act by reacting with cholesterol in protozoa cell membrane, decreasing hydrogen ions availability for the methanogenesis process thus reducing methane production (Patra & Saxena, 2009). Wang *et al.* (2011) reported a significant reduction of methane emission in ml per apparent dry matter disappearance when saponins were added to barley grain-based diet in the *in vitro* studies. Similarly, Guo *et al.* (2008) reported 8% decrease in methane production when 0.4 mg/ml of tea saponins was added to rumen liquor and 50% and 79% decrease in protozoa and fungi respectively. Zhou *et al.* (2011) reported defaunating effects and 12.7% methane reduction with tea saponins. Mao *et al.* (2010) reported 27.7% daily methane production decrease.

2.8.8 Effects of tannins on nutrient utilization

Tannins are water-soluble naturally occurring plant polyphenols Naumann *et al.* (2017), with a capacity to precipitate proteins and form protein complexes. They are divided into hydrolysable and condensed tannins based on their chemical structure and together have antimicrobial, antiparasitic, anthelminthic, anti-irritant, antisecretolytic and antiphlogistic effects. Tannins are toxic to ciliated protozoa, fiber degrading bacteria and methanogen archaea. Supplementation with tannins therefore could effectively reduce methane production by enhancing microbial ecosystem, gut health and performance (Huang *et al.*, 2018).

However, high tannin content are observed to induce a negative response characterized with unpleasant taste or bitterness, toxicity, reduced fermentation and growth depression and also interference with mineral absorption (Naumann *et al.*, 2017; Bueno *et al.*, 2020). This has resulted in limitation of their use (Dai & Faciola, 2019). Tseu *et al.* (2020) in an experiment comparing monensin and tannin effects on feed intake and rumen kinetics observed that inclusion of tannins linearly reduced feed intake, digestibility, rumen disappearing rate and also urinary and retained nitrogen, Min *et al.* (2020) suggesting that increasing the amount of tannins in feeds of ruminants has great potential in reducing methane but must be used with precaution.

2.8.9 Effect of theobromine on nutrient utilization

Theobromine is a naturally occurring alkaloid in theobroma cacao and theobroma species (Alexander *et al.,* 2008). It has a slightly bitter taste and toxic to monogastric and young ruminants. Cocoa shells and oilcakes containing high amounts of theobromine up to 8.0 kg/day are acceptable to ruminants without adverse effects at an inclusion rate of 2% an equivalent to 0.5 kg/day of cocoa residue. Nutritive studies on theobroma plant species report that inclusion of theobromine at 0.05- 0.10% stimulates feed intake and growth in sheep with no hematological abnormalities. High inclusion, however, causes depression in feed intake and weight gain. Cattle fed with theobromine containing feeds were reported to have maintained normal milk yield but the butter fat somehow increased (Alexander *et al.,* 2008).

2.8.10 Effect of shea nut meal supplementation on hematological parameters and blood metabolites of sheep

Hematological studies are important and are used to understand the influence of feeds on animal health under feed trials. Nutrition is known to cause interference in the myriads and other constituents of metabolism in blood (Beigh *et al.,* 2018). In normal circumstances, serum glutamic-oxaloacetic (SGOT) and serum glutamate pyruvate transaminase (SGPT) are supposed to be low in blood, but may increase if the plane of nutrition is low, the liver is damaged and there are diseases such as bloat. Increase in the SGOT and SGPT (protein) is a reflection of the dietary protein level which portrays the health status of the animal (Etim *et al*., 2014).

Hematological studies for shea nut meal have been conducted mainly in rabbits, poultry and sheep, evaluating the varying inclusion levels on hematological parameters.

Ansah *et al.* (2011) reported that up to 10% SNM inclusion did not negatively affect rabbit growth hematological and blood biochemical parameters. Similarly, Alemede and Ogunbajo (2013) reported that varying shea nut meal levels of SNM0, SNM10, SNM15, SNM20 and SNM25% in rabbit feeds recorded low values except for SGOT which was slightly higher for SNM20. The animals fed SNM20 also had better performance compared to other treatment groups. Apparently, there is scarce information on SNM effect on blood metabolites of sheep. However, Ansah *et al.* (2011) reported no significant difference on hemoglobin concentration (Hb), parked cell volume (PCV) and red blood cell count (RBC) when SNM was fed to rabbits at 5 and 10% levels . The control group recorded the highest Hb, PCV and RCB and no significant

difference was reported among the treatment means for WBC, neutrophils, lymphocytes, eosinophils, monocytes, basophils, cholesterol, albumin and total serum protein. Table 2.5 below shows the hematological parameters and serum metabolites of sheep and rabbits.

Table 2.5. Hematological parameters and serum metabolites of sheep and rabbits fed shea nut meal

Parameters	**Sheep**		**Rabbits**				
	Inclusion level						
Inclusion level	11.5 % (1)	23.0% (1)	25% (1)	5% (2)	10% (2)	11.5% (3)	23% (3)
Hemoglobin g/DL	10.53	10.53	10.40	12.83	13.05	10.53	10.67
PCV (%)	31.2	31.2	31.25	38.50	39.17	31.20	31.50
White blood cells	8.37	9.3	-	4.60	4.50	8.37	9.30
Serum total protein gL^{-1}	59.50	61.50	-	6.29	5.48	59.50	61.37
Albumin	19.67	21.17	-	4.87	4.66	19.67	21.17
Globulin	39.83	40.17	-	-		39.83	40.17
Glucose	5.90	5.77	-	-	-	5.90	5.77
Basophils	1.39	1.00	-	0	0	1.39	1.00
Eosinophils	5.64	6.83	5.75	1.00	0.33	5.64	5.84
Cholesterol	1.10	1.23	-	4.53	5.46	-	-
Neutrophils	41.3	38.7	46.20	54.80	53.70	-	-

Source: Ansah *et al.* (2011) :2, Konlan *et al.* (2012) :3 and Obioha (2018) :1. Note. Numbers 1-3 indicate the citations in each column in table.

2.8.11 Effect of shea nut by-products inclusion levels on feed intake, weight gain, nutrient digestibility and performance of sheep

Despite the presence of anti-nutritional factors in shea by-products, studies conducted sofa have reported sufficient presence of substantial nutrient amounts in shea nut cake/meal (Abdul-Mumeen, 2013). Studies done both on shea nut meal and shea nut butter show that inclusion of SNM up to 25% in ruminant diets is possible without causing negative effects (Obioha, 2018). Yusuf *et al.* (2009) reported a no difference in DM intake with shea butter but there was a higher forage intake in the control group, and more concentrate intake in shea butter based diets that also had better performance. Konlan *et al.* (2012) reported a significant difference in DM intake

at varying inclusion levels of SNC in diets of sheep, and Dei *et al.* (2007) observed depression in intake at inclusion level of 3.5% and 4.5% in old birds.

Depression in DM intake has been attributed to presence of anti-nutritional factors that reduce palatability and cause mouth membrane irritation (Dei *et al.*, 2007; Obioha, 2018). Shea nut products digestibility in fish was done by Agbo *et al.* (2015) which declines the use of shea nut products as a protein source because of the low crude protein content, but suggests use as a source of lipid and energy especially as a supplement. Table 2.6 below presents summary of varying inclusion levels effects on intake and digestibility of SNC in different trials.

Table 2.6 Effect of shea nut by-products on feed intake, weight gain, nutrient digestibility and performance of sheep

Parameters	**Shea nut cake/meal**					**Shea nut butter**	
Inclusion level (%)	11.5 (A)	15 (B)	23 (A)	25 (B)	30 (B)	50 (C)	100 (C)
Intake (g/d)	612.01	424.1	627.58	190.4	258.6	109.84	107.33
Weight gain (g/d)	31.22	29.2	39.29	71.4	6.3	167.32	214.29
Crude protein (CP) digestibility g/100g DM	83.66	-	68.99	19.9	-	79.56	77.87
Dry matter (DM) digestibility g/100g DM	77.86	61.5	60.10	-	46.4	80.15	84.75
Feed conversion ratio (FCR)	22.66	-	17.83	-	-	8.40	6.63

Sources: Obioha (2018) :B, Konlan *et al.* (2012) :A, and Yusuf *et al.* (2009) :C. Note. A-C indicate citations as labeled on the columns.

2.8.12 Effect of shea nut meal feeding on cost to benefit ratio

Understanding the costs of production of a feed product is important in knowing whether the feed produced is cost effective in relation to its production costs, feed conversion ratio, performance and thus profitability. Shea nut meal contains anti nutritive compounds that interfere with nutrient utilization in feeds which later affect animal performance. Konlan *et al.*

(2012) reported that shea nut meal improved the growth performance of sheep when fed but at what costs and efficiency?

2.8.13 Effect of shea nut fat supplementation on ruminal microbial population

Protozoa in the rumen are important in the reduction of CO_2 to CH_4 (Martin *et al.,* 2010). Therefore, methane production could be higher when rumen protozoa are greater in numbers than when absent or lower in numbers. Bhatta *et al.* (2012) observed a significant reduction in entodinia population and total protozoa in general when different shea nut byproducts were used in *in vitro* gas production. The reduction was attributed to presence of phenols in shea nut byproducts which are believed to be toxic to entodinia than holotricha (Bhatta *et al.,* 2012).

2.8.14 Effect of shea nut meal supplementation on methane gas production

Bhatta *et al.* (2012) reported a significant reduction in methane gas produced when different levels SNC (12.0), SNE (9.6) and SNSE (11.0) were added without tannin binders in an *in vitro* study. The highest ratio of CH_4 (ml) reduction per ml of the total gas was recorded in SNE (0.482) followed by SNC (0.301) and finally SNSE (0.261). This was attributed to presence of tannins.

In conclusion, it is evident that feed availability remains a challenge and non-conventional feed sources should be explored to enhance the increasingly growing need for feeds that's instigating competition for food and contributing to environmental degradation. Shea nut meal is visibly a potential substitute since its widely available, cheap, boosts afforestation, and has high nutrient content including other plant compounds reported to reduce ruminal methane production. Shea nut meal could thus serve as both protein and energy alternative source for feeds including source of plant compounds for rumen modulation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The experiment was set at Tatton Agriculture Park (TAP) of Egerton University, Njoro Campus. The University is located at Njoro, a town situated at the Western rim of the rift valley, twenty-five KM (16 miles) South West of Nakuru town and approximately 200 KM (113 miles) North West of Nairobi town, the capital city of Kenya. The University coordinates are: 0°22'11.0" S, 35°55'58.0" E. (Latitude:-0.369734; Longitude: 35.932779) (www.egerton.ac.ke). At the time of the experiment, the region was receiving a bimodal rain pattern ranging between 1000 to 1200 mm with variable temperatures between 17 and 22°C (Egerton University weather station unpublished data, 2021).

3.2 Objective One: The effect of SNM increasing inclusion on dry matter intake, nutrient digestibility, growth performance (ADG & FCR) and cost to benefit ratio when fed to growing sheep on Rhodes grass hay (70%) and maize bran (30%) basal diets

3.2.1 Experimental animals

The study used 15 growing sheep (Corriedale ewe) of 5 months with an average weight of 25±0.8 kg. The animals were sourced from Ngongongeri farm of Egerton University. They were first dewormed with albendazole 10% oral suspension and transferred to TAP where the experiment was set. Their management was ideal, based on the guidelines of animal welfare of Kenya.

3.2.2 Housing

Animals were individually housed in slated pens on a common concrete floored house covered with corrugated iron sheets. The pens were large enough to allow normal behavior expression, feeding, watering and cleaning purposes and allowed individual collection of daily feed refusals. The pens were also considerably well-ventilated to allow aeration and excess heat loss, with feeding and drinking facilities inbuilt.

3.2.3 Feed ingredients and experimental diets

Shea nut meal was the supplement in this experiment. Shea nut kernels were bought from farmers of Katakwi district in Uganda, Rhodes grass hay from Ngongongeri farm and maize bran from an agrovet grocery within Njoro. The shea nut kernels were mechanically ram pressed to obtain the meal and Rhodes grass hay was finely chaffed to about 2cm size to ease eating.

Experimental diets were formulated to meet the nutritional requirements of sheep with fat not exceeding 70 g/kg dietary DM following the recommendations of National Research Council (NRC, 2001) and were fed at five levels. The formulated diets contained 70% Rhodes grass hay (RGH) and 30% maize bran (MB) to constitute the basal diet/ control diet (T1). The second diet (T2) contained the basal diet and 5% shea nut meal (SNM) as substitute to MB, the third diet (T3) contained the basal diet and 10% SNM as substitute to MB, the fourth diet (T4) contained basal diet and 15% SNM substitute to MB and the fifth diet (T5) contained the basal diet and 20% SNM substitute to MB.

Animals were fed 3% (750g) of their individual live body weight with the mineral supplement and water offered *ad libitum.* A preliminary period of the experiment consisted of 7 days adaptation period in which all animals were fed the basal diet followed by 14 days back grounding period which was used to establish the feed weight constant for individual animals' intake. The daily feed offered per ingredient feed is as in Table 3.1.

Table 3.1 Dietary treatments and ingredient inclusion levels (g)

	Dietary treatment				
Ingredient (% of DM)	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	T5 (20%)
Rhodes hay (g)	525	525	525	525	525
Maize bran (g)	225	187	150	112	75
Shea nut meal (g)	0	38	75	113	150

3.2.4 Sample collection and chemical analysis

Feed ingredients were sampled and dried overnight in an oven under 60^{o}C to obtain constant weight and milled to pass through 1mm sieve. Subsamples were then drawn from each ingredient

and diets formulated in accordance to their inclusion levels, kept in airtight containers awaiting analysis.

Feed refusals collected from individual animals over 7 day's period were pooled according to treatment groups and thoroughly mixed and subsamples were drawn and oven dried at 60^{o}C to a constant weight. The samples were ground to pass through 1mm sieve and placed in labelled airtight containers also awaiting analysis.

For feces, animals were harnessed with polythene bags in preparation for collection of fecal matter. The bags were harnessed to the animals with the help of straps attached to the bags and to the animal by wrapping through the animals both around the chest and between the fore limbs. Fecal bags were emptied in to a weighed bucket every morning and the bucket with feces reweighed to obtain fecal weight after the weight of the bucket subtracted. Approximately 10% of fecals from individual animals were sampled daily for 7days, and individual samples from same group pooled and kept together in polythene bags that were stored in a deep freezer at -20^{o}C. At the end of the collection period, the sampled portions of the feces were thawed, thoroughly mixed and samples were dried at 100^{o}C to a constant weight, then ground to pass through 1mm sieve and placed in labelled airtight containers awaiting analysis.

Proximate analysis for feed ingredients, diets, refusals and fecal samples to determine dry matter (DM), crude protein (CP), crude fibre (CF), ash and Ether extracts (EE) were determined as per AOAC (2000) 17th edition standard procedure as described in Weende methods while neutral detergent fibre (NDF) and acid detergent fibre (ADF) determined as per Van Soest *et al.* (1991) method. Crude protein was calculated as (N x 6.25). Total tannins (TT) were determined in only shea nut meal and the diets that contained the meal, using the method described in (Iqbal *et al.*, 2011; Porter *et al.*, 1985).

Briefly, finely ground samples to pass through 0.5mm sieve of approximately 200 g were weighed into glass beakers of approximately 25 ml capacity and 10 ml of 70% aqueous ethanol added and mixed well. The beakers were suspended in an ultrasonic water bath (Branson2 3210) and subjected to the ultrasonic treatment for 20 minutes in room temperatures. The beaker contents were shifted into centrifugation tubes and centrifuged at 4^{o}C using a refrigerated centrifuge for 10 minutes at approximately 3000g. The supernatant was collected and kept in

dark bottles on ice. The residue pellets in the tubes were washed with a portion of 5 ml of 70% aqueous ethanol and transferred to the beakers which were again subjected to ultrasonic treatment for 20 minutes. The supernatant was again collected following the same procedure. A 0.5 ml of extracted tannin mixed with 70% aqueous ethanol were then pipetted into a 100mm x 12mm test tube and 3 ml of butanol-HCL reagent and 0.1 ml of ferric reagent added. The tubes containing the samples were shaken by vortexing, and were capped with glass marbles, then placed in a heating block adjusted at 97^{o}C for 60 minutes. Tubes were cooled and the spectrophotometric absorbance read and recorded at 550 nm. Total tannins (% DM) considered as leucocyanindin equivalent was calculated using formulae; (A_{550nm} x 78.26 x dilution factor ++) / (% DM of the sample) (Porter *et al.,* 1985)**.**

3.2.5. Determination of feed intake and apparent nutrient digestibility

Feed offers were measured and refusals from individual animals collected and measured on a daily basis at 08:00 hour before trough cleaning and offer of new feed in the new day. The intake and refusals were collected over 7 day's period. Intake was measured by subtracting refusals from feed offers. Nutrient digestibility was then calculated from the data collected above as per the following method:

Apparent nutrient digestibility = $100\ X \frac{Nutrient\ intake - Nutrient\ excreted}{Nutrient\ intake}$

3.2.6. Determination of sheep performance and cost to benefit analysis of SNM varying levels in sheep diets

Initial weight of experimental animals was measured at the end of the adaptation period, then bi-weekly weighing done i.e. (at two weeks' interval as stabilization period) through day 98, each round weighed in the morning at the same time, before feeding to determine weight gain.

The cost to benefit ratio (CBR) of different inclusion levels of SNM was calculated based on the economics of production and the prevailing market prices of the feed ingredients during the experimental time, with the US dollar exchange rate for Kenya shillings (KES) pegged at $ 1: KES 100 at the time of the experiment. The different methods and parameters below were used to deduce performance and CBR;

Live weight gain (LWG) = $Final\ weight - Initial\ weight$

Average daily gain (ADG) = $\frac{Live\ weight\ gain}{Number\ of\ days\ fed}$

Feed conversion ratio (FCR) = $\frac{Total\ feed\ consumed}{Live\ weight\ gain}$

Cost of feeding per sheep = $Total\ feed\ consumed\ X\ Cost\ of\ feed$

Cost of feed/kg gain = $\frac{Cost\ of\ feeding\ per\ ewe}{Live\ weight\ gain}$

3.2.7. Statistical model

$$Y_{ijk} = \mu + \tau i + \varepsilon_{ijk}$$

where;

Y_{ijk} = observations associated with effect of i^{th} treatment,

μ = the overall mean,

τi = effect of i^{th} treatments,

ε_{ijk} = the random error associated with the Y_{ijk}

3.3. Objective two: The effect of SNM increasing inclusion on rumen fermentation parameters (pH, NH_3-N concentration, VFAs & protozoa count) of growing sheep fed Rhodes grass hay (70%) and maize bran (30%) basal diets

Rumen fluid for determining fermentation parameters was collected at week 10 of the experimental period, using a flexible oral stomach tube as per the procedure described by Wang *et al*. (2016), with the first 1.00 ml discarded to avoid saliva contamination. This was collected at 0, 3-, 6-, 9- and 12-hour post feeding into thermal flasks and taken to the laboratory within 15 minutes time. Rumen inoculum from each of the samples obtained at different sampling times were divided into three portions. The first portion was centrifuged at 16000 g for 15 minutes and the supernatant was acidified with metaphosphoric acid solution made from dissolving 25 g of metaphosphoric acid powder in 100 ml of ultrapure water and stored at -20^{o}C to await VFAs

analysis. The second portion was centrifuged at 10,000 xg for 15 minutes and the supernatant acidified with metaphosphoric acid solution and stored at -40^{o}C awaiting NH_3-N and the third portion was filtered using three layered cheese cloth, fixed with 10% formaline and stored under -20^{o}C awaiting protozoa count.

3.3.1 Ruminal pH analysis

The rumen pH was measured immediately using a mobile pH meter (6.6; tecnal, SP, Brazil).

3.3.2 Ammonia nitrogen concentration analysis

Ruminal ammonia nitrogen concentration (NH_3-N) was analyzed by Kjeldahl distillation (KD) method, without acid digestion. Samples obtained at different sampling time and centrifuged at 1,000xg for 15 mins were distilled with potassium hydroxide (2N) following the procedure used in (Souza *et al*., 2013).

3.3.3 VFAs concentration analysis

The VFAs were analyzed using the 1st portion centrifuged at 16000g for 15 minutes using Gas chromatograph (trace GC ultra-thermos scientific) with Crotonic acid from Shangai kefeng chemical reagent Co., ltd China as an internal standard and individual VFAs were identified by comparing relative retention time with the known standards of acetic, propanoic and butyric obtained from Sigma-Aldrich Co. LLC. USA. The GC temperature conditions were set at injection port temperatures of 220^{o}C, injection volume 1 uL, at a temperature program of 100^{o}C for 1 min, 100^{o}C to 190^{o}C increasing by 20^{o}C/min 190^{o}C for 3 min and total analysis time was 7.5 min, following the procedure described in (Lou *et al*., 2015).

3.3.4 Protozoa count

The sample of rumen fluid that was strained under a two layered phyllo cloth of about 2000um pore measurements and fixed with 10% formalin solution was used for determination of protozoa density count. A sedgewick-Rafter counting chamber under a microscope was used for the count following the methods described in Dehority (1984) and D´Agosto and Carneiro (1999) with modifications of (Sahoo *et al.,* 2004; Rossi *et al.,* 2013).

3.3.5 Statistical model

All data was analyzed using the GLM in SAS software version 9.0 2002 and means separation with LSD using the model equation below;

$$Y_{ijk} = \mu + \tau i + \varepsilon_{ij}$$

where;

Y_{ijk} = observations associated with effect of i^{th} treatment,

μ = the overall mean,

τi = effect of i^{th} treatments,

ε_{ijk} = the random error associated with the Y_{ijk}

3.4 Objective three. The effect of SNM increasing inclusion on the *in-vitro* gas production, methane reduction, metabolizable energy and organic matter digestibility of Rhodes grass hay (70%) and maize bran (30%) basal diets

3.4.1 Rumen liquor collection

An *in vitro* gas experiment was set to determine gas production, *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME) and methane reduction potential of SNM. Rumen fluid was collected on the 5^{th} week of the experimental period, in the morning hours before feed offer using an oral tube and from the control group as the donor animals into a pre-warmed thermal flask and then taken to the laboratory within 15 minutes. The sample was filtered using a four layered cheese cloth.

3.4.2 Preparation of rumen medium

The rumen medium preparation compromised of 150 ml water, 75 ml of macro element made from 5.7 g (1N NaOH), 6.2 g (NH_4HCO3), 0.6 g (Na_2HPO_4) in a liter of distilled water, 75 ml micro element made from 13.2 g ($CaCl_2$ x $6H_2O$), 10.0 g ($MnCl_2$ x $4H_2O$), 1.0 g ($CoCl_2$ x $6H_2O$), 0.8 g (Fe_3Cl_2 x $6H_2O$) in a 100 ml distilled water, 0.375 ml buffer solution made from 35 g (KH_2PO_4), 4 g ($MgSO_4$ x $7H_2O$) in a litter of distilled water, 0.375 ml resazurin solution made from 100 mg resazurin in 100 ml distilled water and 15 ml reducing solution made from 2 ml of

1 N NaOH and 285 mg (Na_2S x $7H_2O$) in 47.5 ml distilled water. They were later kept under CO_2 in a 39^oC water bath with a continuous magnetic stirrer.

3.4.3 Experimental design and ingredient inclusion levels

The experiment was set in a completely randomized design. Feed ingredients grounded to 1-mm particle size were measured in accordance to their inclusion levels (substrate same as diet), Approximately 0.200 g of diet samples were weighed in triplicates into 100 ml pre-warmed (at 39^oC) calibrated glass syringes (Menke, 1988). The inclusion levels of feed ingredients are as indicated in the Table 3.2 below.

Table 3.2 Ingredient inclusion levels (mg) of formulated diets

Ingredient (g)	**Dietary treatments**				
	T1 (0%)	T2 (5%)	T3 (10%)	T4 (15%)	T5 (20%)
Rhodes hay	0.14	0.14	0.14	0.14	0.14
Maize bran	0.06	0.057	0.054	0.051	0.048
Shea nut meal	0	0.003	0.006	0.009	0.012

3.4.4 *In vitro* gas production determination

The readings of gas production were taken at 0 hour before incubation and 3, 6, 12, 24, 48, 72 and 96 hours after incubation. Total gas values were corrected by blanks and gas production for specific hour (T) corrected with the equation;

(ml/200 mg DM) = (XV1-30VTfinal 1-VO-GPO)-X200(CF)/weight in mg DM

where: X= the number of times that the gas is released from the syringe and the volume is set back to 30 ml

VO= the initial volume of gas recorded before incubation started

V1= the volume of the gas recorded before the gas is released from the syringe and the volume is set back to 30 ml

VT final= the final volume of gas recorded at the end of incubation time

GPO= the mean blank value

CF= the correction factor for the standard/ standards = DM

Cumulative gas production was fitted in the exponential equation below**;**

$y = a+b\ (1-exp^{(-ct)}$

Where;

y = Volume of gas produced at time‘t’ (ml), a = gas produced from the immediately soluble fraction (ml), b = gas production from the insoluble fraction, (a+b) = the potential gas production (ml), c = the gas production ratio constant for insoluble fraction (b), (ml/hr), t is incubation time (h) model by Ørskov and McDonald (1979) in NEWAY computer package program.

3.4.5 Methane analysis

Methane analysis was performed by GC- flame ionization detection (FID) using gas chromatograph (trace GC ultra, thermo scientific) which was equipped with the methanator and a flame ionization detector using argon as a gas carrier at a flow rate of 25 ml min^{-1} at an oven temperature of 70^{o}C. Samples were run for 45 minutes. The peak areas and retentions of methane were reported and b the digital processor. The retention time for the methane gas were compared to those of known methane. The percentage of methane gas composition was calculated by expressing each peak area as a percentage of the total area which excludes the area of the solvent peak.

3.4.6 Determination of *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME)

In vitro organic matter digestibility was estimated using the formula: IVOMD (%) = 14.88 + 0.889GP + 0.45CP + XA, where;

GP = 24-hour net gas production (ml/200 g), CP = crude protein (%), XA = ash content (%)

(Metabolizable Energy) was calculated using the equation:

ME (MJ/kg/DM) = 2.20 + 0.136GP + 0.057CP + 0.0029CP^2 (g/kg/DM)

where; GP = 24 net gas production (ml/200 g), CP = crude protein, ME = metabolizable energy (MJ= mega joules, kg = kilogram, DM = dry matter), CP^2 = Crude protein (Menke *et al*., 1979)

3.4.7 Statistical model

$$Y_{ijk} = \mu + \tau i + \varepsilon_{ij}$$

where;

Y_{ijk} = observations associated with effect of i^{th} treatment,

μ = the overall mean,

τi = effect of i^{th} treatments,

ε_{ijk} = the random error associated with the Y_{ijk}

3.5 Statistical analysis

General linear model (GLM) in statistical analysis system (SAS) was used for analysis of all data. Treatments (diets), animals and overall error were sources of variation. All variables except for animals and overall error were considered fixed. Means were separated using the least significance difference (LSD) with significance level determined at $p<0.05$.

CHAPTER FOUR

RESULTS

4.1 Objective one: The effect of SNM increasing inclusion dry matter intake, nutrient digestibility, growth performance (ADG & FCR) and cost to benefit ratio when fed to growing sheep on Rhodes grass hay (70%) and maize bran (30%) basal diets

4.1.1 Chemical composition of feed ingredients and experimental diets with SNM

The nutrient composition of feed ingredients and formulated diets are as presented in Table 4.1. Increasing inclusion of SNM ($p<0.05$) increased ADF, NDF, CP, EE, and ME. Ash content was ($p<0.05$) high in (0%) and reduced with increasing SNM. Total tannins in the diets also increased significantly ($p<0.05$) with SNM increasing inclusion in the diets.

Table 4.1 Chemical composition of feed ingredients and experimental diets (g/kg DM)

Ingredients	**Parameters**								
	DM	Ash	NDF	ADF	CP	EE	TT	ME	CF
Shea nut meal	905	56	589	302	172	304	120	25	-
Maize bran	943	252	365	104	131	37	-	15	-
Rhodes grass hay	919	103	713	418	113	14	-	17	-
Treatments									
T1 (0%)	929^{a}	142^{a}	613^{d}	376^{c}	120^{c}	30.8^{e}	0.00^{d}	15.6^{d}	294^{a}
T2 (5%)	926^{ab}	125^{b}	623^{c}	383^{b}	138^{b}	64.8^{d}	4.00^{d}	16.6^{b}	285^{ab}
T3 (10%)	916^{ab}	111^{c}	639^{b}	390^{a}	141^{ab}	83.2^{c}	6.78^{c}	17.2^{b}	283^{b}
T4 (15%)	913^{ab}	101^{d}	641^{ab}	390^{ab}	149^{a}	102^{b}	8.17^{b}	17.9^{a}	278^{b}
T5 (20%)	910^{b}	88.5^{e}	649^{a}	395^{a}	150^{a}	134^{a}	9.36^{a}	18.3^{a}	279^{b}
SEM	5.343	2.1794	2.5210	1.646	3.211	0.541	0.505	0.175	3.007

SEM =standard error of the means,
^{abcde} Means in the same column with different letter superscripts are significantly different at (p<0.05). DM = Dry mater, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, CP = Crude protein, EE = Ether extract, ME = Metabolizable energy, CF = Crude fiber. TT = total tannins & - Not determined.

4.1.2 Feed intake and apparent nutrient digestibility of Rhodes grass hay and maize bran basal diets in supplementation with increasing SNM

The aim was to ascertain the effect of increasing SNM inclusion on feed intake and nutrient digestibility in sheep. Observations made show a significant decline on supplement and hay intake (p<0.05) with increased SNM inclusion. Total DM intake was significantly (p<0.05) higher at 5% SNM inclusion but was not different (p>0.05) with the control (0%). it then decreased significantly (p<0.05) with SNM inclusion as the lower intake observed at 20%. Nutrient digestibility improved with SNM inclusion (p<0.05). Digestibility was high in 5% and 10% compared to the control 0% (p<0.05) but decreased with further inclusion at 15% and 20% which was lower or comparable to the control. Feed intake and nutrient digestibility data are presented in Table 4.2 below.

Table 4.2 Feed intake (g) and nutrient digestibility (%) of Rhodes grass hay and maize bran basal diets in supplementation with increasing SNM in diets of growing sheep

Parameters	**Treatment diets**					**SEM**
	T1	T2	T3	T4	T5	
Feed Intake (g/d)						
Concentrate mixture	225[a]	225[a]	213[a]	192[b]	181[b]	5
Hay	533[a]	611[a]	364[b]	264[b]	243[b]	47.9
Total DMI	758[a]	836[a]	577[b]	456[bc]	424[c]	47
Digestibility (%)						
DM	60.25[e]	82.45[b]	85.86[a]	71.44[c]	65.70[d]	0.8575
Protein	93.26[a]	98.21[a]	97.06[a]	94.44[a]	93.23[a]	1.6995
NDF	44.16[b]	52.76[a]	52.71[a]	42.17[bc]	41.33[c]	0.8035
ADF	45.27[bc]	65.78[a]	50.17[b]	45.91[cd]	45.19[cb]	1.5619
EE	99.99[a]	99.99[a]	99.88[b]	99.75[c]	98.68[d]	0.0139

SEM= Standard error of the means,
[Abcd] Means in the same column with different letter superscripts are significantly different at (p<0.05). DM= dry matter, ADF=Acid detergent fiber, NDF=Neutral detergent fiber, EE=Ether extract

4.1.3 Performance of growing sheep fed Rhodes grass hay and maize bran basal diets in supplementation with increasing SNM

The objective of this study was to understand the effect of increasing SNM inclusion on the performance of growing sheep. The initial mean weight of the sheep was 25.48 kg and the final mean weight recorded was 34.5 kg. Diet (5%) recorded the highest weight and the least weight was observed for (0%). Final weights however did not differ (p>0.05) across diets. The average daily gains were significantly (p<0.05) improved with SNM inclusion, it was higher for (5%) and lower for (0%) (p<0.05). The FCR was improved (p<0.05) with SNM increasing inclusion and the lowest FCR was observed at 15 & 20% respectively. The results obtained are as presented in Table 4.3.

Table 4.3. Performance of growing sheep fed Rhodes grass hay and maize bran basal diets in supplementation with increasing SNM

Parameters	**Treatments**					**Sig level**	**SEM**
	T1	T2	T3	T4	T5		
Initial weight (kg)	25.4[a]	25.7[a]	25.9[a]	25.3[a]	25.2[a]	0.05	2.345
Final weight (kg)	32.9[a]	38.7[a]	35.6[a]	34.7[a]	34.5[a]	0.01	2.930
Average daily gain (g)	76.9[b]	133[a]	99.3[b]	96.3[ab]	94.9[ab]	0.05	15.414
Total DMI	758[a]	836[a]	577[b]	456[bc]	424[c]	0.05	47
Feed conversion ratio (DMI/kg gain)	9.89[b]	6.66[b]	5.86[b]	4.77[a]	4.97[a]	0.05	0.779

[ab]Means in the same row with different letter superscripts are significantly different at (p<0.05). T1= (0%), T2 = (5%), T3 = (10%), T4 =15%, T5 = (20%), SEM = Standard error of the means

4.1.4 Cost to benefit analysis of SNM increasing inclusion in the diets of growing sheep fed Rhodes grass hay and maize bran basal diets

The aim was to ascertain the effect of SNM increasing inclusion on cost benefit ratio analysis when fed to growing sheep. Results obtained showed a significant (p<0.05) high cost of feed with increasing SNM inclusion. Total feed consumption was (p<0.05) higher at (5%) but was not different (p>0.05) with the control (0%). However, consumption decreased with increasing inclusion, resulting in a significant (p<0.05) reducing cost of feeding per sheep. The lower cost of feeding/sheep was observed at 20%. Cost of feed/kg gain was lower at (15%) but was not (p>0.05) different in all SNM diets. The results are presented in Table 4.4 below;

Table 4.4 Cost: benefit analysis of SNM increasing inclusion in the diets of growing sheep fed Rhodes grass hay and maize bran basal diets

Parameters	**Inclusion levels**					**Sig level**	**SEM**
	T1	T2	T3	T4	T5		
Cost /kg/feed (KES)	15.6^{e}	15.9^{d}	16.2^{c}	16.5^{b}	16.9^{a}	0.05	0.000
Total feed consumed (kg)	74.3^{a}	81.9^{a}	56.5^{b}	44.7^{bc}	41.5^{c}	0.01	4.627
Cost of feeding/sheep (KES)	1156^{a}	1308^{a}	917^{b}	739^{b}	704^{b}	0.01	74.056
Live weight gain (kg)	7.53^{b}	13.0^{a}	9.73^{ab}	9.43^{ab}	9.30^{ab}	0.05	1.511
Cost of feed/kg gain (KES/kg)	154^{a}	107^{b}	95.1^{b}	78.7^{b}	84.3^{b}	0.05	12.835

^{abcde} Means in the same row with different letter superscripts are significantly different at (p<0.05), T1= (0%), T2 = (5%), T3 = (10%), T4 =15%, T5 = (20%), SEM =Standard error of the means.

4.2 Objective two: The effect of SNM increasing inclusion on rumen fermentation parameters (pH, NH_3-N concentration, VFAs & protozoa count) of growing sheep fed Rhodes grass hay (70%) and maize bran (30%) basal diets

4.2.1 Effect of SNM increasing inclusion on mean ruminal pH for subsequent hours post feeding

Rumen pH range in all treatments post feeding was within the optimum of 5.5-6.9. A lower pH was observed for 0% at 6 hour (5.5) and the higher for 20% (6.9) at both 0- and 12-hour post feeding. The pH at 0 hour was not (p<0.05) different across treatments but became (p<0.05) variable post feeding. When pH means were computed, it was observed that pH increased with increased SNM inclusion. The pH means are presented in appendix H and pH value trends obtained post feeding are as presented in the figure 4.1 below:

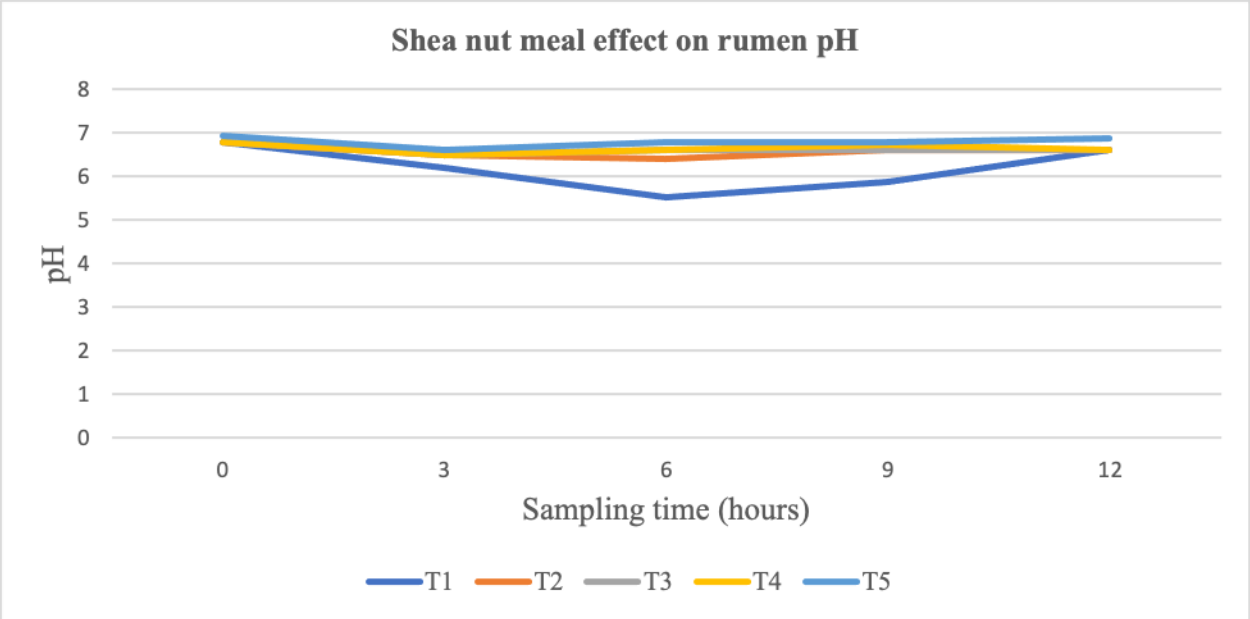

Figure 4.1. Effect of SNM increasing inclusion on the rumen pH of sheep fed Rhodes grass hay and maize bran basal diets post feeding. (T1=0%SNM), (T2=5%SNM), (T3=10%SNM), (T4=15%SNM) & (T5=20%SNM).

4.2.2 Effect of SNM increasing inclusion on the mean Kjeldahl ruminal ammonia nitrogen concentration for subsequent hours post feeding (mg/100 ml)

The purpose was to examine the effect of varying SNM inclusion on rumen NH_3-N concentration post feeding at 0, 3, 6, 9 and 12 hours. It was observed that the concentration of NH_3-N at 0-hour sampling post feeding was significantly $p<0.05$ low in SNM diets compared to the control (0%). However, from hour 3, 6, 9 & 12 NH3-N concentration was improved for 5, 10 & 15% diets except for 20%. The mean NH_3-N concentration was however observed to decrease with SNM increasing inclusion as shown in appendix I. The results of the ammonia concentration trend post feeding are as indicated in the figure 4.2 below:

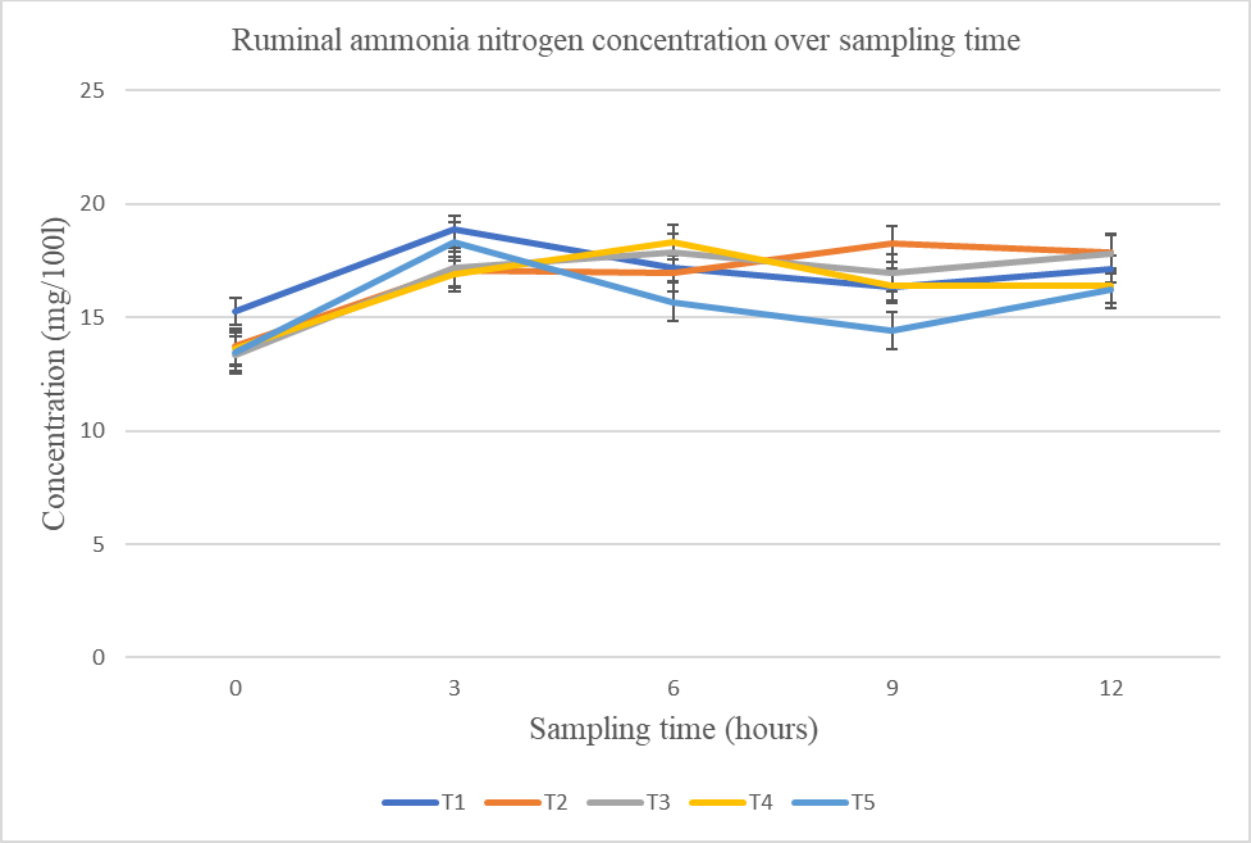

Figure 4.2 Effect of SNM increasing inclusion on rumen NH_3-N concentration in sheep fed Rhodes grass hay and maize bran basal diets post feeding. T1=treatment 1 (0%) SNM, T2=treatment 2 (5%) SNM, T3=treatment 3 (10%) SNM, T4=treatment 4 (15%) SNM and T5=treatment 5 (20%) SNM.

4.2.3 Effect of SNM increasing inclusion on ruminal VFAs concentration post feeding

The aim was to determine whether SNM inclusion in sheep diets had an effect on rumen VFAs concentration as well as providing an explanation why though with a decrease in feed intake, sheep fed with SNM diets still exhibited a better performance. Results obtained showed that SNM (p<0.05) decreased tVFAs concentration with increasing inclusion. The control group (0%) had higher VFAs concentration while the lowest VFAs concentration was observed in 20%. Propionate production however was improved (p<0.05) with SNM increasing inclusion while acetate (p<0.05) reduced, thus improving the molar acetate propionate ratio. The results are presented in Table 4.5 below.

Table 4.5. Effect of SNM increasing inclusion on the concentration of the selected VFAs over time, tVFAs and acetate: propionate ratio

Sampling time							
Acetate	**Diets**					**Sig**	**SEM**
	T1	T2	T3	T4	T5	**level**	
0	43.10^{a}	33.87^{b}	32.53^{b}	32.33^{b}	32.23^{b}	0.05	0.6389
3	51.37^{a}	40.93^{b}	40.87^{b}	41.47^{b}	41.40^{b}	0.05	0.3429
6	55.40^{a}	52.97^{b}	54.10^{ab}	54.10^{ab}	53.37^{b}	0.05	0.5698
9	65.50^{a}	56.07^{b}	56.27^{b}	56.20^{b}	56.53^{b}	0.05	0.7920
12	74.07^{a}	59.27^{b}	59.27^{b}	58.63^{b}	58.23^{b}	0.05	1.3954
Mean	57.88	48.42	48.54	48.46	48.37		
Butyrate							
0	19.80^{a}	17.97^{b}	17.60^{b}	17.57^{b}	17.77^{b}	0.05	0.1461
3	25.00^{a}	18.80^{b}	18.73^{b}	18.73^{b}	18.73^{b}	0.05	0.2595
6	24.37^{a}	20.27^{b}	20.03^{b}	20.47^{b}	20.20^{b}	0.05	0.2211
9	23.50^{a}	22.10^{b}	22.17^{b}	22.53^{ab}	22.27^{b}	0.05	0.3474
12	20.60^{a}	18.83^{b}	18.70^{b}	18.67^{b}	18.70^{b}	0.05	0.1687
mean	22.65	19.59	19.45	19.59	19.53		
Propionate							
0	32.20^{a}	33.87^{a}	34.07^{a}	34.10^{a}	34.27^{a}	0.05	0.7294
3	35.80^{a}	36.97^{a}	36.47^{a}	36.20^{a}	36.90^{a}	0.05	0.6731
6	35.73^{a}	35.93^{a}	36.23^{a}	36.03^{a}	36.13^{a}	0.05	0.9563
9	34.87^{a}	35.43^{a}	35.63^{a}	35.37^{a}	35.77^{a}	0.05	0.7486
12	33.50^{a}	34.10^{a}	34.33^{a}	34.70^{a}	34.07^{a}	0.05	1.4435
Mean	34.42	35.26	35.35	35.28	35.43		
Total VFAs	114.9^{a}	103.5^{b}	103.5^{b}	103.4^{b}	103.3	0.5	0.6033
Ace: prop	1.682^{a}	1.379^{b}	1.375^{b}	1.377^{b}	1.365^{b}	0.5	0.0177

SEM= Standard error of the means, ^{ab}Means in the same row with different letter superscripts are significantly different at (p<0.05). Ace: prop = Acetate propionate ratio.

4.2.4 Effect of SNM increasing inclusion on the mean ruminal protozoan count (ml) for subsequent hours post feeding

The aim was to determine whether SNM exhibits anti protozoan properties. The counts show protozoa were generally high in all treatments at 0-hour post feeding but then decreased in the subsequent hours post sampling. The mean protozoa counts were higher in the control group and the counts decreased (p<0.05) with SNM increasing inclusion in diets. The results of the counts are presented in Table 4.6.

Table 4.6. Effect of SNM increasing inclusion on mean ruminal protozoan count/ ml rumen fluid for subsequent hours post feeding

Hours post feeding	**Diets**					**Sig Level**	**SEM**
	T1	T2	T3	T4	T5		
0	12.33^{a}	8.33^{b}	8.33^{b}	7.00^{b}	6.67^{b}	0.05	0.596
3	6.33^{a}	5.67^{a}	5.67^{a}	5.00^{ab}	3.00^{b}	0.05	0.683
6	6.00^{a}	6.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	0.05	0.699
9	8.33^{a}	8.00^{a}	6.67^{ab}	6.33^{ab}	4.67^{c}	0.05	0.745
12	9.67^{a}	9.00^{a}	8.33^{ab}	8.67^{a}	6.67^{b}	0.05	0.537
Mean	8.53^{a}	7.4^{ab}	6.80^{b}	6.40^{c}	5.07^{c}	0.05	0.469

^{abc}Means in the same row with different letter superscripts are significantly different at (p<0.05). T1= (0%), T2 = (5%), T3 = (10%), T4 = (15%), T5 = (20%), SEM = Standard error mean.

4.3 Objective three: The effect of SNM increasing inclusion on the *in-vitro* gas production, methane reduction, metabolizable energy and organic matter digestibility of Rhodes grass hay (70%) and maize bran (30%) basal diets

4.3.1 Effect of SNM increasing inclusion on the *in vitro* gas production pattern for the 96-hour incubation period

The *in vitro* gas production of increasing SNM inclusion in the basal diets of (Rhodes grass hay and maize bran) is presented in figure 4.3. Shea nut meal inclusion at 5% increased gas

production. However, gas production reduced with further SNM inclusion for 10, 15 and 20%. The lowest gas production was observed at 20%.

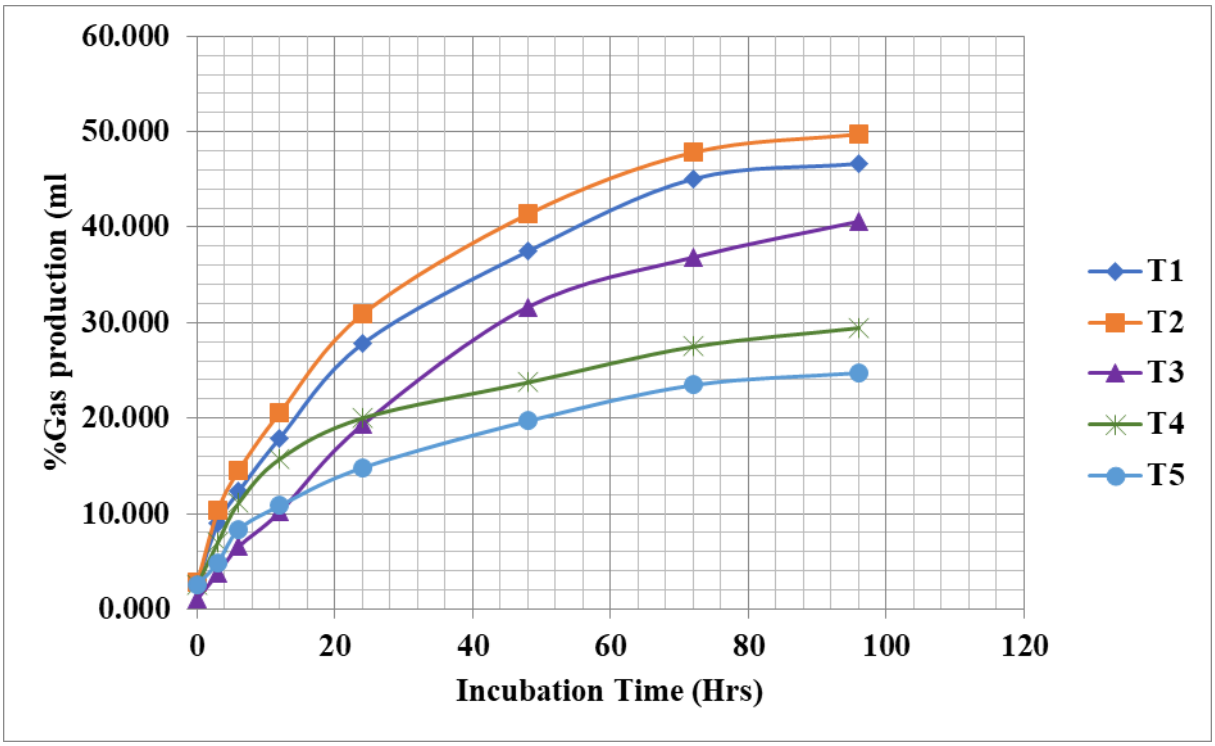

Figure 4.3 Effect of SNM increasing inclusion on gas production during the 96- hour incubation period. T1 (0%), T2 (5%), T3 (10%), T4 (15%) and T5 (20%)

4.3.2 Effect of SNM increasing inclusion on the *in vitro* 24-hour gas production, predicted parameters, organic matter digestibility and metabolizable energy

Table 4.7 shows the calculated and estimated parameters from gas production with increasing SNM inclusion in diets. The 24-hour gas production was highest at (5%) and reduced (p<0.05) with further SNM inclusion. Gas production from the immediately soluble fraction (a) was not (p<0.05) different across treatments but was lowest at (20%) and highest at (15%) SNM inclusion. Gas production from the slowly degradable fraction (b) varied (p<0.05) across treatments, it was higher at (15%) and lower at 20%. Gas production rate (c) was higher (p<0.05) at 0% and decreased with SNM increased inclusion in diets. *In vitro* Organic matter digestibility (IVOMD) was higher in the control (0%) and declined (p<0.05) with increased SNM inclusion. The decline was not significant (p>0.05) at 5% but was at 10, 15 and 20%. The ME was

improved at (5%) and (10%) compared to the control, but declined with SNM increasing inclusion.

Table 4.7. Effect of SNM increasing inclusion on the *in vitro* 24-hour gas production, predicted parameters, organic matter digestibility & metabolizable energy

Parameters	Diets					Sig level	SEM
	T1	T2	T3	T4	T5		
Gas_{24}	9.86^{a}	10.32^{a}	9.86^{a}	4.28^{b}	3.98^{b}	0.05	0.449
a	2.72^{a}	2.78^{a}	1.28^{a}	2.81^{a}	2.47^{a}	0.05	0.547
b	3.89^{bc}	4.17^{b}	8.16^{a}	1.24^{cd}	1.04^{d}	0.05	0.862
c	5.26^{a}	1.45^{ab}	0.23^{b}	1.07^{b}	0.43^{b}	0.05	1.282
a + b	6.61^{b}	6.95^{b}	9.44^{a}	4.05^{c}	3.51^{c}	0.05	0.755
RSD	3.52^{b}	3.83^{ab}	4.25^{a}	1.29^{c}	1.61^{c}	0.05	0.164
IVOMD	43.28^{a}	42.83^{a}	40.46^{b}	35.51^{c}	34.03^{c}	0.05	0.528
ME	4.65^{b}	4.95^{a}	4.82^{ab}	4.27^{c}	4.25^{c}	0.05	0.074

^{abc}Means within the same row with the same letter superscripts are not significantly different (p<0.05), a= the gas production from the immediately soluble fraction (ml), b= the gas production from the insoluble fraction (ml), c= the gas production rate constant from the insoluble fraction (b), a+b= potential gas production, ME= Metabolizable energy, IVOMD= Organic matter digestibility

4.3.3 Effect of SNM increasing inclusion on methane gas reduction

Shea nut meal exerted methanogenic properties when used. The methane for 0.200 g of truly digested substrate was significantly (p<0.05) reduced with increased SNM inclusion by 28.74% (T2), 31.94% (T3), 42.76% (T4) and 256.95% (T5). A higher reduction was observed at 20% and was significantly different from (5, 10 & 15%). The results of SNM effect on methane reduction are presented in Table 4.8.

Table 4.8. Effect of SNM increasing inclusion on methane gas reduction

Parameter	Diets					Sig Level	SEM
	T1	T2	T3	T4	T5		
Total gas production (ml)	46.65[a]	49.79[a]	40.56[b]	29.49[c]	24.77[c]	0.05	1.725
CH_4 (ml)	11.19[a]	8.45[b]	6.63[c]	4.06[d]	2.52[e]	0.05	0.281
% Methane	24.01[a]	17.15[b]	16.34[b]	13.81[bc]	10.34[c]	0.05	1.234
CH_4 (ml) for 0.02 g of truly digested substrate	8.76[a]	5.89[b]	4.52[c]	3.19[d]	2.76[e]	0.05	0.103
% Methane reduction	-	28.74[b]	31.94[b]	42.76[ab]	56.95[a]	0.05	4.692

[abcde] Means in the same row with different letter superscripts are significantly different at (p<0.05) T1= (0%), T2 = (5%), T3 = (10%), T4 =15%, T5 = (20%), SEM =Standard error mean.

Figure 4.4 and 4.5 below show the correlation effect of SNM increasing inclusion in diets on the total gas production and % methane reduction in total gas

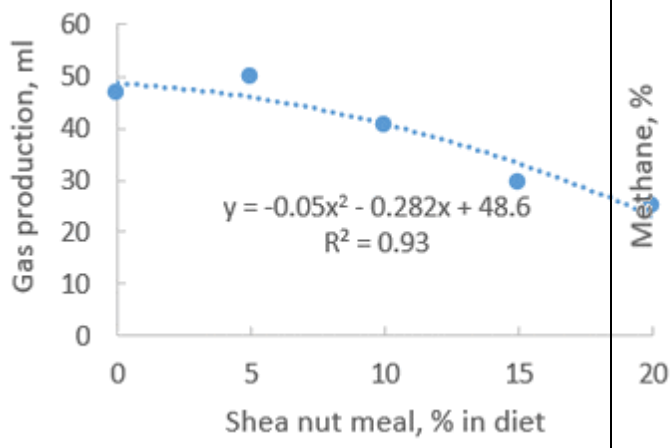

Figure 4.4. Gas production reduced with SNM increasing inclusion in diets

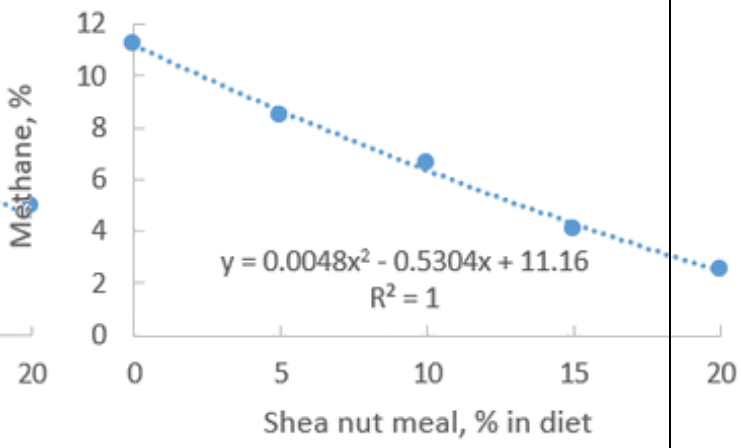

Figure 4.5. Percent methane reduced in total gas with SNM increasing inclusion in diets

Figure 4.6 Percentage methane gas reduced with reduction in total gas production

CHAPTER FIVE

DISCUSSION

5.1 Objective one: The effect of SNM inclusion levels on dry matter intake, nutrient digestibility, growth performance (ADG & FCR) and cost benefit ratio when fed to growing sheep on Rhodes grass hay (70%) and maize bran (30%) basal diets

5.1.1 Chemical composition of feed ingredients and experimental diets containing SNM

The chemical composition and total tannins in shea nut meal (SNM) used in this study were substantially higher from those reported in Bhatta *et al*. (2012), Kumar *et al*. (2015) and Obioha (2018), but were within the range with what was reported in Dei *et al*. (2007) except for ash, DM and ME which were slightly higher. The differences may possibly be attributed to environmental, butterfat extraction rate (processing methods) and nutrient differences, while total tannins were within the range reported in (Dei *et al.,* 2007). Shea nut meal inclusion improved the nutrient content of diets especially for CP EE, NDF, ADF and ME. The improvement in diets is possible because SNM has such high nutrient content. The results concur with Bhatta *et al*. (2012), Konlan *et al*. (2012) and Kumar *et al*. (2015) making SNM a potential ingredient for inclusion in sheep diets as a source of both protein and energy.

5.1.2 Feed intake and apparent nutrient digestibility of Rhodes grass hay and maize bran basal diets in supplementation with increasing SNM

Dry matter intake (DMI) at 5% SNM inclusion (835.67 g/d) was improved but was not significantly different from the control group 0% (758.33 g/d). Dry matter intake then declined with further SNM inclusion at 10 (577 g), 15 (456.33 g), and 20% (423.67 g). This contradicts Konlan *et al*. (2012) findings that reported a significant increase in intake with increased SNM inclusion but coincides with Yusuf *et al.* (2009) that reported intake at 100 g/kg shea nut butter inclusion equaled the control and declined with further higher shea nut butter inclusion. The variances in DMI could be attributed to difference in environment, breed, age, shea nut butter extraction rate that impacted diet compositions. A similar decline in DMI was also observed by Anash *et al*. (2011) when SNC was fed to rabbits, Obioha (2018) when high levels SNC above 25% caused depressed feed intake in west African Dwarf sheep and Dei *et al.* (2007) who reported depression in intake in old birds. The decline in DMI with shea nut by products can be

attributed to high residual fat levels and tannins that reduce feed digestibility, palatability and cause mouth membrane irritation (Dei *et al.*, 2007; Obioha, 2018).

Tannins are reported to induce a negative response on intake due to their bitter taste and also weaken energy metabolism in protozoa causing their reduction that in turn reduces digestibility and subsequently intake (Ku-Vera *et al*., 2020). Fat levels beyond 7% are also reported to reduce DMI (Beauchemin *et al*., 2009). These findings also concur with studies done on coconut oil in which Machmuller and Kreuzer (1999) reported feed refusal when coconut oil was supplemented in diets of sheep above 7%. Higher intake at (5%) could be attributed on the moderate levels of fat and tannins which must have improved palatability, reduced feed dustiness and improved consistency and dispersion in micro nutrients (Yusuf *et al.*, 2009).

Nutrient digestibility significantly improved with SNM. It was high at 5 & 10% SNM. It then declined or was comparable to the control with further inclusion at 15 and 20% SNM. The high improvement in digestibility at low inclusion of 5% and 10% can be attributed to the improvement in diet composition with fat and tannins maintained at favorable limits. The decline with further inclusion could be due to the increasing levels of fat and tannins in the diets beyond required limits. The findings concur with Konlan *et al*. (2012), Obioha (2018) and Yusuf *et al*. (2009) that reported improvements in digestibility with SNM increasing inclusion except at higher inclusion levels of 25%. The observed decline at 15% and 20% inclusions which were lower compared to Konlan *et al*. (2012), Obioha (2018) and Yusuf *et al*. (2009) can possibly be due to the difference in shea nut fat extraction rates and therefore nutrient composition of the formulated diets as SNM used in this study had high residual fat including the concentration of tannins. At low inclusion however, SNM had a positive impact on nutrient digestibility that could be attributed to the increased supply of protein and energy to the rumen microbes, hence enhancing the rumen environment Castillo-González *et al.* (2013) but also a relatively low fat and tannin levels. Mergeduš *et al.* (2018) reported that moderate tannins caused a beneficial ruminal response and improved performance in ruminants.

5.1.3 Performance of growing sheep fed Rhodes grass hay and maize bran basal diets in supplementation with increasing SNM

Average daily gains (ADG) range was (133-76.9 g), higher at 5% (133 g) and significantly lower at 0% (76.9 g). The high ADG in SNM fed groups can be attributed to the improvement in feed intake for those at 5% and increasing high energy in diets at 10%, 15% and 20% as a result of increased fats in SNM diets that improved calorific supply despite the low DMI and also nutrient digestibility. These findings agree with Konlan *et al.* (2012) that also reported improvement in weight gain in sheep fed SNM diets. The FCR range was (4.77-9.89 DMI/kg gain). It improved with increased SNM inclusion. It was lower at 15% (4.77 DMI/kg gain) and higher at 0% (9.89 DMI/kg gain). Improvement in FCR with increased SNM inclusion can be accredited to increased improvement in protein and energy levels of diets with SNM due to its high nutrients and digestibility as well.

Fat is an excellent source of energy and thus is reported to improve the feed energy value (Behan *et al.,* 2019). Fats also of good fatty acid profile are believed to modulate the rumen by maintaining optimum pH levels, improvement of acetate propionate ratio and detoxification of protozoa with subsequent reduction in methane production, this in turn results in improvement in feed conversion ratio and growth performance (Bhatt *et al.,* 2013; Patra 2013; Rasmussen & Harrison, 2011). The presence of tannins as well must have played a role in improved performance as low tannins of 5-10kg^{-1} DM are believed to improve rumen efficiency and general performance Kelln *et al.* (2021), and the tannins supply in diets of this study were within acceptable range.

5.1.4 Cost to benefit analysis of SNM increasing inclusion in the diets of growing sheep fed Rhodes grass hay and maize bran basal diets

The cost/kg formulated diets increased significantly with increased SNM inclusion and was in a range of (15.56 – 16.94 KES). A lower cost was observed in 0% (15.56 KES) which was significantly different from 20% (16.94 KES). The increase in cost with SNM inclusion could be because the shea nuts were sourced towards off season therefore it was overpriced for this study. The cost of feeding per sheep was however reduced with increased SNM inclusion. This was attributed to the reduction in total feed consumption with SNM increased inclusion. Consumption range was (41.5 -74.3 kg/sheep). It was higher in 0% (74.3 kg) and lower in 20%

(41.5) which decreased significantly with inclusion. Live weight/kg gain range was (7.53-13 kg). It was significantly higher at 5% (13 kg) and lower at 0% (7.53 kg). The cost of feed/kg gain ranged between (78.7-154 KES/kg). It was significantly lower in SNM diets compared to the control, with the lowest recorded in 15% (78.7 KES/kg). The reduction in the total feed consumption and hence the cost of feeding per sheep can be attributed to the energy rich SNM diets that compensated for low consumption yet bettered live weight gain. The results concur with Yusuf *et al.* (2009) that reported a lowered cost of feed per sheep fed when shea nut butter fat was fed at 100 g/kg compared to when it was fed at 50 g/kg and in the control group. SNM enhances protein, NDF, ADF and energy. This must have caused a positive nutrient utilization benefit plus tannins bounding proteins in the rumen hence making nutrients much more available in the abomasum and thus bettered performance (Bhatta *et al.,* 2012; Mergeduš *et al.,* 2018; Yusuf *et al.,* 2009).

5.2 Objective two: The effect of SNM increasing inclusion on rumen fermentation parameters (pH, NH_3-N concentration, VFAs & protozoa count) of growing sheep fed Rhodes grass hay (70%) and maize bran (30%) basal diets

5.2.1 Effect of SNM increasing inclusion on Mean Ruminal pH for subsequent hours post feeding

The type of ration fed to ruminants affects the rumen microbes and therefore, the metabolic process, that might in turn cause acute gastrointestinal acidosis especially if rumen pH is decreased to and maintained at a low range of 5.5-5.0 for quite a long (Jaramillo-López *et al.,* 2017). The lowered pH would eventually disrupt the microflora population Faniyi *et al.* (2019), feed intake, digestibility, ruminal NH_3-N concentration and the concentration of the different volatile fatty acids (Atikah *et al.,* 2018; Dai & Faciola, 2019). The pH in this study ranged between 5.5-6.7 in the control group verses 6.5-6.9 in the groups fed SNM diets. It was thus observed that SNM did not negatively affect pH but rather stimulated a positive effect that maintained the rumen pH range close to neutral for safe fiber digestion across sampling time (Jaramillo-López *et al.*, 2017; Kung, 2014).

This result agreed with Venkateswarlu *et al*. (2018) that reported that pH was not affected when SNM was included in sheep diets. Other studies with essential oils such as olive oil, palm oil,

linseed oil, whole soybean oil similar to the SNM oil have been reported to maintain a high pH range of 6.2-6.5 when included in diets of (Fiorentini *et al.*,2015; Vargas *et al.,* 2020). Just like other essential oils, SNM exerts neutralizing properties and does not cause lowered pH observed with grain feeding making it a potential feed ingredient and also an additive in prevention of ruminal acidosis (Jaramillo-Lopez *et al*., 2017).

5.2.2 Effect of SNM increasing inclusion on the Ammonia nitrogen concentration (mg/100 ml) for subsequent hours post feeding

Ruminal NH_3-N concentration of about 5 mg/100 ml is said to be optimum for ruminal microbial activities. However, a higher concentration is believed to improve total bacterial count, feed intake and digestibility (Perdok & Leng, 1990). The concentration of NH_3-N throughout sampling hours was above the 5mg/100 ml minimum levels in all diets. For diets 5%, 10% and 15%, NH_3-N concentration was improved and maintained stable at 9- and 12-hour post feeding compared to the control. This improvement could be attributed to the fact that SNM improved nitrogen content in diets as it has been reported that increase in ruminal ammonia concentrations are allied with the nitrogen in the diets (Meissner *et al.,* 1993). However, the mean NH_3-N was low in SNM diets compared to the control.

The findings agree with Bhatta *et al.* (2012) and Venkateswarlu *et al*. (2018) that also reported a reduced NH_3-N concentration in an *in vitro* experiment. For 20% diet, the NH_3-N concentration was generally lower compared to the control (0%) throughout sampling hours. The low concentrations can be attributed to the effect of high tannins in the diet. The stable NH_3-N concentration in SNM diets of 5, 10 and 15% at 9 and 12 hour compared to the control could be attributed to the stability in the pH as fluctuations in the pH is believed to impact NH_3-N concentration (Dai & Faciola, 2019).

5.2.3 Effect of SNM increasing inclusion on ruminal tVFAs concentration for subsequent hours post feeding and acetate: propionate ratio

Volatile fatty acids are a result of rumen degradation of nutrients, affected by the type of feed and the ruminal pH (Russell, 1998). Total volatile fatty acids (tVFAs) concentration was higher for the control 0% (114.9 ml) compared to 5 (103.5 ml), 10 (103.5 ml), 15 (103.4 ml) and 20% (103.3 ml) SNM diets. This results agrees with Venkateswarlu *et al*. (2018) that also reported a

reduction in tVFAs in an *in vitro* experiment. The reduction in tVFAs can be attributed to the presence of tannins in SNM diets. Tannins bind proteins and carbohydrates, forming complexes and reducing digestibility and thus VFAs production. The claim is supported in reports made by Bhatta *et al.* (2012) in which tVFAs increased when shea nut cakes were incubated together with polyethylene glycol (PEG). The acetate propionate ratio was improved with SNM inclusion. This can be attributed to the presence of fats.

Fats are believed to lower ruminal acetate concentration while favoring propionate production and thus the molar acetate: propionate ratio improvement (Hristove *et al.,* 2013). The low acetate concentration could be attributed to the depression in activity and growth of the fibrolytic bacterial species (Atikah *et al.,* 2021). This outcome generally concurred with other studies in which oil products such as rubber seed oil, flaxseed oil and olive, sunflower, linseed oil respectively tended to significantly change the rumen VFAs profile by increasing propionate proportion verses acetate proportion Pi *et al.* (2019) and Vargas *et al.* (2020) though contradicting the general increase in the tVFAs.

5.2.4 Effect of SNM increasing inclusion on Mean ruminal protozoan count/ ml for subsequent hours post feeding

Rumen protozoa are important in methanogenesis, and its believed that a reduction of their numbers below 10^7 cells/ml could potentially reduce methane production and increase microbial protein flow (Dai & Faciola, 2019). Phytochemicals of some plants such as C-12:0 & C-14:0 medium chain fatty acids and tannins have been reported to be toxic to the protozoans (Faniyi *et al.,* 2019). In this study, mean total protozoa count reduced with increasing SNM inclusion with the lower count observed in 20% (5.07 /ml), which was significantly lower compared to the control 0% (8.53 /ml).

These results agrees with Bhatta *et al.* (2012) and Venkateswarlu *et al.* (2018) that reported that tannins and oils in shea nut by products negatively affected protozoa populations. Other studies using different oils at different levels have also reported similar reductions in protozoa count (Atikah *et al.,* 2021). Bhatt *et al.* (2011) and Majewska *et al.* (2017) reported a linear decline in rumen protozoa population with increased level of coconut oil supplementation. Similarly, Fiorentini *et al.* (2015) and Kim *et al.* (2015) also reported a 50% reduction in the diversity of

ciliate associated methanogens with coconut materials and with palm oil, linseed oil, and whole soybean respectively.

5.3 Objective three: The effect of SNM increasing inclusion on the *in-vitro* gas production, methane reduction, metabolizable energy and organic matter digestibility of Rhodes grass hay (70%) and maize bran (30%) basal diets

5.3.1 Effect of SNM increasing inclusion on the 96-hour *in vitro* gas production (incubation period)

Inclusion of SNM enhanced gas production at 5% compared to the control (0%). However, a decrease in gas production was observed with further increasing inclusion of SNM which was significantly different at 15% and 20%. Initially from 0 to 24 hr, gas production for 15% was not affected but it became pretentious over hours whereas for 20%, gas production was obviously affected throughout the incubation time. The observed improvement in gas production at 5% compared to the control could be attributed to a positive stimulatory interaction between nutrients and plant metabolites present in SNM but must have been at a minimum that can successfully be fed. A decline with increased SNM at 10, 15 and 20% could be as a result of increasing levels of SNM in the diets hence an increase in plant phenolics which must have gone beyond microbial activity (Getachew *et al*., 2004).

These results concur with Kumar *et al*. (2015) and Venkateswarlu *et al*. (2018) that similarly reported a decline in gas production with increasing SNM. Plant phenolics such as tannins, saponins and even oils insert a negative biological effect on microbial growth and activity and therefore impacting nutrient digestibility (Getachew *et al*., 2004). Tannins even form affinities with feed and microbial proteins therefore diminishing nutrient digestibility Huang *et al*. (2018) and Naumann *et al*. (2017) and oils especially those in form of free fatty acids become readily available to microbes thus becoming harmful and reducing digestibility (Atikah *et al.,* 2021; Bhatta *et al.,* 2012; Getachew *et al*., 2004; Martínez *et al*., 2013).

5.3.2 Effect of SNM increasing inclusion on *in vitro* 24-hour gas production, organic matter digestibility and metabolizable energy of Rhodes grass hay and maize bran basal diets

The *in vitro* gas production from the 24-hour incubation was higher at 5% (10.32 ml) but was not significantly different with the control 0% (9.86 ml). The 24-hour gas production then declined

with SNM further inclusion. The observed improvement in gas production at 5% compared to the control is attributed to a positive stimulatory interaction between nutrients and plant metabolites present in SNM but must have been at a minimum that can successfully be fed. A decline in 24- hour gas with further SNM inclusion could be as a result of increased levels of the tannins and fats which must have gone beyond favorable microbial activity levels (Getachew *et al*., 2004).

In vitro organic matter digestibility (IVOMD) ranged between 34.03% to 43.28%. It was higher in the control 0% (43.28%) and significantly declined with increasing SNM inclusion. The lower IVOMD was observed in 20% (34.03%). These findings were in agreement with Venkateswarlu *et al*. (2018) that reported a decline in IVOMD with higher SNM inclusion. The decline in organic matter digestibility can be attributed to the increasing tannins and fats in the diets. Tannins bind proteins and carbohydrates thus forming complexes and reducing on fermentation (Bhatta *et al.,* 2012; Bueno *et al*., 2020). Fats/oil on other hand are believed to weaken energy metabolism in protozoa thus causing their reduction that in turn reduces digestion (Ku-Vera *et al*., 2020).

These results however disagreed with Kumar *et al*. (2015) that reported a no difference in IVOMD when using rumen fluid from buffalos. The differences might have arisen from the variation in diet composition and the type of rumen liquor used. Metabolizable energy (MJ/kg DM) was significantly improved for 5% (4.95) followed by 10% (4.819 MJ/kg DM) compared to the control 0% (4.65 MJ/kg DM). Metabolizable energy then declined significantly with higher SNM inclusion. These findings concedes with Venkateswarlu *et al*. (2018) but the ME values reported in this study were far lower than those reported in Kumar *et al*. (2015) and Venkateswarlu *et al*. (2018) (7.39 MJ/kg DM) and (12.02 MJ/kg DM) respectively.

5.3.3 Effect of SNM increasing inclusion on methane gas reduction *in vitro*

The percentage of methane gas in the total gas produced was reduced with increasing SNM inclusion: 5 (28.74%), 10 (31.94%), 15 (42.76%) and 20% (56.95%). The highest reduction was observed at 20% (56.95%), and was significantly different from (5), (10) and (15%). The reduction in % methane gas can be attributed to the reduction in the total gas production, resulting from the action of tannins and fat in the SNM. These findings are in agreement with

Bhatta *et al.* (2012) report when different shea nut by products reduced methane when they were incubated without polyethylene glycol 6000 (PEG). Similar observations were made by Venkateswarlu *et al.* (2018) and Kumar *et al*. (2015) that reported methane reduction of 28.21% and 64.0% respectively with increasing SNM inclusions. Tannins are naturally occurring and form the major constituents of essential oils. Both tannins and essential oils are believed to exert inhibitory effects on methanogenesis through firstly, inhibition of protozoa growth that are responsible for methanogenesis and also by directly providing a sink to hydrogen (Beauchemin *et al.,* 2009; Kumar *et al.,* 2015; Patra, 2013; Rasmussen & Harrison, 2011).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Most ruminant livestock farmers are low input ranchers relying majorly on natural forages for feeding of their livestock. Occasionally, they do give concentrate supplements such as maize bran. However, due to the high demand for the same concentrates for use in other livestock feeds like pigs and poultry, there has been an increase in prices and inadequate availability. Non-conventional concentrates such as shea nut meal could bridge this gap. The study was therefore conducted to establish the nutrient value of shea nut meal and its impact on growth performance, the cost: benefit ratio, rumen fermentation parameters, gas kinetics and its methane reduction potential.

The following conclusions were drawn from the study:

(i) Shea nut meal contains substantial nutrients (CP, NDF, ADF & EE). It enhances the nutrient composition in diets when added, and also improves digestibility, growth performance, feed conversion ratio and cost benefit ratio when fed to sheep. The best intake and performances were observed at 5% but the cost: benefit ratio improved with increased inclusion and was best observed at 20%.

(ii) Shea nut meal modulates the rumen, by maintaining a high constant pH, optimizes ruminal NH_3-N concentrations, improves acetate propionate ratio and unleashes ant-protozoan properties making it a possible technology in the defaunation and control of ruminal acidosis. The ideal ruminal parameters were observed at 10% SNM inclusion

(iii) Shea nut meal increases gas production at 5% and improves the gas production from both the readily soluble and slowly degradable fraction at 10% compared to the control. Methane percentage concentration in the gas also greatly reduced with SNM and % methane reduction reduced with increasing SNM inclusion making it a potential ingredient for ruminant feed for suppression of enteric methane production.

6.2 Recommendations

Basing on the findings drawn from the conclusions, the following recommendations can be made:

(i) Shea nut meal is an average protein and high energy concentrate, it can be used to improve the protein and the energy value of sheep feeds. However, due to the high concentration of anti-nutritional compounds (tannins) in the meal, it should not be included beyond 10% as digestibility and performance deteriorated

(ii) Shea nut meal could also be used as an additive source of essential oils and perhaps tannins when required in ruminant feed for purposes of rumen modulation especially in controlling rapid fermentation and hence prevention of ruminal acidosis associated with drastic lowering of pH

(iii) Shea nut meal could also be used as a rumen defaunation additive and a rumen methane production suppressant in ruminant feeds.

6.3 Recommendation for further studies

(i) Further studies are recommended to understand SNM effect on VFAs rate of passage and absorption at tissue metabolism before adopted as a commercial feed ingredient as the meal is high in tannins and possibly residual fat depending on the extraction method used during the processing.

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APPENDICES

Appendix A. Abstract of the Publications

Abstract 1.

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Performance and cost benefit of variable inclusion levels of shea nut meal in the diet of growing sheep

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Abstract
Poor forages are the drivers to poor livestock performance. Concentrates or fats can improve nutrient utilization in forages if mixed together. However, conventional concentrates are expensive and in demand for use in pig and poultry feeds. This study investigated the nutrient composition of shea nut meal (SNM) and its effect on the performance of growing sheep when added at different inclusion levels. Fifteen ewes weighing 25±0.8kg were grouped into five (N = 3) and assigned five diets containing 0%, 5%, 10%, 15% and 20% SNM in a completely randomized design. Diets were analyzed for chemical composition and data on intake, nutrient digestibility, average daily gain (ADG), feed conversion ratio (FCR) and cost of feed per kg gain were computed. Results obtained showed SNM (g/kgDM) contains 302 (ADF), 589 (NDF), 172 (CP), 304 (EE), 120 (total tannins) and 25 (ME) and improved 395 (ADF), 649 (NDF), 150 CP, 304 EE, and 18.3 ME in diets. Feed intake was improved at 5% but was not different (p>0.05) from 0% but also declined with increasing inclusion of 10%, 15% and 20%. Nutrient digestibility was improved (p<0.05) except for EE. Average daily gain was also improved and was higher at (5%) and lower at (0%) (p<0.05). The best FCR was observed at (20%) but did not differ in all SNM diets (p>0.05). The cost of feed per kg gain (KES/kg) reduced with increasing SNM inclusion levels. It was concluded that SNM is a possible cheaper energy and protein source for growing sheep. It improved nutrients in diets, intake at 5%, nutrient digestibility, ADG, FCR and reduced the cost of feed per kg gain in sheep feeding.

Keywords: Average daily gain, cost of feed/kg gain, feed conversion ratio, feed intake, nutrient digestibility.

Abstract 2.

Evaluation of Shea nut meal potential to mitigate enteric methane *in vitro*

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Abstract

The worry of unmitigated global warming causing irreversible climate change is on the rise. Sustainable solutions to mitigate methane production from ruminant farming should thus be sought. Shea nut meal (SNM) contains numerous plant compounds proved to reduce enteric methane production in ruminants and its adoption in ruminant feed could become a game changer. This experiment examined total gas production, metabolizable energy, organic matter digestibility and methane mitigation properties of shea nut meal when included at varying levels of SNM0 (0%), SNM5 (5%), SNM10 (10%), SNM15 (15%) and SNM20 (20%) in a basal diet containing Rhodes grass hay and maize bran in an *in vitro* experiment set in a completely randomized design. Results showed total gas production (ml) was improved at 5% (49.8) compared to 0% (46.7) but declined with further SNM inclusions at 10% (40.6), 15% (29.5) and 20% (24.8), Organic matter digestibility (% OMD) was reduced ($p<0.05$) with SNM inclusion of 5% (42.8), 10% (40.5), 15% (35.51), 20% (34.0) compared to 0% (43.3). Metabolizable energy was improved ($p<0.05$) for 5% (4.95) and 10% (4.82) but also declined ($p<0.05$) with SNM further inclusion of 15% (4.27) and 20% (4.25). Methane from the 0.2g of the truly digested substrate was ($p<0.05$) reduced with increasing inclusion by 28.7%, 31.9%, 42.8% and 56.9% in 5%, 10%, 15% and 20% respectively. It was concluded that SNM reduced methanogenesis and low inclusion of 5% can safely be fed without detrimental effects on fermentation.

Key words: *gas production, Metabolizable energy, Methane reduction, organic matter digestibility*

Abstract 3.

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Shea nut meal effect on rumen fermentation parameters of sheep

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Abstract
Although ruminants have a greater capacity to utilize various feed sources with lesser risks, the impact of non-conventional feed on rumen function should be understood. This study investigated the effects of Shea nut meal (SNM) different inclusion levels on rumen parameters of sheep fed 70% Rhodes grass hay and 30% maize bran basal diets. Five diets (D) containing SNM at different levels of D1=0% SNM, D2=5% SNM, D3=10% SNM, D4=15% SNM and D5=20% SNM were fed to fifteen ewes clustered into three, in a completely randomized design (CRD). Rumen samples were collected in the 10th week of the feeding trial at hour 0, 3, 6, 9 and 12 post feeding and analyzed for pH, NH_3-N, volatile fatty acids & protozoa count. SNM increased mean pH, and was in a range of (6.58-6.8) in SNM diets compared D1 (6.2). Mean NH_3-N (mg/100ml) in SNM groups decreased with SNM increasing inclusion. Acetate: propionate ratio (mmol/L) was favored for SNM diets; 5% (1.379), 10% (1.375), 15% (1.377), 20% (1.365) compared to D1 (1.682) but total volatile fatty acids (mmol/L) ($p<0.05$) reduced with SNM increased inclusion. Mean protozoa counts were similarly reduced with SNM increased inclusion; 5% (7.40), 10% (6.80), 15% (6.40), 20% (5.07) compared to D1 (8.53). It was concluded that SNM had no adverse effects on the rumen parameters but rather enhanced the rumen function through improved propionate production and exertion of ant protozoan properties, making it a safe supplement and/or additive sourceof essential oils and perhaps tannins when needed in formulation of ruminant feeds.

Keywords: NH_3-N concentration, pH, protozoa count and volatile fatty acid concentration

Appendix B. Experimental Animals

Appendix C. Pictures of the shea nut different products

a. Full grown shea nut tree with shea nut fruits

b. Un- shelled shea nut kernels

b. Shelled shea nut kernels

d. Shea nut meal

Appendix D: Analysis of variance for the nutrient composition of experimental diets.

Dependent Variable: DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	860.400000	215.100000	2.51	0.1082
Error	10	856.000000	85.600000		
Corrected Total	14	1716.400000			

R-Square	Coeff Var	Root MSE	DM Mean
0.501282	1.006530	9.252027	919.2000

The GLM Procedure

t Tests (LSD) for DM

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	85.6
Critical Value of t	2.22814
Least Significant Difference	16.832

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	929.667	3	1
A			
B A	926.667	3	2
B A			
B A	916.000	3	3
B A			
B A	913.000	3	4
B			
B	910.667	3	5

Dependent Variable: Ash

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5189.400000	1297.350000	91.04	<.0001
Error	10	142.500000	14.250000		

Corrected Total	14	5331.900000

R-Square	Coeff Var	Root MSE	Ash Mean
0.973274	3.320068	3.774917	113.7000

The GLM Procedure

t Tests (LSD) for Ash

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	14.25
Critical Value of t	2.22814
Least Significant Difference	6.8676

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	142.000	3	1
B	125.333	3	2
C	111.333	3	3
D	101.333	3	4
E	88.500	3	5

Dependent Variable: ADF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	655.6000000	163.9000000	20.15	<.0001
Error	10	81.3333333	8.1333333		
Corrected Total	14	736.9333333			

R-Square	Coeff Var	Root MSE	ADF Mean
0.889633	0.737052	2.851900	386.9333

The GLM Procedure

t Tests (LSD) for ADF

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	8.133333

Critical Value of t 2.22814
Least Significant Difference 5.1884
Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	395.000	3	5
A			
A	390.333	3	3
A			
A	390.000	3	4
B	383.333	3	2
C	376.000	3	1

Dependent Variable: NDF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2536.266667	634.066667	33.26	<.0001
Error	10	190.666667	19.066667		
Corrected Total	14	2726.933333			

R-Square	Coeff Var	Root MSE	NDF Mean
0.930080	0.689889	4.366539	632.9333

The GLM Procedure

t Tests (LSD) for NDF

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 19.06667
Critical Value of t 2.22814
Least Significant Difference 7.9439

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	648.667	3	5
A			
B A	641.000	3	4
B			
B	639.000	3	3

C	623.000	3	2
D	613.000	3	1

Dependent Variable: CP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1672.400000	418.100000	13.52	0.0005
Error	10	309.333333	30.933333		
Corrected Total	14	1981.733333			

R-Square	Coeff Var	Root MSE	CP Mean
0.843908	3.976483	5.561774	139.8667

The GLM Procedure

t Tests (LSD) for CP

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	30.93333
Critical Value of t	2.22814
Least Significant Difference	10.118

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	150.000	3	4
A			
A	149.000	3	5
A			
B A	141.000	3	3
B			
B	138.667	3	2
C	120.667	3	1

Dependent Variable: EE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	18156.68267	4539.17067	5165.98	<.0001

Error	10	8.78667	0.87867
Corrected Total	14	18165.46933	

R-Square	Coeff Var	Root MSE	EE Mean
0.999516	1.129001	0.937372	83.02667

The GLM Procedure

t Tests (LSD) for EE

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.878667
Critical Value of t	2.22814
Least Significant Difference	1.7053

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	134.3333	3	5
B	102.0000	3	4
C	83.2000	3	3
D	64.8000	3	2
E	30.8000	3	1

Dependent Variable: Total tannins

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	168.0645333	42.0161333	54.87	<.0001
Error	10	7.6568000	0.7656800		
Corrected Total	14	175.7213333			

R-Square	Coeff Var	Root MSE	Total tannins Mean
0.956426	15.45082	0.875031	5.663333

The GLM Procedure

t Tests (LSD) for Total tannins

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.76568

Critical Value of t 2.22814
Least Significant Difference 1.5919

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	9.3633	3	5
A			
B A	8.1667	3	4
B			
B	6.7800	3	3
C	4.0067	3	2
D	0.0000	3	1

Dependent Variable: ME

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.16186667	3.54046667	38.35	<.0001
Error	10	0.92326667	0.09232667		
Corrected Total	14	15.08513333			

R-Square	Coeff Var	Root MSE	ME Mean
0.938796	1.773116	0.303853	17.13667

The GLM Procedure

t Tests (LSD) for ME

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 0.092327
Critical Value of t 2.22814
Least Significant Difference 0.5528

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	18.2867	3	5
A			
A	17.9867	3	4
B	17.2267	3	3

C	16.5833	3	2
D	15.6000	3	1

Dependent Variable: CF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	493.0666667	123.2666667	4.54	0.0238
Error	10	271.3333333	27.1333333		
Corrected Total	14	764.4000000			

R-Square	Coeff Var	Root MSE	CF Mean
0.645038	1.835436	5.208967	283.8000

The GLM Procedure

t Tests (LSD) for CF

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	27.13333
Critical Value of t	2.22814
Least Significant Difference	9.4765

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	294.000	3	1
A			
B A	285.333	3	2
B			
B	282.667	3	3
B			
B	279.000	3	5
B			
B	278.000	3	4

Appendix E: Analysis of variance for the feed intake and percentage nutrient digestibility of Rhodes grass hay and maize bran basal diets in supplementation with increasing levels of shea nut meal.

Dependent Variable: Digestibility of DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1418.580093	354.645023	160.76	<.0001
Error	10	22.061000	2.206100		
Corrected Total	14	1440.641093			

R-Square	Coeff Var	Root MSE	M Mean
0.984687	2.030774	1.485295	73.13933

The GLM Procedure

t Tests (LSD) for M

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	2.2061
Critical Value of t	2.22814
Least Significant Difference	2.7021

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	85.857	3	3
B	82.450	3	2
C	71.443	3	4
D	65.700	3	5
E	60.247	3	1

Dependent Variable: Digestibility of CP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	62.1452400	15.5363100	1.79	0.2069
Error	10	86.6549333	8.6654933		
Corrected Total	14	148.8001733			

R-Square	Coeff Var	Root MSE	CP Mean
0.417642	3.090802	2.943721	95.24133

The GLM Procedure

t Tests (LSD) for CP

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	8.665493
Critical Value of t	2.22814
Least Significant Difference	5.3554

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	98.210	3	2
A			
A	97.060	3	3
A			
A	94.443	3	4
A			
A	93.260	3	1
A			
A	93.233	3	5

Dependent Variable: Digestibility of ADF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1003.003573	250.750893	34.26	<.0001
Error	10	73.186400	7.318640		
Corrected Total	14	1076.189973			

R-Square	Coeff Var	Root MSE	AF Mean
0.931995	5.403536	2.705299	50.06533

The GLM Procedure

t Tests (LSD) for ADF

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	7.31864
Critical Value of t	2.22814
Least Significant Difference	4.9217

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	65.777	3	2
B	50.173	3	3
B			
C B	45.917	3	4
C B			
C B	45.270	3	1
C			
C	43.190	3	5

Dependent Variable: Digestibility of NDF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	385.8374267	96.4593567	49.80	<.0001
Error	10	19.3693333	1.9369333		
Corrected Total	14	405.2067600			

R-Square	Coeff Var	Root MSE	NF Mean
0.952199	2.984896	1.391738	46.62600

The GLM Procedure

t Tests (LSD) for NDF

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.936933
Critical Value of t	2.22814
Least Significant Difference	2.5319

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	52.760	3	2

	A			
	A	52.710	3	3
	B	44.157	3	1
	B			
C	B	42.173	3	4
C				
C		41.330	3	5

Dependent Variable: Digestibility of EE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.23537333	0.05884333	101.45	<.0001
Error	10	0.00580000	0.00058000		
Corrected Total	14	0.24117333			

R-Square	Coeff Var	Root MSE	EE Mean
0.975951	0.024117	0.024083	99.86133

The GLM Procedure

t Tests (LSD) for EE

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.00058
Critical Value of t	2.22814
Least Significant Difference	0.0438

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	99.99333	3	2
A			
A	99.99000	3	1
B	99.88667	3	3
C	99.75667	3	4
D	99.68000	3	5

Dependent Variable: Concentrate mixture intake

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	4788.933333	1197.233333	15.75	0.0003
Error	10	760.000000	76.000000		
Corrected Total	14	5548.933333			

R-Square	Coeff Var	Root MSE	Concentrate mixture intake Mean
0.863037	4.206078	8.717798	207.2667

The GLM Procedure

t Tests (LSD) for Concentrate mixture intake

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	76
Critical Value of t	2.22814
Least Significant Difference	15.86

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	225.000	3	1
A			
A	225.000	3	2
A			
A	213.333	3	3
B	192.333	3	4
B			
B	180.667	3	5

Dependent Variable: Hay intake

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	319740.9333	79935.2333	11.62	0.0009
Error	10	68806.0000	6880.6000		
Corrected Total	14	388546.9333			

R-Square	Coeff Var	Root MSE	Hay intake Mean
0.822915	20.58638	82.94938	402.9333

The GLM Procedure

t Tests (LSD) for Hay intake

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	6880.6
Critical Value of t	2.22814
Least Significant Difference	150.91

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	610.67	3	2
A			
A	533.33	3	1
B	363.67	3	3
B			
B	264.00	3	4
B			
B	243.00	3	5

Dependent Variable: Total Dry matter intake

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	397051.7333	99262.9333	14.84	0.0003
Error	10	66882.6667	6688.2667		
Corrected Total	14	463934.4000			

R-Square	Coeff Var	Root MSE	Total dry matter intake Mean
0.855836	13.40246	81.78182	610.2000

The GLM Procedure

t Tests (LSD) for Total dry matter intake

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	6688.267
Critical Value of t	2.22814
Least Significant Difference	148.78

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats

	A	835.67	3	2
	A			
	A	758.33	3	1
	B	577.00	3	3
	B			
C	B	456.33	3	4
C				
C		423.67	3	5

Appendix F: Analysis of variance for the performance of sheep fed Rhodes grass hay and maize bran diets in supplementation with increasing levels of SNM.

Dependent Variable: Initial weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.9440000	0.2360000	0.01	0.9995
Error	10	168.6400000	16.8640000		
Corrected Total	14	169.5840000			

R-Square	Coeff Var	Root MSE	Initial weight Mean
0.005567	16.11688	4.106580	25.48000

The GLM Procedure

t Tests (LSD) for Initial weight

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	16.864
Critical Value of t	2.22814
Least Significant Difference	7.471

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	25.867	3	3
A			
A	25.667	3	2
A			
A	25.400	3	1
A			

A	25.267	3	4
A			
A	25.200	3	5

Dependent Variable: Final weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	309.270667	77.317667	1.00	0.4533
Error	10	776.166667	77.616667		
Corrected Total	14	1085.437333			

R-Square	Coeff Var	Root MSE	Final weight Mean
0.284927	25.25329	8.810032	34.88667

The GLM Procedure

t Tests (LSD) for Final weight

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	77.61667
Critical Value of t	2.22814
Least Significant Difference	16.028

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	42.667	3	2
A			
A	35.833	3	5
A			
A	35.267	3	1
A			
A	30.500	3	4
A			
A	30.167	3	3

Dependent Variable: Total weight gain

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	314.5826667	78.6456667	2.17	0.1459
Error	10	362.1933333	36.2193333		

Corrected Total	14	676.7760000

R-Square	Coeff Var	Root MSE	Total weight gain Mean
0.464825	63.08438	6.018250	9.540000

The GLM Procedure

t Tests (LSD) for Total weight gain

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	36.21933
Critical Value of t	2.22814
Least Significant Difference	10.949

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	17.000	3	2
A			
B A	11.300	3	5
B A			
B A	9.867	3	1
B			
B	5.233	3	4
B			
B	4.300	3	3

Dependent Variable: Average daily gain

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	32747.03067	8186.75767	2.17	0.1460
Error	10	37709.08667	3770.90867		
Corrected Total	14	70456.11733			

R-Square	Coeff Var	Root MSE	Average daily gain Mean
0.464786	63.07717	61.40772	97.35333

The GLM Procedure

t Tests (LSD) for Average daily gain

Alpha	0.05
Error Degrees of Freedom	10

Error Mean Square 3770.909
Critical Value of t 2.22814
Least Significant Difference 111.72

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	173.47	3	2
A			
B A	115.30	3	5
B A			
B A	100.70	3	1
B			
B	53.40	3	4
B			
B	43.90	3	3

Dependent Variable: FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	27953.79733	6988.44933	1.78	0.2085
Error	10	39164.64000	3916.46400		
Corrected Total	14	67118.43733			

R-Square	Coeff Var	Root MSE	FCR Mean
0.416485	64.92771	62.58166	96.38667

The GLM Procedure

t Tests (LSD) for FCR

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 3916.464
Critical Value of t 2.22814
Least Significant Difference 113.85

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treats
A	178.30	3	3
A			
B A	97.70	3	4
B A			
B A	81.57	3	1

B	A			
B	A	67.03	3	5
B				
B		57.33	3	2

Appendix G: Analysis of variance for the cost benefit analysis of SNM increasing inclusion in sheep diets fed of Rhodes grass hay and maize bran basal diets.

Dependent Variable: Cost per kg feed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.29616000	0.82404000	Infty	<.0001
Error	10	0.00000000	0.00000000		
Corrected Total	14	3.29616000			

R-Square	Coeff Var	Root MSE	Cost per kg feed Mean
1.000000	0	0	16.24400

The GLM Procedure

t Tests (LSD) for Cost per kg feed

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0
Critical Value of t	2.22814
Least Significant Difference	0

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	16.94	3	5
B	16.52	3	4
C	16.22	3	3
D	15.98	3	2
E	15.56	3	1

Dependent Variable: Total feed consumed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3812.692333	953.173083	14.84	0.0003
Error	10	642.447467	64.244747		
Corrected Total	14	4455.139800			

R-Square	Coeff Var	Root MSE	Total feed consumed Mean
0.855796	13.40348	8.015282	59.80000

The GLM Procedure

t Tests (LSD) for Total feed consumed

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	64.24475
Critical Value of t	2.22814
Least Significant Difference	14.582

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	81.893	3	2
A			
A	74.317	3	1
B	56.547	3	3
B			
C B	44.723	3	4
C			
C	41.520	3	5

Dependent Variable: live wight gain

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	47.3000000	11.8250000	1.73	0.2201
Error	10	68.4600000	6.8460000		

Corrected Total	14	115.7600000

R-Square	Coeff Var	Root MSE	live wight gain Mean
0.408604	26.69884	2.616486	9.800000

The GLM Procedure

t Tests (LSD) for live wight gain

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	6.846
Critical Value of t	2.22814
Least Significant Difference	4.7601

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	13.000	3	2
A			
B A	9.733	3	3
B A			
B A	9.433	3	4
B A			
B A	9.300	3	5
B			
B	7.533	3	1

Dependent Variable: cost of feed/kg gain

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	10797.41404	2699.35351	5.46	0.0135
Error	10	4942.34873	494.23487		
Corrected Total	14	15739.76277			

R-Square	Coeff Var	Root MSE	cost of feed/kg gain Mean
0.685996	21.43639	22.23139	103.7087

The GLM Procedure

t Tests (LSD) for cost of feed/kg gain

Alpha	0.05
Error Degrees of Freedom	10

Error Mean Square 494.2349
Critical Value of t 2.22814
Least Significant Difference 40.445

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	153.88	3	1
B	106.54	3	2
B			
B	95.07	3	3
B			
B	84.31	3	5
B			
B	78.74	3	4

The GLM Procedure

t Tests (LSD) for Total cost of feeding per sheep

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 16453.15
Critical Value of t 2.22814
Least Significant Difference 233.36

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	1308.9	3	2
A			
A	1156.6	3	1
B	917.3	3	3
B			
B	738.9	3	4
B			
B	703.5	3	5

Appendix H: Analysis of variance for the effect of SNM increasing inclusion on ruminal pH over sampling time

Dependent Variable: 0 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.04266667	0.01066667	1.23	0.3580
Error	10	0.08666667	0.00866667		
Corrected Total	14	0.12933333			

R-Square	Coeff Var	Root MSE	0 hour Mean
0.329897	1.363695	0.093095	6.826667

The GLM Procedure

t Tests (LSD) for 0 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.008667
Critical Value of t	2.22814
Least Significant Difference	0.1694

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	6.93333	3	5
A			
A	6.80000	3	2
A			
A	6.80000	3	3
A			
A	6.80000	3	4
A			
A	6.80000	3	1

Dependent Variable: 3 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.27600000	0.06900000	3.14	0.0649
Error	10	0.22000000	0.02200000		
Corrected Total	14	0.49600000			

R-Square	Coeff Var	Root MSE	3 hour Mean
0.556452	2.296037	0.148324	6.460000

The GLM Procedure

t Tests (LSD) for 3 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.022
Critical Value of t	2.22814
Least Significant Difference	0.2698

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	6.6000	3	5
A			
A	6.5000	3	2
A			
A	6.5000	3	3
A			
A	6.5000	3	4
B	6.2000	3	1

Dependent Variable: 6 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.97066667	0.74266667	85.69	<.0001
Error	10	0.08666667	0.00866667		
Corrected Total	14	3.05733333			

R-Square	Coeff Var	Root MSE	6 hour Mean
0.971653	1.457645	0.093095	6.386667

The GLM Procedure

t Tests (LSD) for 6 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.008667
Critical Value of t	2.22814

Least Significant Difference 0.1694

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	6.80000	3	5
B	6.60000	3	3
B			
B	6.60000	3	4
C	6.40000	3	2
D	5.53333	3	1

Dependent Variable: 9 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.69066667	0.42266667	19.81	<.0001
Error	10	0.21333333	0.02133333		
Corrected Total	14	1.90400000			

R-Square	Coeff Var	Root MSE	9 hour Mean
0.887955	2.240174	0.146059	6.520000

The GLM Procedure

t Tests (LSD) for 9 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.021333
Critical Value of t	2.22814
Least Significant Difference	0.2657

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	6.8000	3	5
A			
A	6.7333	3	4
A			
A	6.6000	3	3
A			
A	6.6000	3	2

B	5.8667	3	1

Dependent Variable: 12 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.17066667	0.04266667	1.60	0.2488
Error	10	0.26666667	0.02666667		
Corrected Total	14	0.43733333			

R-Square	Coeff Var	Root MSE	12 hour Mean
0.390244	2.454399	0.163299	6.653333

The GLM Procedure

t Tests (LSD) for 12 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.026667
Critical Value of t	2.22814
Least Significant Difference	0.2971

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	6.8667	3	5
A			
A	6.6000	3	4
A			
A	6.6000	3	1
A			
A	6.6000	3	2
A			
A	6.6000	3	3

Appendix I: Analysis of variance for the effect of SNM increasing inclusion on ruminal NH_3-N concentration over sampling time

Dependent Variable: 0 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.37066667	1.84266667	5.97	0.0101

Error	10	3.08666667	0.30866667
Corrected Total	14	10.45733333	

R-Square	Coeff Var	Root MSE	0 hour Mean
0.704832	4.000800	0.555578	13.88667

The GLM Procedure

t Tests (LSD) for 0 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.308667
Critical Value of t	2.22814
Least Significant Difference	1.0107

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	15.2667	3	1
B	13.7333	3	2
B			
B	13.6000	3	4
B			
B	13.4667	3	5
B			
B	13.3667	3	3

Dependent Variable: 3 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	9.14933333	2.28733333	7.02	0.0059
Error	10	3.26000000	0.32600000		
Corrected Total	14	12.40933333			

R-Square	Coeff Var	Root MSE	3 hour Mean
0.737295	3.230653	0.570964	17.67333

The GLM Procedure

t Tests (LSD) for 3 hour

Alpha	0.05
Error Degrees of Freedom	10

Error Mean Square 0.326
Critical Value of t 2.22814
Least Significant Difference 1.0387
Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	18.8667	3	1
A			
A	18.3333	3	5
B	17.2000	3	3
B			
B	17.0667	3	2
B			
B	16.9000	3	4

Dependent Variable: 6 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	12.15333333	3.03833333	6.74	0.0067
Error	10	4.50666667	0.45066667		
Corrected Total	14	16.66000000			

R-Square	Coeff Var	Root MSE	6 hour Mean
0.729492	3.903006	0.671317	17.20000

The GLM Procedure
t Tests (LSD) for 6 hour

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 0.450667
Critical Value of t 2.22814
Least Significant Difference 1.2213
Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	18.3333	3	4
A			
B A	17.8667	3	3
B A			
B A	17.1667	3	1
B			

B	16.9333	3	2
C	15.7000	3	5

Dependent Variable: 9 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	23.25333333	5.81333333	19.12	0.0001
Error	10	3.04000000	0.30400000		
Corrected Total	14	26.29333333			

R-Square	Coeff Var	Root MSE	d Mean
0.884381	3.348352	0.551362	16.46667

The GLM Procedure

t Tests (LSD) for 9 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.304
Critical Value of t	2.22814
Least Significant Difference	1.0031

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	18.2667	3	2
B	16.9333	3	3
B			
B	16.4000	3	4
B			
B	16.3333	3	1
C	14.4000	3	5

Dependent Variable: 12 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.11600000	1.77900000	1.21	0.3670
Error	10	14.75333333	1.47533333		
Corrected Total	14	21.86933333			

R-Square	Coeff Var	Root MSE	12 hour Mean
0.325387	7.105887	1.214633	17.09333

The GLM Procedure

t Tests (LSD) for 12 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.475333
Critical Value of t	2.22814
Least Significant Difference	2.2097

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	17.9000	3	2
A			
A	17.8000	3	3
A			
A	17.1333	3	1
A			
A	16.4000	3	4
A			
A	16.2333	3	5

Appendix J: Analysis of variance for the effect of SNM increasing inclusion on ruminal Butyrate concentration levels over time.

Dependent Variable: 0 hour Butyrate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	10.08400000	2.52100000	48.48	<.0001
Error	10	0.52000000	0.05200000		
Corrected Total	14	10.60400000			

R-Square	Coeff Var	Root MSE	0 hour Butyrate Mean
0.950962	1.254318	0.228035	18.18000

The GLM Procedure

t Tests (LSD) for 0 hour Butyrate

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 0.052
Critical Value of t 2.22814
Least Significant Difference 0.4149

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	19.8000	3	1
B	17.9667	3	2
B			
B	17.8000	3	3
B			
B	17.7667	3	5
B			
B	17.5667	3	4

Dependent Variable: 3 hour Butyrate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	95.14666667	23.78666667	127.43	<.0001
Error	10	1.86666667	0.18666667		
Corrected Total	14	97.01333333			

R-Square	Coeff Var	Root MSE	3 hour Butyrate Mean
0.980759	2.163853	0.432049	19.96667

The GLM Procedure

t Tests (LSD) for 3 hour Butyrate

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 0.186667
Critical Value of t 2.22814
Least Significant Difference 0.786

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	25.0000	3	1
B	18.8333	3	3
B			

B	18.8000	3	2
B			
B	18.6000	3	4
B			
B	18.6000	3	5

Dependent Variable: 6 hour Butyrate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	41.12666667	10.28166667	70.10	<.0001
Error	10	1.46666667	0.14666667		
Corrected Total	14	42.59333333			

R-Square	Coeff Var	Root MSE	6 hour Butyrate Mean
0.965566	1.817900	0.382971	21.06667

The GLM Procedure

t Tests (LSD) for 6 hour Butyrate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.146667
Critical Value of t	2.22814
Least Significant Difference	0.6967

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	24.3667	3	1
B	20.4667	3	4
B			
B	20.2667	3	2
B			
B	20.2000	3	5
B			
B	20.0333	3	3

Dependent Variable: 9 hour Butyrate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.97733333	0.99433333	2.75	0.0888

Error	10	3.62000000	0.36200000
Corrected Total	14	7.59733333	

R-Square	Coeff Var	Root MSE	9 hour Butyrate Mean
0.523517	2.672480	0.601664	22.51333

The GLM Procedure

t Tests (LSD) for 9 hour Butyrate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.362
Critical Value of t	2.22814
Least Significant Difference	1.0946

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	23.5000	3	1
A			
B A	22.5333	3	4
B			
B	22.2667	3	5
B			
B	22.1667	3	3
B			
B	22.1000	3	2

Dependent Variable: 12 hour butyrate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	8.48666667	2.12166667	24.86	<.0001
Error	10	0.85333333	0.08533333		
Corrected Total	14	9.34000000			

R-Square	Coeff Var	Root MSE	12 hour Butyrate Mean
0.908637	1.529417	0.292119	19.10000

The GLM Procedure

t Tests (LSD) for 12 hour Butyrate

Alpha	0.05

Error Degrees of Freedom 10
Error Mean Square 0.085333
Critical Value of t 2.22814
Least Significant Difference 0.5314
Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	20.6000	3	1
B	18.8333	3	2
B			
B	18.7000	3	3
B			
B	18.7000	3	5
B			
B	18.6667	3	4

Appendix K: Analysis of variance on the effect of SNM increasing inclusion on ruminal propionate concentration levels over time.

Dependent Variable: 0 hour propionate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	8.68000000	2.17000000	1.36	0.3148
Error	10	15.96000000	1.59600000		
Corrected Total	14	24.64000000			

R-Square	Coeff Var	Root MSE	0 hour propionate Mean
0.352273	3.748751	1.263329	33.70000

The GLM Procedure
t Tests (LSD) for 0 hour propionate

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 1.596
Critical Value of t 2.22814
Least Significant Difference 2.2983
Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	34.267	3	5
A			

A	34.100	3	4
A			
A	34.067	3	3
A			
A	33.867	3	2
A			
A	32.200	3	1

Dependent Variable: 3 hour propionate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.86000000	0.71500000	0.53	0.7194
Error	10	13.59333333	1.35933333		
Corrected Total	14	16.45333333			

R-Square	Coeff Var	Root MSE	3 hour propionate Mean
0.173825	3.197179	1.165905	36.46667

The GLM Procedure

t Tests (LSD) for 3 hour propionate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.359333
Critical Value of t	2.22814
Least Significant Difference	2.1211

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	36.9667	3	2
A			
A	36.9000	3	5
A			
A	36.4667	3	3
A			
A	36.2000	3	4
A			
A	35.8000	3	1

Dependent Variable: 6 hour propionate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.44400000	0.11100000	0.04	0.9964
Error	10	27.43333333	2.74333333		
Corrected Total	14	27.87733333			

R-Square	Coeff Var	Root MSE	6 hour propionate Mean
0.015927	4.599133	1.656301	36.01333

The GLM Procedure

t Tests (LSD) for 6 hour propionate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	2.743333
Critical Value of t	2.22814
Least Significant Difference	3.0133

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	36.233	3	3
A			
A	36.133	3	5
A			
A	36.033	3	4
A			
A	35.933	3	2
A			
A	35.733	3	1

Dependent Variable: 9 hour propionate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.42400000	0.35600000	0.21	0.9260
Error	10	16.81333333	1.68133333		
Corrected Total	14	18.23733333			

R-Square	Coeff Var	Root MSE	9 hour propionate Mean
0.078082	3.661509	1.296662	35.41333

The GLM Procedure

t Tests (LSD) for 9 hour propionate

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 1.681333
Critical Value of t 2.22814
Least Significant Difference 2.359

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	35.767	3	5
A			
A	35.633	3	3
A			
A	35.433	3	2
A			
A	35.367	3	4
A			
A	34.867	3	1

Dependent Variable: 12 hour propionate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2.30266667	0.57566667	0.09	0.9828
Error	10	62.51333333	6.25133333		
Corrected Total	14	64.81600000			

R-Square	Coeff Var	Root MSE	12 hour propionate Mean
0.035526	7.323570	2.500267	34.14000

The GLM Procedure

t Tests (LSD) for 12 hour propionate

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 6.251333
Critical Value of t 2.22814
Least Significant Difference 4.5487

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	34.700	3	4
A			
A	34.333	3	3
A			
A	34.100	3	2

A			
A	34.067	3	5
A			
A	33.500	3	1

Appendix L: Analysis of variance on the effect of SNM increasing inclusion on ruminal acetate concentration levels over time.

Dependent Variable: 0 hour acetate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	262.7106667	65.6776667	53.63	<.0001
Error	10	12.2466667	1.2246667		
Corrected Total	14	274.9573333			

R-Square	Coeff Var	Root MSE	0 hour acetate Mean
0.955460	3.178801	1.106647	34.81333

The GLM Procedure

t Tests (LSD) for 0 hour acetate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.224667
Critical Value of t	2.22814
Least Significant Difference	2.0133

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	43.1000	3	1
B	33.8667	3	2
B	32.5333	3	3
B	32.3333	3	4
B	32.2333	3	5

Dependent Variable: 3 hour acetate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	250.5626667	62.6406667	177.62	<.0001
Error	10	3.5266667	0.3526667		
Corrected Total	14	254.0893333			

R-Square	Coeff Var	Root MSE	3 hour acetate Mean
0.986120	1.374458	0.593857	43.20667

The GLM Procedure

t Tests (LSD) for 3 hour acetate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.352667
Critical Value of t	2.22814
Least Significant Difference	1.0804

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	51.3667	3	1
B	41.4667	3	4
B			
B	41.4000	3	5
B			
B	40.9333	3	2
B			
B	40.8667	3	3

Dependent Variable: 6 hour acetate mean

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	10.32400000	2.58100000	2.65	0.0963
Error	10	9.74000000	0.97400000		
Corrected Total	14	20.06400000			

R-Square	Coeff Var	Root MSE	6 hour acetate Mean
0.514553	1.828296	0.986914	53.98000

The GLM Procedure

t Tests (LSD) for 6 hour acetate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.974
Critical Value of t	2.22814
Least Significant Difference	1.7955

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	55.4000	3	1
A			
B A	54.1000	3	4
B A			
B A	54.0667	3	3
B			
B	53.3667	3	5
B			
B	52.9667	3	2

Dependent Variable: 9 hour acetate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	204.9573333	51.2393333	27.23	<.0001
Error	10	18.8200000	1.8820000		
Corrected Total	14	223.7773333			

R-Square	Coeff Var	Root MSE	9 hour acetate Mean
0.915899	2.360663	1.371860	58.11333

The GLM Procedure

t Tests (LSD) for 9 hour acetate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.882
Critical Value of t	2.22814
Least Significant Difference	2.4958

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	65.500	3	1

B	56.533	3	5
B			
B	56.267	3	3
B			
B	56.200	3	4
B			
B	56.067	3	2

Dependent Variable: 12 hour acetate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	558.0360000	139.5090000	23.88	<.0001
Error	10	58.4133333	5.8413333		
Corrected Total	14	616.4493333			

R-Square	Coeff Var	Root MSE	12 hour acetate Mean
0.905242	3.904920	2.416885	61.89333

The GLM Procedure

t Tests (LSD) for 12 hour acetate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	5.841333
Critical Value of t	2.22814
Least Significant Difference	4.397

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	74.067	3	1
B	59.267	3	2
B			
B	59.267	3	3
B			
B	58.633	3	4
B			
B	58.233	3	5

Appendix M: Analysis of variance on the effect of SNM increasing inclusion on rumen tVFAs concentration and acetate propionate ratio

Dependent Variable: tVFAs

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	320.6914667	80.1728667	73.42	<.0001
Error	10	10.9194667	1.0919467		
Corrected Total	14	331.6109333			

R-Square	Coeff Var	Root MSE	tVFAs Mean
0.967071	0.988487	1.044963	105.7133

The GLM Procedure

t Tests (LSD) for tVFAs

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.091947
Critical Value of t	2.22814
Least Significant Difference	1.9011

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	114.9600	3	1
B	103.4733	3	2
B			
B	103.4533	3	3
B			
B	103.3933	3	4
B			
B	103.2867	3	5

Dependent Variable: Ace: prop

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.22718293	0.05679573	60.73	<.0001
Error	10	0.00935200	0.00093520		

Corrected Total	14	0.23653493	

R-Square	Coeff Var	Root MSE	Ace: prop Mean
0.960462	2.129994	0.030581	1.435733

The GLM Procedure

t Tests (LSD) for Ace: prop

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.000935
Critical Value of t	2.22814
Least Significant Difference	0.0556

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	1.68167	3	1
B	1.37967	3	2
B			
B	1.37700	3	4
B			
B	1.37533	3	3
B			
B	1.36500	3	5

Appendix N: Analysis of variance on the effect of SNM increasing inclusion on ruminal protozoan count/ml.

Dependent Variable: 0 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	61.06666667	15.26666667	5.33	0.0146
Error	10	28.66666667	2.86666667		
Corrected Total	14	89.73333333			

R-Square	Coeff Var	Root MSE	0 hour Mean
0.680535	19.84129	1.693123	8.533333

The GLM Procedure

t Tests (LSD) for 0 hour

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 2.866667
Critical Value of t 2.22814
Least Significant Difference 3.0802

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	12.333	3	1
B	8.333	3	2
B			
B	8.333	3	3
B			
B	7.000	3	4
B			
B	6.667	3	5

Dependent Variable: 3 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	19.73333333	4.93333333	4.11	0.0318
Error	10	12.00000000	1.20000000		
Corrected Total	14	31.73333333			

R-Square	Coeff Var	Root MSE	3 hour Mean
0.621849	21.33984	1.095445	5.133333

The GLM Procedure

t Tests (LSD) for 3 hour

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 1.2
Critical Value of t 2.22814
Least Significant Difference 1.9929

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	6.3333	3	1
A			
A	5.6667	3	2
A			
A	5.6667	3	3
A			
A	5.0000	3	4

B	3.0000	3	5

Dependent Variable: 6 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6.26666667	1.56666667	0.94	0.4797
Error	10	16.66666667	1.66666667		
Corrected Total	14	22.93333333			

R-Square	Coeff Var	Root MSE	6 hour Mean
0.273256	24.51255	1.290994	5.266667

The GLM Procedure

t Tests (LSD) for 6 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.666667
Critical Value of t	2.22814
Least Significant Difference	2.3487

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	6.000	3	1
A			
A	6.000	3	2
A			
A	5.000	3	3
A			
A	5.000	3	4
A			
A	4.333	3	5

Dependent Variable: 9 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	25.73333333	6.43333333	2.61	0.0998
Error	10	24.66666667	2.46666667		
Corrected Total	14	50.40000000			

R-Square	Coeff Var	Root MSE	9 hour Mean

0.510582	23.09651	1.570563	6.800000

The GLM Procedure

t Tests (LSD) for 9 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	2.466667
Critical Value of t	2.22814
Least Significant Difference	2.8573

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	8.333	3	1
A			
A	8.000	3	2
A			
B A	6.667	3	3
B A			
B A	6.333	3	4
B			
B	4.667	3	5

Dependent Variable: 12 hour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	15.06666667	3.76666667	1.09	0.4138
Error	10	34.66666667	3.46666667		
Corrected Total	14	49.73333333			

R-Square	Coeff Var	Root MSE	12 hour Mean
0.302949	21.99093	1.861899	8.466667

The GLM Procedure

t Tests (LSD) for 12 hour

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	3.466667
Critical Value of t	2.22814
Least Significant Difference	3.3873

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	9.667	3	1
A			
A	9.000	3	2

A			
A	8.667	3	4
A			
A	8.333	3	3
A			
A	6.667	3	5

Dependent Variable: mean protozoa

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	19.56266667	4.89066667	22.10	<.0001
Error	10	2.21333333	0.22133333		
Corrected Total	14	21.77600000			

R-Square	Coeff Var	Root MSE	mean protozoa Mean
0.898359	6.878081	0.470461	6.84000

The GLM Procedure

t Tests (LSD) for h

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.221333
Critical Value of t	2.22814
Least Significant Difference	0.8559

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	8.5333	3	1
B	7.4000	3	2
B			
C B	6.8000	3	3
C			
C	6.4000	3	4
D	5.0667	3	5

7.16 Appendix O: Analysis of variance on the effect SNM increasing inclusion on gas production over 96 hours incubation period.

Dependent Variable: 0

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	6.26251693	1.56562923	1.60	0.2491
Error	10	9.79482000	0.97948200		
Corrected Total	14	16.05733693			

R-Square	Coeff Var	Root MSE	0 Mean
0.390010	42.20297	0.989688	2.345067

The GLM Procedure

t Tests (LSD) for 0

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.979482
Critical Value of t	2.22814
Least Significant Difference	1.8005

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	2.8200	3	1
A			
A	2.7930	3	2
A			
A	2.5313	3	5
A			
A	2.5017	3	4
A			
A	1.0793	3	3

Dependent Variable: 3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	58.51124093	14.62781023	4.90	0.0190
Error	10	29.86432800	2.98643280		
Corrected Total	14	88.37556893			

R-Square	Coeff Var	Root MSE	3 Mean
0.662075	37.35474	1.728130	4.626267

The GLM Procedure

t Tests (LSD) for 3

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	2.986433
Critical Value of t	2.22814
Least Significant Difference	3.1439

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	7.482	3	2
A			
A	6.157	3	1
A			
B A	4.463	3	4
B			
B	2.686	3	3
B			
B	2.343	3	5

Dependent Variable: 6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	4.20341667	1.05085417	0.55	0.7030
Error	10	19.07225867	1.90722587		
Corrected Total	14	23.27567533			

R-Square	Coeff Var	Root MSE	6 Mean
0.180593	38.57967	1.381023	3.579667

The GLM Procedure

t Tests (LSD) for 6

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.907226
Critical Value of t	2.22814
Least Significant Difference	2.5125

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	4.222	3	2
A			
A	4.114	3	4
A			
A	3.430	3	5
A			

A	3.341	3	1
A			
A	2.790	3	3

Dependent Variable: 12

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	25.26303093	6.31575773	3.49	0.0497
Error	10	18.11013667	1.81101367		
Corrected Total	14	43.37316760			

R-Square	Coeff Var	Root MSE	f Mean
0.582458	29.85622	1.345739	4.507400

The GLM Procedure

t Tests (LSD) for 12

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.811014
Critical Value of t	2.22814
Least Significant Difference	2.4483

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	6.094	3	2
A			
B A	5.619	3	1
B A			
B A C	4.651	3	4
B C			
B C	3.639	3	3
C			
C	2.534	3	5

Dependent Variable: 24

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	116.6053991	29.1513498	48.12	<.0001
Error	10	6.0578807	0.6057881		
Corrected Total	14	122.6632797			

R-Square	Coeff Var	Root MSE	24 Mean

0.950614	10.35988	0.778324	7.512867

The GLM Procedure

t Tests (LSD) for 24

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.605788
Critical Value of t	2.22814
Least Significant Difference	1.416

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	10.3237	3	2
A			
A	9.8623	3	1
A			
A	9.1120	3	3
B	4.2847	3	4
B			
B	3.9817	3	5

Dependent Variable: 48

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	165.2432251	41.3108063	12.41	0.0007
Error	10	33.2985393	3.3298539		
Corrected Total	14	198.5417644			

R-Square	Coeff Var	Root MSE	48 Mean
0.832284	22.18372	1.824789	8.225800

The GLM Procedure

t Tests (LSD) for 48

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	3.329854
Critical Value of t	2.22814
Least Significant Difference	3.3198

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	12.317	3	3
A			
A	10.487	3	2

A			
A	9.680	3	1
B	4.893	3	5
B			
B	3.753	3	4

Dependent Variable: 72

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	33.75774627	8.43943657	2.05	0.1624
Error	10	41.09561467	4.10956147		
Corrected Total	14	74.85336093			

R-Square	Coeff Var	Root MSE	72 Mean
0.450985	37.88508	2.027205	5.350933

The GLM Procedure

t Tests (LSD) for 72

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	4.109561
Critical Value of t	2.22814
Least Significant Difference	3.688

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	7.577	3	1
A			
B A	6.464	3	2
B A			
B A	5.182	3	3
B			
B	3.778	3	5
B			
B	3.753	3	4

Dependent Variable: 96

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	11.14216107	2.78554027	2.01	0.1689
Error	10	13.85764867	1.38576487		
Corrected Total	14	24.99980973			

R-Square	Coeff Var	Root MSE	96 Mean
0.445690	56.02620	1.177185	2.101133

The GLM Procedure

t Tests (LSD) for 96

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.385765
Critical Value of t	2.22814
Least Significant Difference	2.1416

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	3.7507	3	3
A			
B A	1.9700	3	4
B A			
B A	1.9187	3	2
B			
B	1.5917	3	1
B			
B	1.2747	3	5

Appendix P: Analysis of variance on the effect of SNM increasing inclusion on *in vitro* gas production of 24-hour incubation, predictable parameters, invitro organic matter digestibility and ME.

Dependent Variable: 24 hour gas production

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	116.6053991	29.1513498	48.12	<.0001
Error	10	6.0578807	0.6057881		
Corrected Total	14	122.6632797			

R-Square	Coeff Var	Root MSE	24 hour gas production Mean
0.950614	10.35988	0.778324	7.512867

The GLM Procedure

t Tests (LSD) for f

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.605788
Critical Value of t	2.22814
Least Significant Difference	1.416

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	10.3237	3	2
A			
A	9.8623	3	1
A			
A	9.1120	3	3
B	4.2847	3	4
B			
B	3.9817	3	5

Dependent Variable: a

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5.04725693	1.26181423	1.40	0.3015
Error	10	8.99164600	0.89916460		
Corrected Total	14	14.03890293			

R-Square	Coeff Var	Root MSE	a Mean
0.359519	39.32551	0.948243	2.411267

The GLM Procedure

t Tests (LSD) for a

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.899165
Critical Value of t	2.22814
Least Significant Difference	1.7251

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	2.8100	3	4
A			

A	2.7790	3	2
A			
A	2.7177	3	1
A			
A	2.4743	3	5
A			
A	1.2753	3	3

Dependent Variable: b

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	99.9715200	24.9928800	11.21	0.0010
Error	10	22.2950233	2.2295023		
Corrected Total	14	122.2665433			

R-Square	Coeff Var	Root MSE	b Mean
0.817652	40.34818	1.493152	3.700667

The GLM Procedure

t Tests (LSD) for b

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	2.229502
Critical Value of t	2.22814
Least Significant Difference	2.7164

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	8.164	3	3
B	4.171	3	2
B			
C B	3.892	3	1
C			
C D	1.240	3	4
D			
D	1.038	3	5

Dependent Variable: c

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	4	50.7826136	12.6956534	2.57	0.1028
Error	10	49.3388540	4.9338854		
Corrected Total	14	100.1214676			

R-Square	Coeff Var	Root MSE	c Mean
0.507210	131.5586	2.221235	1.688400

The GLM Procedure

t Tests (LSD) for c

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	4.933885
Critical Value of t	2.22814
Least Significant Difference	4.041

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	5.263	3	1
A			
B A	1.447	3	2
B			
B	1.073	3	4
B			
B	0.426	3	5
B			
B	0.232	3	3

Dependent Variable: a + b

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	69.09605093	17.27401273	10.09	0.0015
Error	10	17.11713600	1.71171360		
Corrected Total	14	86.21318693			

R-Square	Coeff Var	Root MSE	a + b Mean
0.801456	21.40677	1.308325	6.111733

The GLM Procedure

t Tests (LSD) for a + b

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	1.711714
Critical Value of t	2.22814
Least Significant Difference	2.3802

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	9.439	3	3
B	6.949	3	2
B			
B	6.609	3	1
C	4.050	3	4
C			
C	3.512	3	5

Dependent Variable: RSD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	22.01599427	5.50399857	67.96	<.0001
Error	10	0.80985467	0.08098547		
Corrected Total	14	22.82584893			

R-Square	Coeff Var	Root MSE	rsd Mean
0.964520	9.819631	0.284579	2.898067

The GLM Procedure

t Tests (LSD) for RSD

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.080985
Critical Value of t	2.22814
Least Significant Difference	0.5177

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats

	A	4.2513	3	3
	A			
B	A	3.8250	3	2
B				
B		3.5197	3	1
	C	1.6077	3	5
	C			
	C	1.2867	3	4

Dependent Variable: IVOMD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	215.0027976	53.7506994	64.28	<.0001
Error	10	8.3624633	0.8362463		
Corrected Total	14	223.3652609			

R-Square	Coeff Var	Root MSE	IVOMD Mean
0.962561	2.331467	0.914465	39.22273

The GLM Procedure

t Tests (LSD) for IVOMD

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.836246
Critical Value of t	2.22814
Least Significant Difference	1.6637

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	43.2773	3	1
A			
A	42.8307	3	2
B	40.4587	3	3
C	35.5123	3	4
C			
C	34.0347	3	5

Dependent Variable: ME

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.20602467	0.30150617	18.50	0.0001
Error	10	0.16296667	0.01629667		
Corrected Total	14	1.36899133			

R-Square	Coeff Var	Root MSE	ME Mean
0.880959	2.781431	0.127658	4.589667

The GLM Procedure

t Tests (LSD) for ME

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.016297
Critical Value of t	2.22814
Least Significant Difference	0.2322

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	4.9520	3	2
A			
B A	4.8197	3	3
B			
B	4.6513	3	1
C	4.2717	3	4
C			
C	4.2537	3	5

Appendix Q: Analysis of variance for effect of SNM increasing inclusion on Methane reducing potential.

Dependent Variable: total gas

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1402.984707	350.746177	39.29	<.0001
Error	10	89.262467	8.926247		

Corrected Total	14	1492.247173

R-Square	Coeff Var	Root MSE	total gas Mean
0.940183	7.811207	2.987682	38.24867

The GLM Procedure

t Tests (LSD) for total gas

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	8.926247
Critical Value of t	2.22814
Least Significant Difference	5.4354

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	49.787	3	2
A			
A	46.650	3	1
B	40.557	3	3
C	29.487	3	4
C			
C	24.763	3	5

Dependent Variable: methane (ml)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	139.7131333	34.9282833	192.90	<.0001
Error	10	1.8106667	0.1810667		
Corrected Total	14	141.5238000			

R-Square	Coeff Var	Root MSE	methane (ml) Mean
0.987206	6.437508	0.425519	6.610000

The GLM Procedure

t Tests (LSD) for methane (ml)

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.181067

Critical Value of t 2.22814
Least Significant Difference 0.7741
Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	11.1867	3	1
B	8.4500	3	2
C	6.6333	3	3
D	4.2567	3	4
E	2.5233	3	5

Dependent Variable: % methane in total gas

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	298.7158933	74.6789733	19.86	<.0001
Error	10	37.6086000	3.7608600		
Corrected Total	14	336.3244933			

R-Square	Coeff Var	Root MSE	% methane in total gas Mean
0.888178	11.77518	1.939294	16.46933

The GLM Procedure

t Tests (LSD) for % methane in total gas

Alpha 0.05
Error Degrees of Freedom 10
Error Mean Square 3.76086
Critical Value of t 2.22814
Least Significant Difference 3.5281
Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	24.053	3	1
B	17.147	3	2
B			
B	16.340	3	3
B			
B	14.467	3	4

C	10.340	3	5

Dependent Variable: methane of 0.2 truly digestible substrate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	72.74657333	18.18664333	471.56	<.0001
Error	10	0.38566667	0.03856667		
Corrected Total	14	73.13224000			

R-Square	Coeff Var	Root MSE	methane of 0.2 truly digestible substrate Mean
0.994726	3.890332	0.196384	5.048000

The GLM Procedure

t Tests (LSD) for methane of 0.2 truly digestible substrate

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	0.038567
Critical Value of t	2.22814
Least Significant Difference	0.3573

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	8.8700	3	1
B	5.8933	3	2
C	4.5167	3	3
D	3.1933	3	4
E	2.7667	3	5

Dependent Variable: % methane reduction

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	5155.195307	1288.798827	23.20	<.0001
Error	10	555.601533	55.560153		
Corrected Total	14	5710.796840			

R-Square	Coeff Var	Root MSE	% methane reduction Mean
0.902710	23.67660	7.453868	31.48200

The GLM Procedure

t Tests (LSD) for % methane reduction

Alpha	0.05
Error Degrees of Freedom	10
Error Mean Square	55.56015
Critical Value of t	2.22814
Least Significant Difference	13.561

Means with the same letter are not significantly different.

t Grouping	Mean	N	treats
A	56.953	3	5
B	39.900	3	4
B			
B	31.817	3	3
B			
B	28.740	3	2
C	0.000	3	1