EFFECTS OF OPTIMAL AND SUB-OPTIMAL FEEDING ON APPARENT DIGESTIBILITY, NITROGEN BALANCE AND METHANE EMISSION IN BORAN STEERS FED RHODES GRASS HAY

By

ANDREW MUTHAMA MBITHI, BVM

Department of Animal Production

A thesis submitted in partial fulfillment of the requirements for degree of Master of Science in Animal Nutrition and Feed Science, University of Nairobi.

DECLARATION

This thesis is my original work and has not been presented for award of a degree in this or any other university.

Andrew Muthama Mbithi (BVM)

J56/76133/2014

Signature……**Date**…….

This thesis has been submitted to Graduate School with our approval as academic supervisors

Prof C. K. Gachuiri (BVM, MSc, PhD)

Department of Animal production,

University of Nairobi

Signature…… ……**Date**……

Dr. J.P. Goopy, (BSc (Hons I) App Sci, PhD).

Livestock systems and environment (LSE) section,

International Livestock Research Institute.

Signature……  ……. **Date**…….……

DEDICATION

This work is dedicated to my lovely mum; Agnes Mbithi, daughter; Ruby Mueni and siblings; Rose, Mike and Joseph.

ACKNOWLEDGEMENT

Firstly, I thank God for seeing me through this academic journey. Much regards to my supervisors; Prof. Charles Gachuiri and Dr. John Goopy for instilling wisdom and guidance in this work. I will forever be indebted for their tireless efforts and time apportioned towards this endeavor.

My gratitude goes to the University of Nairobi, Department of Animal Production teaching and non-teaching staff for the assistance offered to me during the coursework and my project. To my classmates, I will forever be thankful for keeping me company during this academic journey.

I also appreciate my colleague (Mr. Showman) and the staff at Mazingira-ILRI for the coordinated efforts and execution of duties especially during the feeding trials that that resulted in a smooth flow of activities. Special thanks go to Dr. Daniel Korir, the research assistant at Mazingira-ILRI. You took me through all the procedures which entailed my research and walked with me through each step.

I also wish to thank ILRI for giving me the opportunity and hosting me in an environment conducive for research. Ultimately the funders of my project GIZ/BMZ are well appreciated, your monetary facilitation made everything to proceed without a hitch.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ACROYNMS

ADF	Acid Detergent Fiber
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
BMZ/GIZ	Federal Ministry of Economic co-operation and Development-Germany
CH_4	Methane
CO_2	Carbon dioxide
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EPA	Environment Protection Agency
FAO	Food and Agricultural Organization
GHG	Greenhouse gas
Gt	Giga tone
IACUC	Institutional Animal Care and Use Committee
ILRI	International livestock research institute
IPCC	Intergovernmental panel on climate change
LSD	Least Significant Difference
LW	Live weight
MER	Maintenance Energy Requirement
Mmol	Millimoles
N	Nitrogen
NDF	Neutral detergent fiber
NH_3	Ammonia
OM	Organic matter

ABSTRACT

The objective of this study was to determine effects of optimal and suboptimal feed intakes on apparent digestibility, nitrogen balance and methane emissions in Boran steers fed Rhodes grass hay. The study was carried out at the International Livestock Research Institute (ILRI), Nairobi campus using Boran steers (n=12; 150.0 ± 12.5 kg LW) in a completely randomized design. The steers were stratified by live weight and allocated to four treatments replicated three times. The experimental diet was made of Rhodes grass (*Chloris gayana)* hay (CP: 7.1%) with 4 levels of intake. The four experimental diets (treatments) were; Diet 1; calculated at 120% Maintenance Energy Requirement (MER), Diet 2; 100% MER, Diet 3; 80% MER and diet 4; 60% MER and were fed for five weeks. Daily feed intake, fecal, urine and methane outputs were monitored. Samples of the diet and fecal matter were collected and analysed for dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and nitrogen (N) content. Urine samples were analysed for total N. The results from the study showed that methane production increased with increase in energy intake: 64.13, 55.1, 52.9 and 38.63 g CH_4/100kg LW per day at 120% MER, 100% MER, 80% MER, 60% of MER respectively. Nitrogen efficiency (%) was significantly different between the treatments 28.6, 41.74, 48.16 and 51.9 for 120% MER, 100% MER, 80% MER, 60% of MER respectively. Apparent digestibility of DM, OM, CP, NDF and ADF was not affected ($P>0.05$) by the treatment. The steers on the 60% MER and 80% MER treatment groups lost weight; -500 g/100kg LW and -245 g/100kg LW respectively. The steers on 100% MER and 120% MER treatment groups gained mean weight of 284g and 565 g/100kg LW daily during the five weeks of the trial. It was concluded that restricted feeding affects nitrogen efficiency and enteric methane gas production but had no effect on apparent digestibility of the Rhodes grass hay.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Feed availability in the tropics is seasonal being scarce during droughts and plenty during and after the rains. As such, animals are subjected to either low or high amounts of feed intake. Underfeeding is common in the tropics during long dry periods with low quantity roughages (Grimaud *et al*., 1998). Amount of feed consumed by ruminant animals has different effects on digestibility. Previous studies have shown that high feed intake by ruminants results in both increased dry matter (DM) and organic matter (OM) apparent digestibility (Galyean and Owens, 1991; Lechner-Doll., 1991). In these studies, the authors reported that high feed intake led to increased retention time of the feed particles in the rumen, allowing the microbes to digest them adequately.

Low feed intake has been reported to have varying effects on digestibility. Grimaud and Doreau (1995) reported that there was no variation in digestibility with low intakes of forage-based diets in non-lactating cows. This contrasted with the findings of Grimaud *et al*., (1998) who reported a decrease in digestibility for both *Bos indicus* and *taurus* fed at reduced amounts of feed. Grimaud *et al*., (1999) stated that decrease in digestibility in animals on low intake could be explained by increased metabolic loses in feces but not particle retention time.

The amount of feed consumed has been reported to affect the amount of enteric methane produced by the ruminants. Several studies have suggested that high feed intake results in high methane production (Nkrumah *et al*., 2006; Fitzsimons *et al*., 2013). Mercadante *et al*., (2015) reported that there was no evidence that the efficient ruminants (taking low levels of feed) will produce less methane. They concluded that the enteric methane emissions did not differ between the low and high intake in their study.

Most published studies on tropical feeds have provided little information on effects of levels of feed intake on digestibility, nitrogen balance and methane production. Therefore, this study explored the effects of different amounts of Rhodes grass hay intake on digestibility, nitrogen balance, and enteric methane emissions in growing Boran steers.

1.2 Problem statement

Sub-optimal feeding is usually common in tropics especially during the dry seasons. During these dry seasons, the feeding of the livestock is characterized by use of the low-quality diets. Low quality diets are associated with low supply of nutrients to livestock and hence low digestibility and less supply of nutrients to the animal. This ultimately leads to slow growth rate and low productivity of livestock. There is limited information on effects of suboptimal feeding of livestock, particularly cattle in the tropics. The current study was undertaken to bridge the existing gap of knowledge help animal nutritionists to understand the right amount of tropical diet to feed cattle for optimal performance.

1.3 Justification

The amount of feed consumed by livestock usually affects digestibility of nutrients and this has an impact on the productivity of the animal. Ruminants are important livestock species which contribute to production of meat and milk, both of which improve the livelihood of many people in the tropics. Boran cattle are one of the common beef breeds in the tropics which is adaptable to the harsh climatic conditions of these regions. Ruminants which have low feed intake are said to have high nitrogen efficiency due to recycling of the nitrogen. There is however limited information on recycling in the sub-optimally fed ruminants. High nitrogen efficiency leads to less N excretion to the environment and hence low nitrous oxide

(a greenhouse gas). Rhodes grass hay is one of the common feed resources found in the tropics during the dry season and hence it was readily available to be in the current study. Methane emission is importance factor to consider in livestock production because it is one of the greenhouse gases which lead to climate change and contribute to energy inefficiency in ruminants.

1.4 Objectives

1.4.1 Main objective

To determine the effects of feed intake on digestibility, nitrogen balance and methane emissions in Boran steers fed Rhodes grass hay.

1.4.2 Specific objectives

1) To determine the effect of feeding different amounts of Rhodes grass hay on digestibility in Boran steers

2) To evaluate effects of feeding different amounts of Rhodes grass hay on enteric methane emission from Boran steers

3) To determine the effect of feeding different amounts of Rhodes grass hay on nitrogen balance in Boran steers

1.5 Hypotheses

1) Feed intake level does not have any effect on the apparent digestibility in Boran steers
2) Feed intake level does not have any effect on enteric methane emission from Boran steers
3) Feed intake level does not have any effect on nitrogen balance in Boran steers

CHAPTER TWO: LITERATURE REVIEW

2.1 Livestock production in the tropics

The contribution of agriculture to the gross domestic product (GDP) of sub-Saharan Africa as a whole is estimated to be 32% (Thornton *et al.,* 2010) while the livestock sector contributes 25% to the region's agricultural GDP, mainly through meat, milk, eggs, wool, hides and skin (Otte and Chilonda, 2002). According to Winrock international (1992), if draught power and manure (the non-monetized contribution of livestock) were to be included, the contribution would increase to about 35%. Livestock sector employs 1.3 billion people globally and directly supports around 600 million poor small holder farmers in the developing world, sub-Saharan Africa included (Thornton *et al.,* 2010).

Sub-Saharan Africa has one of the fastest human population growth rates globally, 2.6% per annum (Otte and Chilonda, 2002) and hence there is need to increase livestock productivity to feed this growing population. The global growth of this sector is however rapid and is being driven by other factors like urbanization and increasing incomes in the developing countries (Delgado, 2005).

2.2 Constraints of livestock production in tropics

Constraints to livestock development in tropical countries include inappropriate policies, scarce livestock feeds, devastating diseases, degraded lands and water resources and poor access to markets and climate change (Masikati, 2010). Feed shortages during the dry season constitute the greatest challenge in terms of quantity and quality. The main constraint to increasing livestock productivity and output is the lack of adequate supplies of good quality livestock feed in the dry season at a competitive price (Kassam *et al*., 2009).

Low soil fertility for forage production and weak market chains for livestock and livestock products are some of the additional challenges to the livestock production. These constraints are however, within farmers' capacity to mitigate (Masikati, 2010).

2.2.1 Feed quantity in tropics

Severity of feed shortages is worsened during the dry season due to the seasonal nature of rainfall patterns in many parts of Africa leading to fluctuations in forage quality and quantity (Morton and Matthewman, 1996). Moreover, population pressure and emerging new markets created by urbanization have caused an increase in land under cultivation at the expense of grazing land (Morton and Matthewman, 1996) further exacerbating the problem.

According to Amenu *et al*., (2011), grazing land is restricted to waste land, roadsides, edges of cropping fields and riverbanks, as well as fallow land resulting in low quantity of feed for the livestock. The crop residues which are by-products are of low quality and therefore cannot supply enough nutrients to increase productivity (Amenu *et al.,* 2011). Lukuyu *et al.,* 2009 reported fluctuations in livestock feed availability in East Africa with the greatest feed scarcity being felt during the dry season in Rwanda, Uganda and Kenya. Lack of knowledge, inadequate extension and sometimes ignorance leads to serious problems related to feed shortages (Lukuyu *et al.,* 2009).

Increased livestock production in smallholder mixed-crop–livestock systems faces many constraints at the level of the farm and the value chain. Feed limitation, which is common in tropical livestock farming systems, maximum herd output can only be achieved with small herd sizes (Oosting *et al.,* 2014). Katongole *et al.,* (2012) observed that feed scarcity to livestock is a real challenge in both the urban and rural dwellings as the livestock keeping

increasingly becomes popular in tropics. Seasonal decrease of feed in the tropics follow the pattern of rainfall availability and vegetation growth (Ayantunde *et al.,*2005). Keeping many animals and overgrazing during the wet season in pastoral areas of Zimbabwe where majority of the livestock are kept resulted into insufficient feed for the dry season (Ncube and Mpofu 1994).

2.3 Opportunities of livestock production in tropics

The use of multipurpose legume trees can provide high quality feed and improve soil fertility (Lenné and Thomas, 2006). Opportunities, however, exist for improving livestock production in communal areas and some of the possible technologies are not new (Lenné and Thomas, 2006). There is need for promotion of strategies to widen the feed resource base, promote feed conservation and improve nutritive value (Mutibvu *et al.,* 2012).

Various ways of improving livestock health and nutrition management to reduce mortality could also be employed. It appears that what is required is a proper demonstration on implementation of proven technologies and practices like feed preservation through hay and silage making (Mutibvu *et al.,* 2012). According to FAO (2010), the high livestock numbers in the tropics also provide a good opportunity for increased productivity. Of the total estimated global ruminant livestock, 24.5 % are found in African tropics (FAOSTAT 2010).

2.4 Apparent digestibility in ruminant animals

High digestibility of feeds is key to increased productivity of ruminants, and for beef cattle leads to increased Average Daily Gain (ADG). This is important to the producer since the beef cattle attain the market weight faster (Sufyan, 2018). There are however several factors

which affect digestibility in ruminants ranging from the animal and feed characteristics among others (Sufyan, 2018).

Age is an important factor that affects the digestibility of nutrients in ruminants with very young animals unable to digest roughage until their rumen becomes functional (Jung and Allen, 1995). Old ruminants also have impaired chewing ability because of the worn-out teeth resulting in reduced mastication of feed. Moreover, the reduced enzyme secretion in these old ruminants further decreases feed digestibility (Jung and Allen, 1995). The amount of feed consumed also affects digestibility. Higher feed consumption leads to increased passage of digesta and consequently, the digestibility declines due to less retention time in the rumen (Grimaud *et al.,* 1998). Sudden changes of feed composition also decrease the digestibility because of the rumen micro-organism are exposed to different environment hence reduction in their activity (Grimaud *et al.,* 1998).

Other feed factors like the stage of harvest and the variety for the forages within the same species greatly affect the digestibility of feed (Sufyan, 2018). High fiber content lowers the feed digestibility (Jung and Allen, 1995). Processing of the feed (e.g. grinding), leads to increased digestibility because of increased surface area for microbial and enzymatic action within the ruminant digestive system (Sufyan, 2018). Digestibility of a feed determines the amount that is actually absorbed by an animal and therefore the availability of nutrients for growth, reproduction.

Apparent digestibility is estimated by subtracting nutrients contained in the feces from nutrients consumed. Therefore, it does not account for nutrients lost as methane gas or as metabolic waste products excreted in the feces (Grimaud *et al.,* 1998). The measurement of

apparent digestibility is less complex than measuring true digestibility and, therefore, more suited to the requirements of diagnostic livestock systems research (ILCA, 1990).

The amount of feed consumed by ruminants has been reported to have different effects on its apparent digestibility. Previous studies have shown that high levels of feeding in ruminants resulted in both increased dry matter (DM) and organic matter (OM) apparent digestibility (Galyean and Owens, 1991). A study done by Lechner-Doll *et al*., (1991) on ruminants grazed on thorn bush savannah pasture reported that high feed intake levels increased mean retention time of the feed particles in the rumen. This allowed the rumen microbes to digest the feed particles adequately. Increased rumen liquid volume (18.6 liters to 23.6 liters), reported in these experiments, resulted into an increased microbial number due to enough energy supply for their synthesis and hence more feed particles were digested. In contrast, Galyean and Owens (1979) observed a decline of DM apparent digestibility when cross bred steers were subjected to high feed intake.

Low feeding levels have been reported to have varying effects on apparent digestibility. Grimaud and Doreau (1995) reported that there was no variation in apparent digestibility with low feed intakes in ruminants. In contrast, Grimaud *et al*., (1998) in a study with *Bos indicus* and *Bos taurus* reported a decrease in apparent digestibility in the underfed animals.

Contrary to what was observed in animals on high feed intake, Grimaud *et al*., (1999) observed that particle retention time was not important in digestion in underfed ruminants. They further observed that decrease in apparent digestibility could be as a result of increased metabolic loses (due to endogenous loss of epithelial cells) through feces or reduction in protozoal population which are responsible for fiber digestibility.

2.5 Livestock and GHG emissions

Livestock is a major source of anthropogenic greenhouse emissions, responsible for up to 14.5% of the total global GHG emissions based on Global Livestock Environment Assessment Model (GLEAM) (Steinfeld *et al.*, 2006). The livestock sector is also responsible for 33% CH_4, 66% nitrous oxide and 9% CO_2 of the anthropogenic emissions (Steinfeld *et al.*, 2006). Africa is the second largest source of enteric methane (Table 2-1) with 15% global emissions from livestock, after Asia (~33%) and Latin America with 23.9% (O'Mara, 2011). These proportions notably correspond with the ruminant numbers in these regions with emissions mainly linked to cattle because of their large numbers, large body size and low efficiency of production as compared to small ruminants (Herrero *et al.*, 2011). The monogastric also significantly contribute to GHG emissions through Nitrous oxide deposited in manure (Steinfeld *et al.*, 2006).

2.6 Enteric fermentation

Enteric fermentation is a digestive process by which carbohydrates are broken down by microorganisms through a series of steps into simple molecules for absorption into the bloodstream of an animal (Murray *et al.,* 1999). The molecules are the volatile fatty acids which majorly include; acetate, butyrate and propionate. Their proportions depend of the type of diet and type of rumen microbes. Methanogenesis occurs to produce methane and carbon dioxide (Figure 2-1). Of the global GHG emissions, enteric fermentation contributes 40% (UNFAO, 2006).

Enteric methane is mostly produced in the rumen (87-90%) with small percentage in the large intestines (Murray *et al.,* 1999, Dini *et al,* 2012) as by-product of ruminal digestion (Broucek, 2014). The rate of fermentation is affected by animal factors like salivation, chewing and

digesta kinetics (Wilson and Kennedy, 1996, Vargaand and Kolver, 1997). Over 200 species of microorganisms are present in the rumen, although only about 10% of these play an important role in digestion (Johnson and Johnson, 1995). About 95% of methane is belched by the animal (Chagunda *et al.*, 2009), however, a small percentage of methane produced in the large intestine is passed out as fart (Murray *et al.*, 1999).

The IPCC reports that methane is more than 20 times more effective at trapping heat in the atmosphere than CO_2 though it is produced in smaller amounts (Johnson and Johnson, 1995). Decreasing enteric CH_4 production from ruminants without altering animal production is essential both as a strategy to reduce GHG emissions and as means of improving feed conversion efficiency (Martin *et al.*, 2010). The statistics show that there is a big opportunity to mitigate the enteric methane emissions from livestock through precision feeding.

2.7 Methane emissions

The methane concentration in the atmosphere is increasing at a rate of 22 Million tons/year because of the increasing livestock numbers (Jardine *et al.,* 2003). The dangers of the methane increase in the atmosphere are global warming and decrease in livestock production efficiency (Jardine *et al.,* 2003). The greenhouse effect of methane gas leads to absorption of infrared radiation and trapping heat in the atmosphere. The reduced livestock production efficiency as a result of increased methane production leads to less of animal products e.g. milk and meat and hence global food insecurity (Jardine *et al.,* 2003).

The primary sources of methane are either natural or anthropogenic. The natural sources of methane emissions include: Wetlands, Oceans, termites and burning (Johnson and Johnson, 1995). It is estimated that these sources contribute 160 million tons of global methane

production per year. The anthropogenic sources of methane production include the methane from energy/refuse (Coal, Charcoal, Gas and Oil, Landfills and wastewater) and agriculture (rice, livestock, manure and burning). Methane from these sources is as result of human activities and hence can be mitigated easily (Johnson and Johnson, 1995). The estimated global methane production from these sources is 155 Million tons and 165 million tons per year. The total global methane production is approximately 550 million tons per year FAO (2006).

Among the livestock animals, ruminants are the primary emitters of methane. The rumen which is a large forestomach occupies a total stomach capacity of 70% which is about 100 to 150 Liters in cattle (Shibata and Terada, 2010). According to FAO (2006), the beef and dairy cattle lead among the ruminants in enteric methane emission, a greenhouse gas which causes global warming through absorption of infrared radiation in the atmosphere (Lashof *et al.,* 1990). The methane emission from ruminants is majorly from the enteric fermentation by rumen micro-organisms (Kristensen, 2011). Factors like the level of feed intake, energy consumption, feed quality, animal size, growth rate, level of production, genetics, environment and health affect the production of methane (Hegarty *et al.,* 2007, 2004, Shibata and Terada, 2010).

Up to 89% of methane emissions from ruminants are produced in the rumen during fermentative digestion of feed and the remaining portion produced in the lower Gastro-Intestinal Tract (GIT) fermentation and from residual microbial fermentation in feces excreted (Hook *et al*., 2010). The methane produced represents 2-12% of gross energy loss from the feed taken in by ruminants (Johnson and Johnson, 1995, Murray et al., 1999, Boadi and Wittenberg, 2002, Pinares-Patiño et al., 2007).). This loss depends on feed quality, feed

intake, feed composition and processing (Johnson and Johnson, 1995). Higher enteric emissions per unit feed intake are observed in low quality diets with low apparent digestibility (Mc Geough *et al.*, 2010).

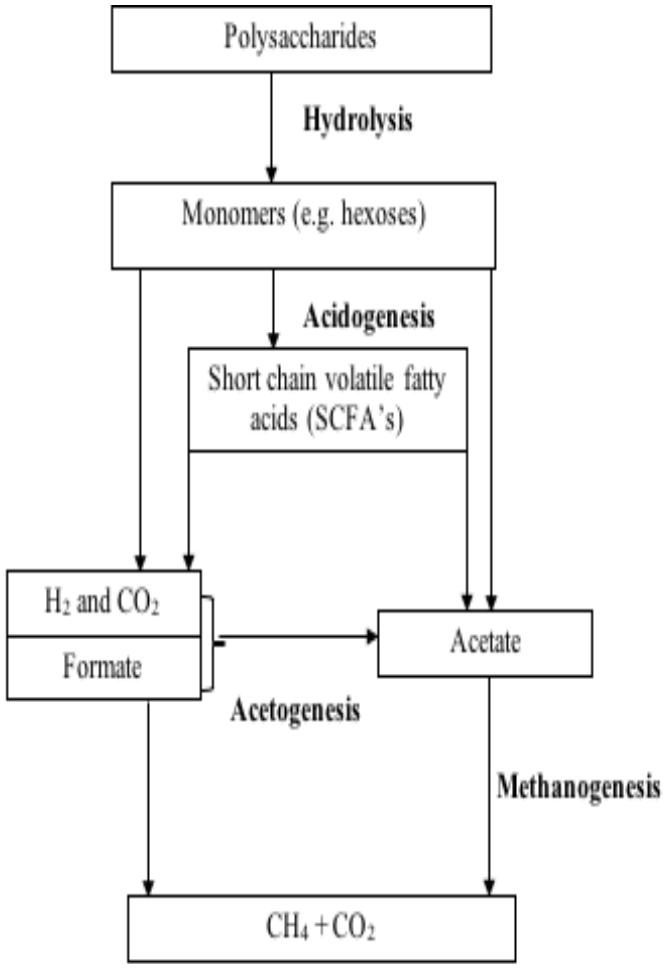

Figure 2-1: Microbial methanogenic degradation of plant fibers
(Adapted from wur.nl, 2010)

Table 2-1: Regional emissions of methane from enteric fermentation by ruminants (million tons of CO_2 -equiv./yr.)

Region	Million tons of CO_2 -equiv./yr.)
Latin America	460
Africa	280
China	259
India	218
Asia (except China and India)	175
Western Europe	160
North America	136
Non-EU former Soviet Union	97
Australia/New Zealand	88
Eastern Europe	28
Middle East	27.3
Global	1928.3

*NON-EFU (NON-EUROPEAN FORMER SOVIET UNION)

(Adapted from EPA 2006)

2.8 Factors affecting methane production

Several factors that affect the rumen methanogens and thus methane production include amount and quality of feed consumed, diet composition, rumen pH, volatile fatty acids (Propionate, acetate and butyrate), environmental stresses, breed and species of the animal (Kumar *et al.,* 2009). A study by Singh and Mohini (1999) showed an increase in methane production at different intakes of berseem (*Trifolium alexandrium)* and legumes. High feed intake increased the passage rate of digesta and hence short residence time in the rumen. This further decreases the rumen microbes' sufficient opportunity of feed degradation and instead

the digestion occurs more in the small intestines. An in-vitro analysis of methane production done by Das and Singh (1999) showed that the quality of diet affects the amount of methane produced. In their study, there was decrease of methane from 51 to 42% when wheat straw was substituted with berseem (*Trifolium alexandrium),* a high-quality forage (Benchaar *et al.,* 2001). A diet mainly made up of legumes have lower portions of carbohydrates and hence faster passage rate and the fermentation is shifted towards higher production of propionate and hence lower methane production (Johnson and Johnson, 1995).

Different breeds and species of the ruminants which host the microbes differ largely in terms of methane production. The methane production in Holstein and Haryana cross-bred cattle was reported to be higher than the exotic breeds (Lal *et al.,* 1987). The higher methane emissions reported in Indian cattle than in buffalos suggest that there is an inter-species difference in methane production since the host environment is different (Mohini and Singh, 2001; Srivastava and Garg, 2002).

Rumen pH also plays a major role in influencing the activity of methanogens. Rumen pH of between 7.0–7.2 is usually optimal for methanogen activity (Kumar *et al.,* 2009). The diet composition also influences the activity of methanogens, with high roughage: concentrate ratio leading to higher methane production (Kumar *et al.,* 2009). Singh and Singh (1997) reported reduction in the number of methanogens when the high concentrate: roughage (75:25) diet was fed to cattle. When the concentrates are high in the diet, the methane production can decrease from 12% to 3% (Johnson and Johnson, 1995).

2.8.1 Effect of feed intake on enteric methane production

High diet intake leads to increased methane production due to increased release of hydrogen

ions in the rumen as result of increased rumen fermentation (Nkrumah *et al*., 2006). The hydrogen ions combine with carbon dioxide through the process of methanogenesis to form methane gas (Nkrumah *et al*., 2006). Poor quality diets have high fiber content which results into increased residency time and low digestibility in the rumen (Fitzsimons *et al*., 2013). Jones *et al.,* (2011) reported an increase in enteric methane production with increase in feed intake in grazing Angus beef cows.

Blaxter and Clapperton (1965) and Johnson and Johnson (1995) reported decrease in methane production when sheep were fed three times the maintenance energy requirements. In their study, they concluded that the low diet digestibility could have also been accompanied by increased passage rate of feed particles and hence lack of enough time for the methanogens to act on the feed to produce methane gas (Moss *et al.,* 2000). Similarly, Johnson and Johnson, (1995) noted that methane production from cattle computed as proportion of gross energy intake decreased by 1.6% for each double intake.

A study by Winders *et al.,* (2018) conducted on growing cross bred steers, fed at 75% of the adlibitum intake reported that daily methane production was significantly different ($p<0.01$) between the optimal (156g/steer) and sub-optimal (126g/steer) intakes. Beauchemin and McGinn (2006) reported lower daily methane production (30g/100kg LW) in Angus beef heifers under low DM intake.

2.9 Mitigation options for methane emissions from ruminants

Fermentation in the rumen contributes about 87% of the total methane emissions from the ruminants. Mitigation techniques and strategies have hence concentrated to reduce the emissions from this source.

2.9.1 Dietary interventions

High levels of concentrates supplementation has been shown to increase apparent digestibility of low-quality diets. This results into reduced feed retention time in the rumen and low methane emissions associated with change in fermented substrate from fiber to starch (Blaxter and Clapperton, 1965). Supplementation has also been shown to improve N retention therefore reducing N excreted by ruminants and hence nitrous oxide production from manure (Del Curto *et al.*, 1990, Bohnert *et al.*, 2002).

Feed processing e.g. grain grinding and chopping of straw enhances apparent digestibility by increasing surface area of the feed upon which microbes can attach enhancing digestion leading to shorter retention time in the rumen (Moss *et al.*, 2000). Shorter retention time in the rumen reduces the amount of methane emitted per unit feed ingested by animals by reducing the rate in which the methanogenogenesis will occur (Pinares-Patiño *et al.*, 2011). Improved pasture management practices like grazing pasture at optimal stage when there is less cell wall content in pastures reduces methane production during digestion in the rumen (Eckard *et al.,* 2010).

2.9.2 Other interventions

The methods of methane mitigation based on vaccination, enzyme inhibitors, phage, homoacetogens, defaunation, feed supplements and animal selection have been extensively documented (Buddle *et al.,* 2011). These approaches are further being investigated, and from the many efforts done towards enteric methane abatement, it is likely that a combination of more than one technique suffices in significant reduction of enteric methane emissions. Different systems of farming however require different methods of methane abatement (Beauchemin *et al.,* 2008). Practically, there are only few methane mitigation measures that

are sustainable due to their cost effectiveness to farmers as also level of increase in production efficiency. Sufficient research is required to better understand how manipulation of diet composition and intake can reduce enteric methane emission (Buddle *et al.*, 2011).

2.10 Nitrogen efficiency in ruminants

According to Calsamiglia *et al*., 2010, ruminants have low nitrogen efficiency averaging 25% expressed as ((g N absorbed/g N intake)∗100). Efficiency of between 15-20% has been reported for different feeds and feeding practices (Calsamiglia *et al.*, 2010), an indicator that these have a high influence on nitrogen use efficiency in ruminants.

Ammonia (NH_3) loss in ruminants during microbial synthesis in the rumen has led to the inefficiencies with up to 60% of dietary nitrogen lost through this route (Reynolds and Kristensen, 2008). Other losses occur in form of microbial nucleic acid nitrogen which is not available for host animal use; this accounting for 15-20% of the total microbial nitrogen (Chen *et al.*, 1990). Other source of inefficiency has been described post ruminal resulting from energy and amino acid imbalances in the tissues, with excess amino acid deaminated in the liver and excreted in urine (Hof *et al.*, 1994).

It has been postulated that cattle have evolved to be efficient in utilizing nitrogen when maintained on a low N intake because of improved urea recycling to the rumen and ammonia capture when ruminants are underfed (Calsamiglia *et al.*, 2010). According to Singh *et al.*, (2008), a certain percentage of underfeeding leads to better utilization of nitrogen in cross-bred calves under subtropical conditions.

Due to scarcity of forage, underfeeding in the tropics will normally be associated with low

quality diets (low in nitrogen). Studies have supported the hypothesis in which cattle with low intakes have lower nitrogen losses as a proportion of nitrogen intake mainly because of N recycling back to the rumen (Bunting *et al.*, 1989, Scott and Hibberd, 1990, Lintzenich *et al.*, 1995, Koster *et al.*, 1996). Ruminants' nitrogen use efficiency is directly related to dietary nitrogen level, total OM intake, quality of protein, rate of bypass and lignification and animal's metabolic protein requirements (Bunting *et al.*, 1989). Feed and feeding systems manipulation can significantly improve efficiencies in ruminants (Bunting *et al.*, 1989).

2.11 Nitrous oxide

The N in manure is metabolized in a number of steps once excreted to yield nitrous oxide among other metabolites, with urea in urine being the main contributor as it is readily broken down (Powell *et al.,* 2014).). Urea ($CO(NH_2)_2$) is formed in the liver from unutilized ammonia in the rumen being absorbed into portal circulation (Powell *et al.,* 2014).

2.11.1 Mitigation measures against the nitrous oxide emission in ruminants

Nitrous oxide is an important greenhouse gas which leads to climate change, it's emission from the ruminants can be reduced through dietary interventions which involve optimum feeding levels with high N efficiency (Eckard *et al.,* 2010).

Nitrous oxide is a highly potent GHG with Global Warming Potential of 295 times that of carbon dioxide (IPCC, 2016). The mitigation measures instituted against it could also increase animal productivity besides lowering global warming and risks of climate change (Eckard *et al.,* 2010).

CHAPTER THREE: MATERIAL AND METHODS

3.1 Experimental site

The study was carried out at ILRI campus, Nairobi, Kenya, from May to August 2016. Experimental protocol was reviewed and approved by Institutional Animal Care and Use Committee (No. IACUC-RC2014-05). Animals were cared in accordance with acceptable code of practice for animals in research.

3.2 Animals and experimental design

Twelve Boran yearling steers (150.0±12.5 kg LW) approximately 15±0.2 months old were housed in individual open partitioned pens (2M × 3M) during the intake measurements. The animals were vaccinated against Foot and Mouth Disease (FMD) and Clostridial infection using 3ml of Fotivax®) and 5ml of Jovaclost® (clostridial toxoid 6 in 1). Both vaccines were administered subcutaneously.

The steers were also treated with an anthelmintic (15ml of 10% Albendazole, orally), washed with an acaricide (Amitraz 12.5% m/v.) and ear tagged one week before the commencement of the trial. They were then transferred to pens measuring (1M×2M) in an enclosed unit during the total collection period. There was continuous lighting and clean water was supplied ad libitum. The experimental design was completely randomized. Steers were randomly stratified by live weight and allocated to four treatments replicated three times. The trial took five weeks.

3.3 Diets, feeding and sampling

3.3.1 Diets

Table 3-1: Diets fed at different treatments

Treatments	1	2	3	4
Intake (% of MER*)	60%	80%	100%	120%
Feed offered (Kg/day)	1.6	2.43	2.8	3.19
DMI (Kg/day)	1.30	1.96	2.27	2.58
Energy (Kcal/day)	2545.85	3849.15	4448.86	5056.41
Protein (g/kg)/day	92.5538	139.935	161.738	183.825

*MER*is the Maintenance Energy Requirement. MER calculation was based on the energy requirement of growing steers (NRC, 2000).*

The experimental diet was made up of Rhodes grass hay (91.5 % DM, 7.1% CP)

3.3.2 Feeding

The animals were fed at 100% MER during the 2-week period prior to the start of feeding trial. The feeding trial period consisted 5 weeks of feeding and 2 weeks of total collection of feces and urine. Weighed amount of feed was offered to each animal once daily at 0930hrs. Water was offered ad-libitum while the mineral requirements for the steers were met through supplementation with salt lick (Maclick®). Any feed refusals were collected and weighed before the animals were supplied with fresh feed the following day.

Plate 3-1: Boran steer feeding in a (2m × 3m) pen

3.3.3 Data Collection and Sampling

Daily feed intake, weekly live weights, total urine and fecal excretion were determined. Enteric methane emissions were determined in 22.5-hour cycle in the gas measurement chambers twice for each animal during the trial.

Feed intake and refusals were recorded daily, body weight of each animal taken weekly in the morning prior to feeding using a digital weigh scale (Gallagher Weigh Scale W210, Australia) for 3 weeks. Daily sampling of the feed was done at feeding to give a representative sample for nutritional analysis. The refusals were bulked by treatment after weighing then mixed and a grab-sample of about 200g taken and stored in zipped polythene bags at -18^0C. Ultimately, the daily feed and refusal samples were bulked and mixed per treatment and subsamples taken for subsequent processing and analysis.

Total fecal excretion was determined by collecting and weighing all the feces from each animal at 24-hour interval for one week. This total collection was for the purpose of

determining the apparent digestibility of nutrients. Feces were mixed thoroughly and a sub-sample of approximately 500 g transferred to aluminium tin foils then dried in the forced-air oven (Genlab Oven, Genlab Ltd. UK.) at 50˚C until a constant weight was obtained for at least two consecutive days. Dried samples were cooled in desiccators, final weight taken, covered with lids then packed in zipped polythene bags and finally stored at room temperature (25 ˚C) for further processing.

Total urine voided was determined over 24-hour periods daily for one week using external catheters connected to a 5-litre barrel containing 100 ml of 10 % Sulphuric acid (Plate 2). Total volume of urine was determined and then a 100ml sample taken and placed in plastic sample bottles for total nitrogen determination.

Plate 3-2: Boran steer during the total collection of urine and fecal samples

3.4 Laboratory analyses

Dried fecal and feed samples were ground through 1-mm sieve using a hammer mill (MF10 basic, IKA, Germany) for determination of chemical composition

3.4.1 Dry Matter, Organic matter and Ash determination

Dry Matter content was determined by drying 2 g of sample in a forced air oven (Genlab Oven, Genlab Ltd, UK.) at 105^0C for 24 h, whereas Ash was determined by combustion of 2 g of the samples in a muffle furnace (Isotemp. Programmable Muffle Furnace 240, Cole-Parmer, Instrument Co., US. at 550^0C for 8h) following AOAC (1990) Method no. 924.05. The OM was then obtained by subtracting ash weight from DM content.

3.4.2 Total Nitrogen

The total N content in feed, fecal and urine samples was determined by micro Kjeldhal procedure AOAC (1990) Method no. 988.05 using selenium pellets as the catalyst.

3.4.3 NDF and ADF procedure determination

The neutral and acid detergent fibers were determined using the Filter Bag Technique-ANKOM 2000 (www.ankom.com).

3.5. Measurement of methane gas

This procedure included three steps; (1) Preparation of chambers, introduction of the animals and initiating measurements, (2) Retrieving data from the Control Computer (CC) and the Picarro Computer (PICAR) and (3) Calculation of emissions.

3.5.1 Preparation of chambers, introduction of animals and measurements

Before introducing the animals into the respiratory chambers (Plate 3), the chambers were cleaned and functioning of automatic waterers was confirmed. The exhaust pumps were then checked to ensure that each was running at the set speed. Next, confirmation of whether the chiller was running was done by ensuring that the pressure gauge reading was between 1.5-2.

Prior to introduction of the animal, their ration was weighed and placed in the chambers. Once the animals were in the chamber, the following parameters for each chamber were recorded: chamber number, animal ID, exhaust fan speed, exhaust flowrate and starting time. The animals were left in the chambers for a period of 22. 5hrs.At the end of the period, the front door of the chamber was opened, the feeding trough removed, and the animals let out.

The time each chamber was opened was recorded on the chamber measurements logbook. The chambers were cleaned prior to the introduction of the next animal.

Plate 3-3: Methane animal respiratory chamber

3.5.2 Retrieving data from the Control and the Picarro computers

Done according to Mazingira laboratory manual book (2016).

3.5.3 Calculation of daily methane emission and energy lost as methane

Done according to Mazingira laboratory manual book (2016).

3.6 Feed apparent digestibility

Total tract DM, OM CP, ADF and NDF apparent digestibility were calculated from the

average nutrient concentrations in diets fed and feces excreted over a 14-d period. This was calculated as:

Apparent digestibility (%) = ((nutrient intake – nutrient excreted) / (nutrient intake)) x 100.

3.7 Nitrogen balance

Nitrogen balance calculations were carried out according to Maynard and Loosli, (1969) based on the equation: N animal products = N feed - N excreted. It was then modified as shown in the equation below in the present study:

N balance (g/d) = N intake (g/d)- (Fecal N (g/d)-Urine N (g/d))

3.8 Statistical analysis

The data was entered in Excel 2010 spreadsheet, Windows 2010. The means of each parameter measured were analysed by one-way Analysis of Variance (ANOVA) procedure using the package Genstat software (Genstat, 14th edition). P-values were used to determine effects of different levels of feed intake on apparent digestibility, nitrogen balance and enteric methane emission. The differences between means was tested at $P<0.05$ and separated using Tukey's test.

CHAPTER FOUR: RESULTS

4.1 Chemical composition of Rhodes grass (*Chloris gayana*) hay

Chemical composition of Rhodes grass hay used in the study is shown in Table 4-1. The Dry matter (DM), Organic matter (OM), Crude protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) content were 915.33 g/kg, 875.57 g/kg, 71.25 g/kg, 739.20 g/kg and 452.82 g/kg respectively.

Table 4-1: Mean chemical composition of Rhodes grass hay (*Chloris gayana*) (g/kg on DM basis)

Nutrient	Chemical composition	SD
DM (g/kg)	915.33	0.16
OM (g/kg DM)	875.57	0.33
CP (g/kg DM)	71.25	0.18
NDF (g/kg DM)	739.20	0.13
ADF (g/kg DM)	452.82	0.08
ME*(MJ/kg DM)	8.2	0.26

DM= Dry matter, **OM**= organic matter, **CP=**Crude protein, **NDF**=Neutral Detergent Fiber, **ADF**=Acid Detergent Fiber, **ME**=Metabolizable Energy

ME*, Calculated value

4.2 Feed Intake

The mean nutrient intakes for the different treatments are shown in Table 4-2. Dry matter (DM), Organic matter (OM), Crude protein (CP), Acid Detergent Fiber (ADF) intakes differed significantly ($P<0.001$) between all treatment groups.

Table 4-2: Least square means and Standard error of mean (SEM) for daily intake (g/100kgd-1) and Apparent digestibility (g/kg) DM, OM, CP, NDF, ADF and methane production in Boran steers fed on grass hay (*Chloris gayana*) at different levels of MER.

	Diet				SEM	P-Value
Treatments	**60%**	**80%**	**100%**	**120%**		
			Intakes (g/d/100kg LW)			
Intake as fed	1604^{a}	2425^{b}	2802^{c}	3185^{d}	39.8	<0.001
DM	1299^{a}	1964^{b}	2270^{c}	2580^{d}	32.2	<0.001
OM	1117^{a}	1689^{b}	1948^{c}	2173^{d}	27.8	<0.001
CP	90.9^{a}	137.7^{b}	174.7^{c}	250.4^{d}	3.14	<0.001
NDF	971^{a}	1468^{b}	1679^{c}	1708^{c}	23.4	<0.001
ADF	588.2^{a}	889.4^{b}	1004.2^{c}	1024.8^{d}	14.51	<0.001
ME (MJ/d/100kg LW)	10.7^{a}	16.1^{b}	18.6^{c}	21.2^{d}	1.01	<0.001
			Apparent digestibility (g/kg)			
DM	576.2	582.0	587.8	587.3	10.95	0.864
OM	773.8	774.5	771.9	755.3	9.08	0.397
CP	619	620.2	623.8	625.9	13.25	0.077
NDF	618.3	638.9	633.4	609.1	12.70	0.331
ADF	573.5	562.4	580.5	527.1	16.18	0.102
			Daily methane production			
g/100kg LW	36.63^{a}	52.9^{b}	55.1^{c}	64.13^{d}	5.11	<0.001
% of feed intake	2.28	2.18	1.96	2.01	0.9	<0.001
Energy loss as CH_4 (MJ / kg DM)	1.55^{a}	1.48^{b}	1.33^{c}	1.37^{c}	0.5	<0.001

Means in a row without similar superscript letter are not significantly different (p>0.05)

DM= Dry matter, **OM**= organic matter, **CP=**Crude protein, **NDF**=Neutral Detergent Fiber, **ADF**=Acid Detergent Fiber, **ME**=Metabolizable Energy

4.3 Apparent digestibility

The apparent digestibilities of the different diets is shown in Table 4-2. There was no significant effect (P>0.05) of treatments on digestibility of DM (P=0.864), OM (P=0.397), CP (P=0.077), NDF (P=0.331) and ADF (P=0.102).

4.4 Nitrogen balance

The mean Nitrogen balance for the animals on different treatment groups is shown in Table 4-3. The Nitrogen intake increased (P <0.001) with increased intake of DM and was 14.55, 22.03, 27.95, 40.07 g/d/100kg LW for 60%, 80%, 100% and 120% MER respectively. Both fecal N and urine N losses increased (P <0.001) with feed and nitrogen intake. The Nitrogen retention (g/d/100kg LW) was significantly different between treatments with the highest retention at 120% MER (20.85 g/d/100kg LW). The 60% MER, 80% MER, 100% MER treatments had N retention of 4.16, 9.22 and 13.47 g/d/100kg LW respectively. There was no negative N balance for any of the treatment groups.

4.5 Nitrogen efficiency

The mean nitrogen intake, nitrogen retention and efficiency for the animals on different treatment groups is shown in Table 4-3. All the parameters were significantly different (P <0.001) between the treatment groups. The efficiency was 28.60%, 41.74%, 48.16%, 51.96% for 60% MER, 80% MER, 100% MER and 120% MER treatment groups respectively.

Table 2-3: Nitrogen intake, retention and efficiency of utilization for various treatments in Boran steers fed on Rhodes (*Chloris gayana*) grass hay) at different levels of MER.

	Diet				SEM	P-Value
Treatments	**60%**	**80%**	**100%**	**120%**		
Nitrogen balance (g /100kg LW/d)						
TNI	14.55[a]	22.03[b]	27.95[c]	40.07[d]	1.226	<0.001
FN	5.54[a]	8.14[b]	9.4[c]	12.75[d]	0.339	<0.001
UN	4.84[a]	4.67[a]	5.07[a]	6.47[b]	0.318	<0.001
NR	4.16[a]	9.22[b]	13.47[c]	20.85[d]	0.545	<0.001
NR as % of total N intake	28.60[a]	41.74[b]	48.16[c]	51.96[c]	2.094	<0.001
NR as % of N absorbed	46[a]	66[b]	72[c]	76[c]	2.926	<0.001

[a]*Means within a row with similar superscript letter are not significantly different (p>0.05)*

TNI-Total Nitrogen intake, **FN**-Fecal Nitrogen, **UN**-Urinary Nitrogen, **NR**-Nitrogen Retained

4.6 Live weights

The effect of dietary treatments on live weight changes of the steers is shown in Fig 4-2. The steers on 100% MER and 120% MER had an average daily gain (g/d/100kg LW) of 284 and 565 respectively. The sub-optimally fed steers lost weight at 500g/d/100kg LW and 245g /d/100kg LW for 60% MER and 80% MER respectively.

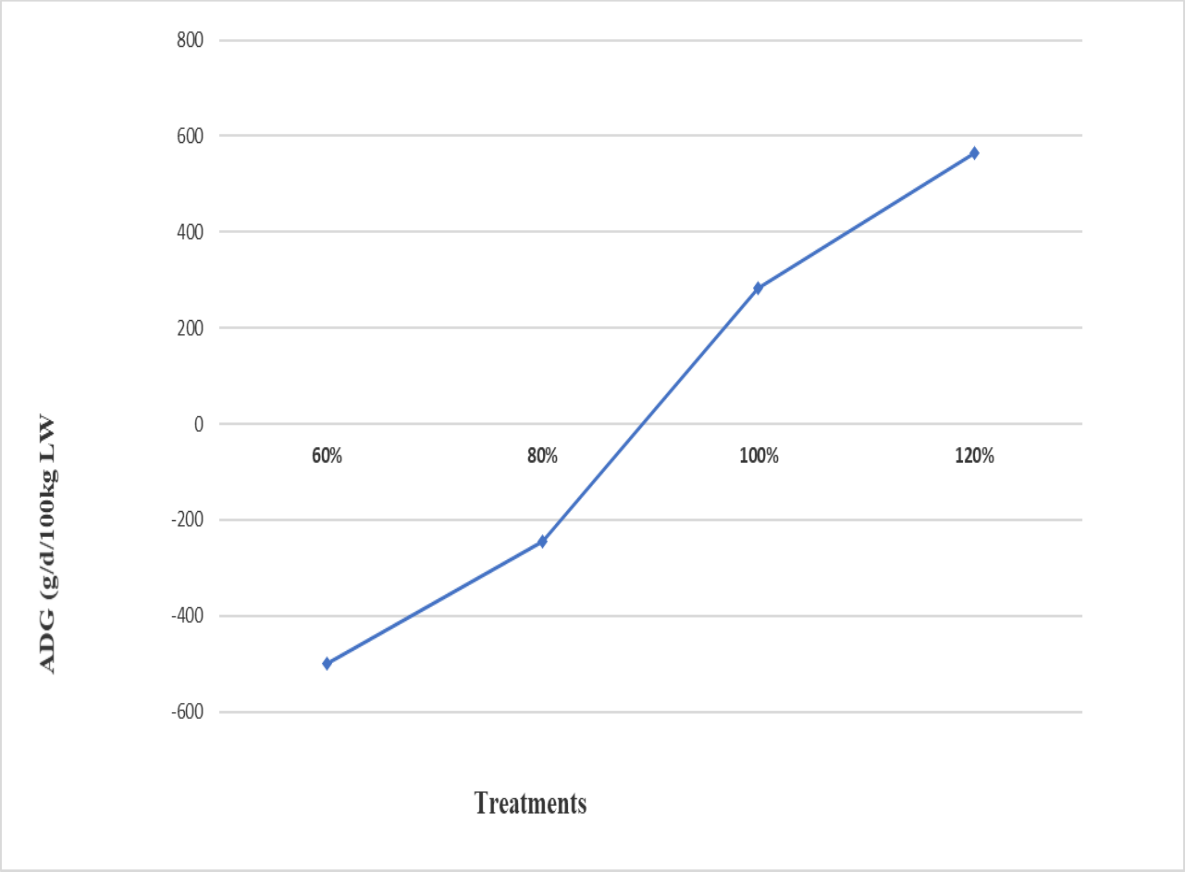

Figure 4-1: Live weight changes (% 100kg/d LW) for Boran steers fed on Rhodes grass *(Chloris gayana)* hay at different levels of MER.

4.7 Enteric methane emission

The enteric methane emissions for the steers on different treatments is shown in Fig 4-1. The dietary treatments had a significant effect on the enteric methane emission ($P < 0.001$). The emissions increased consistently with increased feed intake. The means for the different treatment groups were: 38.63, 52.90, 55.10, 64.13 g CH_4/100kg LW for 60%, 80%, 100% and 120% MER.

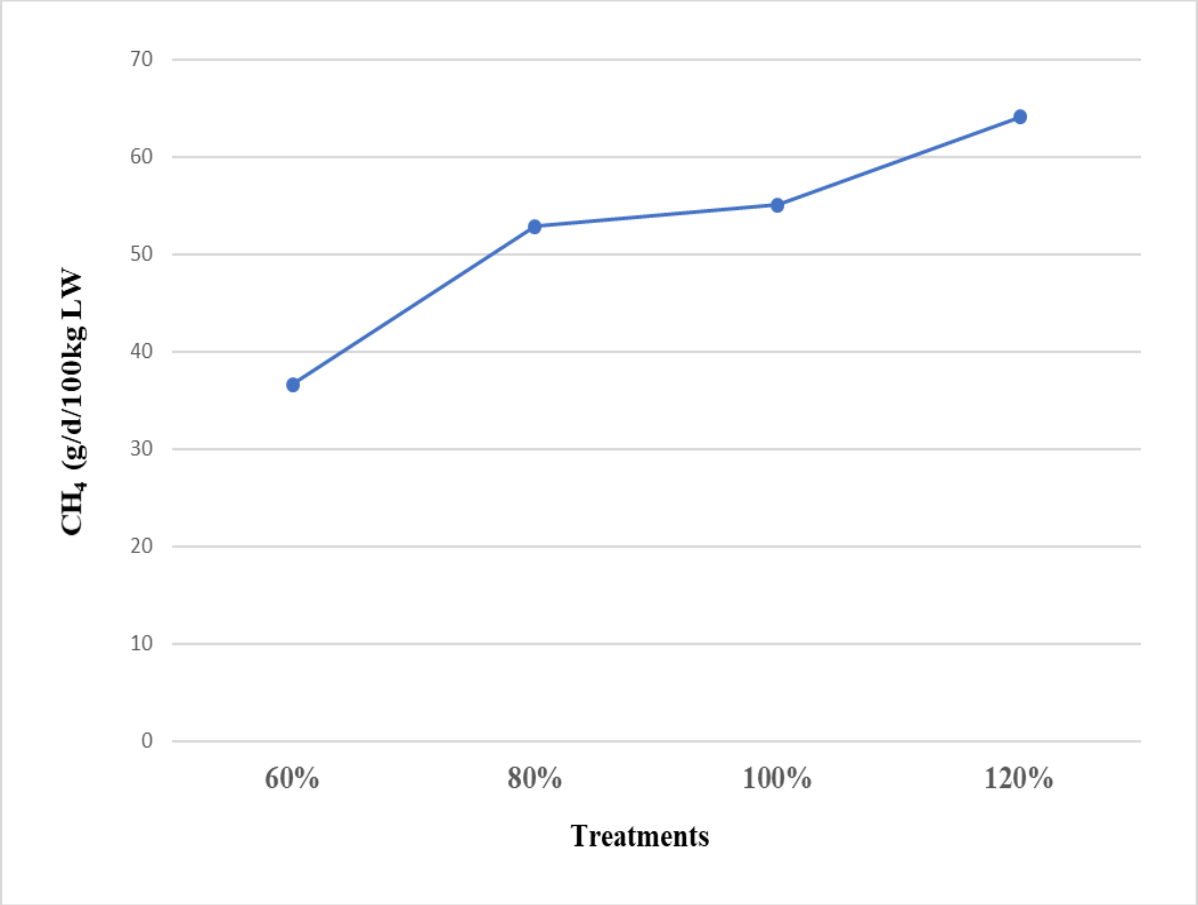

Figure 4-2: Enteric Methane production (g/d/100kg) for Boran steers fed on Rhodes grass (*Chloris gayana*) hay at different levels of MER.

CHAPTER FIVE: DISCUSSION

5.1 Rhodes grass (*Chloris gayana*) hay

The DM content (915 g/kg) of the Rhodes grass hay used in the present study was within the range of a well cured hay ensuring freedom from mold and low susceptibility to spoilage (Heuzé *et al.,* 2016). The crude protein content (71.25 g/kg DM) was within reported levels. In a study done in Ethiopia by Duguma *et al.* (2014) and in Tanzania by Mtenga *et al.,* (1990), a similar CP content of (75 g/kg DM) of Rhodes grass hay was reported. In contrast, Osuga *et al., (*2012) and Mupangwa *et al*., (2000) reported CP values of 61.9g/kg DM and 50.9 g/kg DM respectively. This disparity could be attributed to the fact that the Rhodes grass hay used in the present study was harvested at an early age when CP levels are high. Haffar and Alhadrami (1997), Mbwile 1997(a) and Qingxiang (2002) reported that the age at harvesting and post-harvest handling procedures influence the quality of grasses. A study done by Mbwile and Uden (1997) showed CP content of rhodes grass decreased from 170 g/kg DM to 90 g/kg DM within a 4 to 10 weeks. NRC (1981) and Leng and Nolan (1984) concluded that 70 g/kg DM CP is required for optimal rumen function.

The NDF content of the Rhodes grass hay from the present study was 739.2 g/kg DM. This value was expected since it corroborates with the results of NDF content reported from previous studies. Heuzé *et al.,* (2016) reported NDF content of Rhodes grass hay as 757.0 g/kg DM while Wilman and Moghaddam (1998) reported 793 g/kg DM. Abdulrazak *et al.,* (2015) reported as 718 g/kg DM. Rhodes grass has high amount of NDF and hence low digestibility compared to that of most temperate forages (Heuzé *et al.,* 2016). The NDF of a grass is a major determinant of digestibility and when it is high, the content of the inner cells of a feed become relatively inaccessible to the rumen micro-organisms (Wilman *et al.,* 1998).

The ADF and ME of the Rhodes grass hay in the present study was 452.82 g/kg DM and 8.2 MJ/kg DM respectively. Heuzé *et al.,* (2016) reported the 412 g/kg DM and 8.1 MJ/kg DM of ADF and ME respectively, in agreement with the results of the present study.

5.2 Apparent digestibility

The treatment had no significant (P>0.05) effect on DM, OM, CP NDF and ADF apparent digestibility of Rhodes grass hay (Table 4-2). The DM apparent digestibility of the Rhodes grass hay in the present study was low due to inaccessibility of the plant cells by the rumen micro-organisms (Wilman *et al.,* 1998). The observed nutrient digestibilities in the present study were within the range reported from previous studies.

Mero and Uden (1998) in their study with sheep to determine the effect of feeding different levels of tropical grass on nutrient digestibility reported OM digestibility of Rhodes grass as 600g/kg at low diet intake. They further reported that there was no significant difference in the digestibility of NDF with the increase in feed intake. Similar results have been reported by Mbwile and Udén, (1997b) who observed no difference in in-vivo digestibility of DM, OM, CP, NDF and ADF in Fresian cows which were fed Rhodes grass at different intake levels above MER.

The lack of difference in the digestibility of nutrients in the present study could be attributed to absence of any changes in the ruminal mean solid and liquid retention times and rumen particle size (Grimaud and Doreau 1995). A study by of Naresh *et al.* (1984) reported no difference in digestibility of nutrients (DM, CP, EE, CF and NFE), where male cross bred cattle were fed above MER (1.47 kg DM/100 kg BW) in comparison to the sub optimally fed cattle (1.65 and 1.84 kg/100 kg BW). Other authors have worked on sub optimal feed intakes

to elucidate the effect on digestibility. Graham (1964); Keenan *et al. (*1969) with sheep and Agabriel *et al.* (1995), Grimaud and Doreau (1995) with cattle reported no change in digestibility of nutrients at sub optimal feeding levels compared with optimal in agreement with present study.

Grimaud *et al.,* (1998, 1999) and Doreau *et al.,* (2000) in cattle and Atti *et al.,* (2002) in sheep reported a decrease in digestibility of nutrients at intakes below maintenance energy requirements. They hypothesized (without the *in-situ* measurements of microbial activity) that the decrease could be as a result of low bacterial growth due to limiting feed factors, or low expression of microbial degradation potential. Grimaud *et al.,* (1999) reported decrease in digestibility of nutrients in zebu cows (BW, 208kg) underfed a rice straw-based diet. From this study, the digestibility of DM, OM, NDF and ADF decreased from 596 g/kg, 645 g/kg, 733 g/kg, 730 g/kg to 487 g/kg, 537g/kg, 646 g/kg and 636 g/kg below maintenance energy requirements respectively. In Conclusion, feeding at **d**ifferent MER did not affect the digestibility of nutrients in the present study. There is need for more studies on effects of different amounts of feed intake on nutrient digestibility. The results from the previous digestibility studies are contradicting.

5.3 Nitrogen excretion and balance

Nitrogen balance was positive for all treatment groups and differed significantly ($P<0.01$). The steers fed at 60% MER, 80% MER,100% MER and 120% MER had a daily mean fecal N excretion (g/d/100kg LW) of 5.54, 8.14, 9.4 and 12.75 respectively. The fecal N excretion increased proportionally with the level of N intake. These results concur with the previous observations in ruminants where the amount of fecal N was proportional to the amount of

DM (N) fed. A study done by Peripolli *et al.,* 2010 with sheep fed on pasture reported a similar linear relationship of fecal N excretion and the DM (N) intake.

Lignified N and metabolic N could also have contributed to the fraction of the fecal N across all the groups and could be higher in steers at high feed intakes than those on low feed intakes (Currier *et al.,* 2004a). Metabolic N loss occurs because of mucosal epithelial cell loss through the feces while the lignified N as a result of poor-quality feed which is poorly digested in the rumen. Currier *et al*., 2004(a) reported that 5.35g N/kg DMI of metabolic N loss in cross-bred steers consuming low quality grass straw (CP, 4%). The diet in the current study was of relatively good quality (CP, 7%) and hence this value could have affected the computations of fecal N.

The urine N (g/d/100kg LW) excretion for the steers under the treatments: 60% MER, 80% MER, 100% MER and 120% MER was 4.84, 4.67, 5.07 and 6.47 respectively. The dietary N loss through ammonia during the microbial synthesis in rumen (Reynolds and Kristensen, 2008b) contributes to loss of N through urine. According to Reynolds and Kristensen (2008b), the animals which had low feed intakes could have been capturing the N optimally both from the diet and recycling from the portal circulation because of relatively low amounts of the N intakes. The urine N loss was lower than the fecal N loss. The urine N loss however followed a similar pattern as fecal N loss with the lowest occurring in group with low N intake and vice versa.

There was significant difference ($P<0.01$) in N balance between treatments in the current study. The N balance of the steers increased linearly with the increase in feed (N) intake. At 60% MER, 80% MER,100% MER and 120% MER, the N balance was 4.16, 9.22, 13.47 and 20.85 respectively. The N intakes (g/d/100kg LW) for the treatment groups 60% MER, 80% MER,100% MER and 120% MER were 14.55, 22.03, 27.95 and 40.07 respectively. The fact

that the N balance remained positive in all treatment groups could be attributed to the fact that the steers mobilized body reserves to meet the N requirements for both maintenance and growth.

5.4 Nitrogen efficiency

This parameter expresses the N balance as the proportion of N intake and hence it's an accurate assessment of efficiency of nitrogen utilization by animals. The steers in the treatment groups 60% MER, 80% MER, 100% MER and 120% MER had N efficiency of 28.6% 41.74%, 48.16% and 51.96 % respectively and differed significantly ($P<0.01$). The N efficiency increased proportionally with the increase in amount of feed intake. The low N efficiency value for lowest MER could be attributed to mobilization of N from the body reserves of the steers to cater for the maintenance requirement. Singh *et al.,* 2008 reported a similar N efficiency pattern of 11.13% and 22.59 % for 60% and 80% energy restricted fed cross-bred calves (BW, 159.7kg) which fed on concentrate mixture and wheat straw diet. Grimaud and Doreau (1995) observed low N efficiency in lactating cows (BW, 747kg) fed on low amounts of forage-based diet. George *et al.,* (2005) also reported significantly different N efficiencies in steers with different DM intakes. From the present study, it can be deduced that there is a poor utilization of N when ruminants are sub-optimally fed a low-quality diet.

5.5 Body weight changes

Positive and negative body weight changes were observed in the present study. The steers on the 120% MER, 100%, 80% and 60% MER treatment groups had daily weight changes (g/100kg LW) of 565, 284 -245 and -500 respectively. The increase in weights for the groups of steers fed at 120% and 100% MER in this study is a result of adequate energy intake and hence meeting the maintenance and growth requirements. Abate *et al.,* (1981) fed Fresian and Ayrshire heifers on adlibitum Rhodes grass during the dry season and reported a mean weight

gain of 200g/day. They concluded that Rhodes grass can meet the maintenance requirements of heifers and to some extent, those for growth.

The weight loss for the treatments groups of steers fed at 80% and 60% MER was expected from the calculated requirements of these two treatment groups. According to NRC (2000) the overall nutrient intake was low. The weight loss could therefore be attributed to low energy intake and hence mobilization of body reserves in order to meet the basal metabolic requirements of the steers. Singh *et al.,* 2008 observed a daily weight loss of 250–300 g/d in restricted-fed cross-bred calves. Murphy and Loerch (2014) observed body weight losses in cross-bred growing steers which were restricted fed. The loses averaged between 150 to 250g per day. The diet was concentrate based. From these studies, a similar trend is seen in body weight losses when animals are restricted fed irrespective of the diet composition.

5.6 Enteric methane emission

Enteric methane production (g/100kg LW) by the boran steers in the present study was significantly affected by the treatment. The daily methane production ($g/100kgd^{-1}$) at 60%, 80%, 100% and 120% MER was 38.63 g, 52.9 g, 55.10g and 64.13g respectively. The calculated daily energy lost as methane (MJ/kg DMI) was 1.55, 1.48, 1.33 and 1.37 for treatments 60%, 80%, 100% and 120% MER. The amount of methane produced increased with increasing MER. The high intake leads to a release of proportional amount of hydrogen ions in the rumen due to increased rumen fermentation. The hydrogen ions combine with carbon dioxide through the process of methanogenesis to form methane gas (Nkrumah *et al*., 2006).

Poor quality diets have high fiber content (NDF of more than 300g/kg DM) (NRC, 2001).

High fiber results in increased residency time and low digestibility in the rumen increasing rate of methanogenesis (Fitzsimons *et al*., 2013). Jones *et al.,* (2011) reported an increase in enteric methane production with increase in feed intake in grazing Angus beef cows. These studies concur with results of the present study in that, the amount of enteric methane production is directly proportional to the amount of diet presented to the animal.

In contrast, Blaxter and Clapperton (1965) and Johnson and Johnson (1995) reported decreased methane production when sheep were fed three times the maintenance energy requirements. Moss *et al.,* 2000, observed that the low diet digestibility could have also been accompanied by increased passage rate of feed particles and hence lack of enough time for the methanogens to act on the feed to produce methane gas. Similarly, Johnson and Johnson, (1995) noted that methane production from cattle computed as proportion of gross energy intake decreased by 1.6% for each double intake.

The low methane production at low diet intake in present study could be as a result of reduced fermentation process due to reduced rumen microbial activity hence less hydrogen ions (Nkrumah *et al*., 2006). Fewer hydrogen ions were available to combine with carbon dioxide through the processes of acetogenesis and methanogenesis to form methane gas. A more recent study by Winders *et al.,* (2018) was conducted on growing cross bred steers, one group fed adlibitum and the other limit fed (75% of the adlibitum intake). It was reported that daily methane production was significantly different ($p<0.01$) between the optimal (156g/steer) and sub-optimal (126g/steer) intakes. Beauchemin and McGinn (2006) reported lower daily methane production (30g/100kg LW) in Angus beef heifers under low DM intake. The heifers in their study were restricted-fed at 65% of adlibitum diet intake.

The energy use efficiency is low under suboptimal diet intake since most of methane is lost as energy which could be used for production by the steers. Johnson and Johnson (1995) reported an estimate of 2% energy lost daily as methane, this being expressed as proportion of the gross energy intake in ruminants. Hammond *et al.,* 2014 reported 2MJ/kg DMI daily average loss of energy as methane. These values corroborate with the results for the energy lost as methane from the current study.

From these results, it can be deduced that it is more efficient to produce at optimal feeding since there is less methane emission per unit (kg) body weight gain. The 100% treatment group produced relatively high amounts of enteric methane per unit body weight gain and hence it is not best energy intake level for the steers. The increased production efficiency in ruminants especially the beef animals (with low efficiency of production) is one of the methods of mitigation against enteric methane emissions (Johnson *et al.,* 1996). The current study also shows that there are high energy losses per DMI in form of methane emissions at intakes below MER. The energy use efficiency in cattle fed below maintenance is poor and methane production per DMI increases at low feed intake levels. Better knowledge of factors that determine methane emission could help reduce environmental impacts and improve dietary energy utilization by ruminants.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

This study was conducted to determine the effect of optimal and sub-optimal feeding on feed digestibility, nitrogen balance and methane emissions in Boran steers fed different amounts of Rhodes grass hay. It was hence concluded that;

1. Restricted feeding under or above maintenance energy requirements does not have any effect on digestibility of nutrients in boran steers.
2. Nitrogen balance in boran steers is proportional to the amount of feed (energy) intake. Nitrogen balance increases with increase in the amount of feed intake both at the optimal and suboptimal feeding.
3. The increased level of energy (feed) intake is proportional to enteric methane emission from Boran steers. The methane production per kg of DMI is however higher at low levels of energy intakes, both at optimal and sub optimal feeding.

6.2 RECOMMENDATION

The results of the current study show that there is a need to further investigate relationship between type and amount of forage intakes and methane emission from cattle in the tropics. This will help to establish the best types and amounts of diet intakes which result to better performance and less methane emission from the tropical cattle.

CHAPTER SEVEN: REFERENCES

Abate A., Kayongo M.H & Karue C. N (1981). Dry matter, protein, energy and fiber intake by dairy heifers grazing Rhodes grass (*Chloris gayana*) pasture. Journal of Animal Feed Science and Technology, *6 (1)*, 15-26.

Abdulrazak S. A, Njuguna E. G & Karau P. K (2015). The effect of supplementing Rhodes grass (*Chloris gayana*) hay with Acacia tortilis leaves and pods mixture on intake, digestibility and growth performance of goats. Journal of Livestock Research for Rural Development. *17,* 134.

Agabriel J, Petit M, Ollier A & Chilliard Y (1995). Effects of underfeeding on body reserves variations and on energy efficiency of lactating Charolais cows. Ann. Zootech*, 44,* 317 (Supplement).

Alford A.R, Hegarty R.S, Parnell P. F, Cacho O. J, Herd R. M & Griffith G. R (2006). The impact of breeding to reduce residual feed intake on enteric methane emissions from the Australian beef industry. Australian Journal of Experimental Agriculture, *46, 813*–820.

Amenu K, Markemann A, oßler R, SiegmundSchultze M & Z´arate A.V (2011). Perceived Feed and Water Constraints for Livestock Production in Lume and Siraro Districts, Ethiopia. Proceedings on International Research on Food Security, Natural Resource Management and Rural Development, 2011.

Andueza D, Picard F., Jestin M. & Aufrère J. (2012). The effect of feeding animal ad libitum vs. at maintenance level on the in vivo digestibility of mown herbage from two permanent grasslands of different botanical composition, Grass and Forage Science, *68(3),* 418-426.

AOAC (1990). Official Methods of Analysis, 15th Ed. Assoc. Off. Anal. Chem., Arlington, VA.

Ashby K. D, Casey T. A, Rasmussen M. A & Petrich J. W (2001). Steady-state and time-resolved spectroscopy of F420 extracted from methanogen cells and its utility as a marker for fecal contamination. Journal of Agricultural and Food Chemistry, *49(3),* 1123–1127.

Atti N, Kayouli C, Mahouachi M, Guesmib A & Doreau M. (2002). Effect of a drastic and extended underfeeding on digestion in Barbary ewe. Journal of Animal Feed Science and Technology, *100,* 1–14.

Ayantunde A.A, Fernández-Rivera S & McCrabb G (2005). Coping with feed scarcity in smallholder livestock systems in developing countries. Animal Sciences Group, Wageningen UR, Wageningen, The Netherlands, University of Reading, Reading, UK, ETH (Swiss Federal Institute of Technology), Zurich, Switzerland, and ILRI (International Livestock Research Institute), Nairobi, Kenya. 306 pp.

Balch W. E, Fox G. E & Magrum L. J (1979). Methanogens: re-evaluation of a unique biological group. Microbiological Reviews, *43(2),* 260–296.

Bayush T, Adugna T & Trygve B. (2008). Livestock Production and Feed Resource Constraints in Akaki and Lume Districts, Central Ethiopia. Oulook on Agriculture*, 37(1),* 15-21.

Beauchemin K. A. Kreuzer M, O'Mara F. & McAllister T.A (2008). Nutritional management for enteric methane abatement: a review. Australian Journal of Experimental Agriculture, *48,* 21–27.

Beauchemin K. A., McGinn S. M & Petit, H. V. (2007). Methane abatement strategies for cattle: Lipid supplementation of diets. Canadian Journal of Animal Science*, 87,* 431–440.

Beauchemin K.A & McGinn S.M (2006). Enteric methane emissions from growing beef cattle as affected by diet and level of intake. Canadian Journal of Animal Science, *86(3)*, 401-408.

Beauchemin K.A & McGinn S.M (2006). Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. Journal of Animal Science, *84,* 1489–1496.

Benchaar C & Greathead H. (2011). Essential Oils and Opportunities to Mitigate Enteric Methane Emissions from Ruminants. Journal of Animal Feed Science and Technology, 166-167, 338-355.

Benchaar C, Pomer C & Chiquette J (2001). Evaluation of dietary strategies to reduce methane production in ruminants: a modeling approach. Canadian Journal of Animal Science, *81,* 563–574.

Bird S. H, Hegarty R. S & Woodgate R (2008). Persistence of defaunation effects on digestion and methane production in ewes. Australian Journal of Experimental Agriculture, *48(2),* 152-155.

Blaxter K.L & Clapperton J.L. (1965). Prediction of the amount of methane produced by ruminants. British Journal of Nutrition, *19*, 511-22.

Boadi, D.A & Wittenberg, K.M. (2002). Methane Production from Dairy and Beef Heifers Fed Forages Differing in Nutrient Density Using the Sulphur Hexafluoride (SF6) Tracer Gas Technique. Canadian Journal of Animal Science, *82*, 201-206.

Bohnert D.W, Schauer C.S & DelCurto T. (2002). Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: cow performance and efficiency of nitrogen use in wethers. Journal of Animal Science, *80(6),* 1629–1637.

Broucek J (2014). Production of Methane Emissions from Ruminant Husbandry: A Review. Journal of Environmental Protection, *5*, 1482-1493.

Bruinenberg M. H., Valk H., Korevaar H & Struik P. C. (2002). Factors affecting digestibility of temperate forages from semi natural grasslands: a review. Journal

of Grass and Forage Science, *57,* 292–301.

Buddle B.M, Denis M, Attwood G.T, Altermann E, Janssen P.H, Ronimus R.S, Pinares-Patiño C.S, Muetzel S & Neil Wedlock D (2011). Strategies to reduce methane emissions from farmed ruminants grazing on pasture. The Veterinary Journal, *188(1),* 11-17.

Bunting L. D, Boling J. A, MacKown C. T. & Davenport G. M (1989). Effect of Dietary Protein Level on Nitrogen Metabolism in the Growing Bovine. Journal of Animal Science, *67(3)*, 810–819.

Calsamiglia S, Ferret A, Reynolds C. K & Kristensen N. B (2010). Strategies for optimizing nitrogen use by ruminants. XIth International Symposium on Ruminant Physiology, Clermont-Ferrand (France), *4(7)*, 1184-1196.

Carulla J. E, Kreuzer M, Machmüller A & Hess H.D (2005). Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in foragefed sheep. Australian Journal of Agricultural Research, *56(9),* 961-970.

Carulla J. E, Kreuzer M, Machmüller A & Hess H.D (2005). Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Australian Journal of Agricultural Research, *56(9)*, 961-970.

Cassandro M, Mele M, Stefanon B (2013). Genetic aspects of enteric methane emission in livestock ruminants. Italian Journal of Animal Science, *12,* e73.

Chagunda M.G, Römer D.M & Roberts D.J (2009). Effect of Genotype and Feeding Regime on Enteric Methane, Non-Milk Nitrogen and Performance of Dairy Cows during the Winter-Feeding Period. Journal of Livestock Science, *122*, 323-332.

Coffey M.T (1996). Environmental challenges as related to animal agriculture—swine. In: Kornegay, E.T. (Ed.), Nutrient Management of Food Animals to Enhance and Protect the Environment. CRC, BocaRaton, FL, pp. 29–39.

Colucci P.E, MacLeod G.K, Grovum W.L, McMillan I & Barney DJ (1990). Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. Journal of Dairy Science, *73(8*), 2143-2156.

Currier *et al*., (2004a). Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage: Effects on cow performance and the efficiency of nitrogen use in wethers. Journal of Animal Science, *82*, 1508–1517.

Das A & Singh G.P (1999). Effect of different proportions of berseem and wheat straw on in vitro dry matter digestibility and total gas production. Indian Journal of Animal Nutrition, *16,* 60–64.

de Klein, C.A & Eckard R.J (2008). Targeted technologies for nitrous oxide abatement from animal agriculture. Australian Journal of Experimental Agriculture, *48,* 14–20.

DelCurto T, Cochran R.C, Harmon D.L & Beharka A.A (1990). Supplementation of dormant tallgrass-prairie forage. I. Influence of varying supplemental protein and(or) energy levels on forage utilization characteristics of beef steers in confinement. Journal of Animal Science, *68 (2)*, 515-531.

Delgado C.L (2005). Rising demand for meat and milk in developing countries. Grassland: a global resource-wagenigenacademic.com.

Detmann E, Paulino M.F, Filho S.D & Huhtanen P (2014). Nutritional aspects applied to grazing cattle in the tropics: a review based on Brazilian results. Semina: Ciências Agrárias, Londrina, *35(4),* 2829-2854.

Dini, Y, Gere, J, Briano C, Manetti M, Juliarena P & Picasso V (2012). Methane Emission and Milk Production of Dairy Cows Grazing Pastures Rich in Legumes or Rich in Grasses in Uruguay. Animals Journal, *2*, 288-300.

Dohme F, Machmüller A, Wasserfallen A & Kreuzer M (2000). Comparative efficiency

of various fats rich in medium-chain fatty acids to suppress ruminal methanogenesis as measured with RUSITEC. Canadian Journal of Animal Science, *80(3),* 473-484.

Doreau M, Grimaud P, Atti N & Nozièrea P (2003). Consequences of underfeeding on digestion and absorption in sheep. Small Ruminant Research, *49(3),* 289-30.

Doreau M., Grimaud P & Michalet-Doreau B. (2000). The effects of underfeeding on digestion in ruminants. INRA Production Animal*, 13,* 247–255.

Duguma B, Eshete G, Zewdu T & Tolera A (2014). Comparison of Nutritive Value of Alfalfa, Rhodes Hay, Cynodon Pasture and Linseed Cake –Maize Mixture at Hawassa College of Agriculture, Ethiopia. Academic Journal of Nutrition, *3(2),* 19-21.

Eckard R.J, Grainger C & de Klein C.A (2010). Options for the abatement of methane and nitrous oxide from ruminant production. Journal of Livestock Science, *130*, 47-56.

Fitzsimons C, Kenny D. A, Deighton M. H, Fahey A. G.& McGee M (2013). Methane emissions, body composition, and rumen fermentation traits of beef heifers differing in residual feed intake. Journal of Animal Science, *91(12),* 5789–5800.

Galyean M.L & Owens F.N (1991). Effects of Diet Composition and Level of Feed Intake on Site and Extent of Digestion in Ruminants. Proceedings of the Seventh International Symposium on Ruminant Physiology 483-514.

Galyean M.L, Owens F.N (1979). Level of Feed Intake and Site and Extent of Digestion of High Concentrate Diets by Steers. Journal of Animal Science, *49(1),* 199–203.

Galyean M.L, Owens F.N (1991). Effects of Diet Composition and Level of Feed Intake on Site and Extent of Digestion in Ruminants. Proceedings of the Seventh International Symposium on Ruminant Physiology pp, 483-514.

Galyean M.L, Wagner D.G & Owens F.N (1979). Corn Particle Size and Site and Extent of Digestion by Steers. Journal of Animal Science, *49(1),* 204–210.

Garcia J. L, Patel B. K & Ollivier B (2000). Taxonomic, phylogenetic, and ecological diversity of methanogenic Archaea. Anaerobe Journal, *6(4),* 205–226.

George, S.K., Dipu, M.T., Mehra, U.R., Singh, P & Verma, A.K (2005). Growth of crossbred bulls affected by level of feed intake. Indian Journal of Animal Nutrition, *22,* 81–84.

Gerber P. J, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A & Tempio G (2013). Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO).

Graham N.M. (1964). Energetic efficiency of fattening sheep. I. Utilization of low fiber and high fiber food mixtures. Australian Journal of Agricultural Research*, 15(1),* 100 – 112.

Grainger C, Clarke T, McGinn S.M, Auldist MJ, Beauchemin K.A, Hannah M.C, Waghorn G.C, Clark H & Eckard R.J (2007). Methane emissions from dairy cows measured using the sulfur hexafluoride (SF_6) tracer and chamber techniques. Journal of Dairy Science 2007*, 90,* 2755-2766.

Grimaud P & Doreau M (1995). Effect of extended underfeeding on digestion and nitrogen balance in nonlactating cows. Journal of Animal Science, *73(1),* 211–219.

Grimaud P, Richard D, Kanwé A, Durier C & Doreau M (1998). Effect of undernutrition and refeeding on digestion in *Bos taurus* and *Bos indicus* in a tropical environment. Journal of Animal Science, *67*, 49-58.

Grimaud P, Richard D, Vergeron M.P, Guilleret J.R & Doreau M (1999). Effect of Drastic Undernutrition on Digestion in Zebu Cattle Receiving a Diet Based on

Rice Straw. Journal of Dairy Science, *82*, 974-81.

Guan H, Wittenberg K.M, Ominski K.H, Krause D.O (2006). Efficacy of ionophoresin cattle diets for mitigation of enteric methane. Journal of Animal Science, *84*,1896–1906.

Haas Y.D, Windig J. J, Calus M.P, Dijkstra J, de Haan M, Bannink A & Veerkamp R. F (2011). Genetic parameters for predicted methane production and potential for reducing enteric emissions through genomic selection. Journal of Dairy Science, *94(12*), 6122-6134.

Haffar I & Alhadrami G (1997). Effect of various bale treatments on physical quality and chemical composition of rhodes grass (Chloris gayana) hay. Journal of Grass Forage Science, *52 (2),* 199-206.

Hammonda K.J, Humphriesa D.B, Westburyb D.J, Thompsona A, Cromptona L.A, Kirtona P, Greena C, Reynolds C.K (2014). The inclusion of forage mixtures in the diet of growing dairy heifers: Impacts on digestion, energy utilisation, and methane emissions. Journal of Agriculture, Ecosystems and Environment, *197,* 88–95.

Hayes B.J, Lewin H.A & Goddard M.E (2013). The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity and adaptation. Trends in Genetics Journal, *29(4),* 206-214.

Hayler R, Steingass H & Drochner W (1998). Effect of various feedstuffs rich in tannin content on rumen methanogenesis in-vitro using the Hohenheim gas test. Proceedings of the Society of Nutritional Physiology, *7,* 35.

Hegarty R.S, Goopy J.P, Herd R.M & McCorkell B (2007). Cattle selected for lower residual feed intake have reduced daily methane production. Journal of Animal Science, *85,* 1479–1486.

Herd R.M & Arthur P.F (2009). Physiological basis for residual feed intake. Journal of Animal Science, *87(E. Suppl.),* E64–E71.

Herd R.M, Arthur P.F, Hegarty R.S & Archer J.A (2002). Potential to reduce greenhouse gas emissions from beef production by selection for reduced residual feed intake. 7th World Congress on Genetics Applied to Livestock Production, August 19-23, 2002, Montpellier, France.

Heuzé V, Tran G, Boudon A & Lebas F (2016). Rhodes grass (*Chloris gayana*). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/480.

Hobson P. N & Stewart C. S (1997). The Rumen Microbial Ecosystem, Chapman and Hall, London, UK,

Hof G, Tamminga S & Lenaers P (1994). Efficiency of protein utilization in dairy cows. Journal of Livestock Production Science, *38*, 169-178.

Hook S.E, Wright A.G & McBride B.W (2010). Methanogens: Methane Producers of the Rumen and Mitigation Strategies. Hindawi Publishing Corporation Archaea, 11 pp.

Huhtanen P.1, Rinne M & Nousiainen J.A (2009). A meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. Journal of Dairy Science, *92(10),* 5031-5042.

Hulshof R.B, Berndt A, Gerrits W.J, Dijkstra J & Van Zijderveld S. M (2012). Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. Journal of Animal Science, *90(70),* 2317-2323.

ILCA (International Livestock Centre for Africa), 1990. Livestock systems research manual. Working Paper *1(1).* ILCA, Addis Ababa, Ethiopia. Pp 287.

ILRI (International Livestock Research institute), 1995. Livestock Policy Analysis. ILRI

Training Manual 2. ILRI, Nairobi, Kenya. pp. 264.

Jahnke H. E, Tacher G, Keil P & Rojat D (1988). Livestock production in tropical Africa with special reference to the tsetse-afferent zone. Livestock production in tsetse affected areas of Africa, ILCA/ILRAD, Nairobi, Kenya.

Jardine C.N, Boardman B, Osman A, Vowles J & Palmer J (2003). Methane UK. Report. Environmental Change Institute, University of Oxford, Oxford, 96

Johnson K.A & Johnson D.E (1995). Methane emissions from cattle. Journal of Animal Science, *73(8)*, 2483-2492.

Jones F.M, Phillips F.A, Naylor T & Mercer N.B (2011). Methane emissions from grazing Angus beef cows selected for divergent residual feed intake. Journal of Animal Feed Science and Technology, *166– 167*, 302– 307.

Jung H. G & Allen M. S (1995). Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. Journal of Animal Science, *73,* 2774-2790.

Kassam A, Kueneman E, Kebe B, Ouedraogo S & Youdeowei A (2009). Enhancing Crop-Livestock Systems in Conservation Agriculture for Sustainable Production Intensification: A Farmer Discovery Process Going to Scale in Burkina Faso. Integrated Crop Management Volume.7

Katongole C.KC. K, Kasozi J.NJ. N, Lumu R, Bareeba F, Presto M, Ivarsson E, Lindberg J.E (2012). Strategies for coping with feed scarcity among urban and peri-urban livestock farmers in Kampala, Uganda. Journal of Agriculture and Rural Development in the Tropics and Subtropics, *113 (2),* 165–174.

Keenan D.M, McManus W.R & Freer M (1969). Changes in the body composition and efficiency of mature sheep during loss and regain of live weight. Journal of Agricultural Science (Cambridge*), 72,* 139–147.

Korir D, Goopy J.P, Gachuiri C, Butterbach-Bahl K (2016). Supplementation with

Calliandra calothyrsus improves nitrogen retention in cattle fed low-protein diets. Journal of Animal Production Science, *56,* 619-626.

Kosgey I.S, Rowlands G.J, Van Arendonk J.A & Baker R.L (2008). Small ruminant production in smallholder and pastoral/extensive farming systems in Kenya, *77*, 11-24.

Koster H. H, Cochran R. C, Titgemeyer E. C, Vanzant E. S, Abdelgadir I. & St-Jean G (1996). Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. Journal of Animal Science, *74,* 2473-2481.

Kristensen T, Mogensen L, Knudsen M.T & Hermansen J.E (2011). Effect of Production System and Farming Strategy on Greenhouse Gas Emissions from Commercial Dairy Farms in a Life Cycle Approach. Journal of Livestock Science, *140,* 136-148.

Kumar S, Puniya A.K., Puniya M, Dagar S. S, Sirohi S.K, Singh K, Griffith G.W (2009). Factors affecting rumen methanogens and methane mitigation strategies. World Journal of Microbiology and Biotechnology, *25(9),* 1557-1566.

Kurihara M.1, Magner T, Hunter RA & McCrabb G.J (1999). Methane production and energy partition of cattle in the tropics. British Journal of Nutrition, *81(3*), 227-234.

Lal M, Khan M.Y & Kishan J (1987). Comparative nutrient utilization by Holstein Fresian crossbred cattle and buffaloes fed on wheat straw-based ration. Journal of Animal Nutrition, 4, 177–180.

Lashof D. A & Ahuja D. R (1990). "Relative contributions of greenhouse gas emissions to global warming". Nature Journal 344 *(6266),*529–531.

Lazzarini I, Detmann E, Paulino P.F, Filho S.C, Valadares R.F, Oliveira F.A, Silva P.T & Santiago W.L (2013). Nutritional performance of cattle grazing on low-quality tropical forage supplemented with nitrogenous compounds and/or starch. Revista Brasileira de Zootecnia, 42*(9),* 664-674.

Lechner-Doll M, Kaske M & Engelhardt W.V (1991). Factors affecting the mean retention time of particles in the forestomach of ruminants and camelids. Proceedings of the Seventh International Sychnmposium on Ruminant Physiology, 455-482.

Lechner-Doll M, Rutagwenda T, Schwartz H.J, Schultka W & Engelhardt W.V (1990). Seasonal changes of ingesta mean retention time and forestomach fluid volume in indigenous camels, cattle, sheep and goats grazing a thornbush savannah pasture in Kenya. The Journal of Agricultural Science, *115(03)*, 409 – 420.

Leng R.A & Nolan J.V (1984). Nitrogen Metabolism in the Rumen. Journal of Dairy Science, *67*(5), 1072‑1089.

Lenné J.M & Thomas D (2006). Integrating crop–livestock research and development in Sub-Saharan Africa Option, imperative or impossible? Outlook on Agriculture Journal*, 35,* 167–175.

Lima M.A, Pessoa M.C & Ligo M.A (2006). Primeiro inventário brasileiro de emissões antrópicas de gases de efeito estufa. In: Relatórios de referência: Emissões de metano da pecuária. Brasília: Ministério da Ciência e Tecnologia.

Lintzenich B.A, Vanzant E.S & Cochran R.C (1995). Influence of processing supplemental alfalfa on intake and digestion of dormant bluestem-range forage by steers. Journal of Animal Science, *73(4*), 1187-95.

Lukuyu B.A, Kitalyi A, Franzel S, Duncan A & Baltenweck I (2009). Constraints and options to enhancing production of high-quality feeds in dairy production in Kenya, Uganda and Rwanda. ICRAF Working Paper, 95, Nairobi, Kenya: World

Agroforestry Centre.

Machmüller A & Kreuzer M (1999). Methane suppression by coconut oil and associated effects on nutrient and energy balance in sheep. Canadian Journal of Animal Science*, 79(1),* 65-72.

Marini J.C & Van Amburgh M.E (2005). Partition of Nitrogen Excretion in Urine and the Feces of Holstein Replacement Heifers. Journal of Dairy Science, *88(5),* 1778-1784.

Masikati P (2010). Improving the water productivity of integrated crop-livestock systems in the semi-arid tropics of Zimbabwe: an ex-ante analysis using simulation modeling. Retrieved January 17, 2012, from http://www.zef.de/fileadmin/webfiles/downloads/zefc_ecology_development/eds_78_masikati_text.pdf.

Maynard L.A & Loosli J.K (1969). Animal Nutrition: 7th Edition. McGraw-Hill Book Co. Inc., New York: London, Sydney.

Mazingira laboratory Manual (2016). Chamber Measurement of Enteric Methane gas. ILRI Nairobi, Kenya.

Mbwile R. P & Udén P (1997a). Effects of age and season on growth and nutritive value of Rhodes grass (Chloris gayana cv. Kunth). Journal of Animal Feed Science and Technology, *65 (1-4),* 87-98.

Mbwile R. P & Udén P (1997b). The effect of feeding level on intake and digestibility of Rhodes grass (*Chloris gayana*) by dairy cows. Journal of Animal Feed Science and Technology, *66 (1-3),* 181-196.

Mc Geough E. J, O'Kiely P, Hart K. J, Moloney A. P, Boland T. M & Kenny D. A (2010). Methane emissions, feed intake, performance, apparent digestibility and

rumen fermentation of finishing beef cattle offered whole-crop wheat silages differing in grain content. Journal of Animal Science, *88(8),* 2703–2716.

McCaughey W. P, Wittenberg K, & Corrigan D (1999). Impact of pasture type on methane production by lactating beef cows. Canadian Journal of Animal Science, *79(2),* 221-226.

McCrabb G. J, Berger K. T, Magner T, May C & Hunter R. A (1997). Inhibiting methane production in Brahman cattle by dietary supplementation with a novel compound and the effects on growth. Australian Journal of Agricultural Research, *48(3),* 323 – 329.

McGinn S.M, Beauchemin K.A, Coates T, Colombatto D (2004). Methane emissions from beef cattle: effect of monensin, sunflower oil, enzymes, yeast and fumaric acid. Journal of Animal Science, *82,* 3346–3356.

McGuffey R.K, Richardson L.F, Wilkinson J.I (2001). Ionophores for dairy cattle: current status and future outlook. Journal of Dairy Science, *84,* 194–203.

Meltzer M. I (1995). Livestock in Africa: The economics of ownership and production, and the potential for improvement. Journal of Agriculture and Human Values, *12(2*), 4-18.

Mercadante M.E, Caliman A.N, Canesin R.C, Bonilha S.F, Berndt A, Frighetto R.T, Magnani E & Branco R.H (2015). Relationship between residual feed intake and enteric methane emission in Nellore cattle. R. Bras. Zootec, *44(7),* 255-262.

Mero R. N & Uden P (1998). Promising tropical grasses and legumes as feed resources in Central Tanzania III: Effect of feeding level on digestibility and voluntary intake of four grasses by sheep. Journal of Animal Feed Science and Technology, 70, 79-95.

Mohini M & Singh G.P (2001). Methane production on feeding jowar fodder-based ration in

buffalo calves. Indian Journal of Animal Nutrition, *18,* 204–209.

Morton J & Matthewman R (1996). Improving Livestock Production Through Extension: Information Needs, Institutions and Opportunities. Journal of Natural Resource Perspectives*, 12,* 1-8.

Moss A.R, Givens D. I & Garnsworthy P. C (1995). The effect of supplementing grass silage with barley on digestibility, in Sacco degradability, rumen fermentation and methane production in sheep at two levels of intake. Journal of Animal Feed Science and Technology*, 55,* 9-33.

Moss A.R, Jouany J.P & Newbold J (2000). Methane production by ruminants: It's contribution to global warming. Ann. Zootech, *49(3)*, 231-253.

Mtenga L.A & Kitaly A. J (1990). Growth performance and carcass characteristics of Tanzanian goats fed Chloris gayana hay with different levels of protein supplement. Small Rumunant Research, *3 (1),* 1-8.

Mupangwa J.F, Ngongoni N.T, Topps J.H & Hamudikuwanda H. (2000). Effects of Supplementing a Basal Diet of *Chloris gayana* Hay with One of Three Protein-rich Legume Hays of *Cassia rotundifolia*, *Lablab purpureus* and *Macroptilium atropurpureum* Forage on Some Nutritional Parameters in Goats. Tropical Animal Health and Production, *32(4),* 245–256.

Murphy T.A & Loerch S.C (2014). Effects of restricted feeding of growing steers on performance, carcass characteristics, and composition. Journal of Animal Science, *72(9),* 2497-507.

Murray P.J, Moss A, Lockyer D.R & Jarvis S.C (1999). A Comparison of Systems for Measuring Methane Emissions from Sheep. Journal of Agricultural Science, *133*, 439-444.

Mutibvu T, Maburutse B.E, Mbiriri D.T & Kashangura M.T (2012). Constraints and

opportunities for increased livestock production in communal areas: A case study of Simbe, Zimbabwe. Journal of Livestock Research and Rural Development, *24(9),* 1-17.

Naresh K, Khatta V.K, Virk A.S & Gupta P.C (1984). Nutrient utilization under restricted feeding regime in cattle. Asian Journal of Dairy Research, *3*, 235–236.

National Academies of Sciences, Engineering & Medicine (2016). Nutrient Requirements of Beef Cattle: Eighth Revised Edition. Washington, DC: The National Academies Press.

Nkrumah D, Okine E. K, Mathison G. W, Schmid L.C, Basarab J. A, Price M. A, Wang Z & Moore S. S (2006). Relationship of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of Animal Science, *84 (1),* 145-153.

Nolan J.V, Hegarty R. S, Hegarty J.A, Godwin I. R & Woodgate R (2010). Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. Journal of Animal Production Science, *50(8)*, 801-806.

NRC (2000), Nutrient requirements of beef cattle: Update 2000, 7th edition. The National Academic Press: Washington, DC.

O'Mara F.P (2011). The significance of livestock as a contributor to global greenhouse gas emissions today and in the near future. Journal of Animal Feed Science and Technology, *166-167*, 7-15.

Oenema O, Oudendag D, Gerard L, & Velthof (1997). Nutrient losses from manure management in the European Union. Journal of Livestock Science, *112*, 261-272.

Oosting S.J, Udo H.M & Viets T.C (2014). Development of livestock production in the tropics: farm and farmers' perspectives. Animal Journal*, 8(8),* 1238-1248.

Osuga I.M, Abdulrazak S.A, Muleke C.I & Fujihara T (2011). Effect of supplementing Rhodes grass hay (*Chloris gayana*) with *Berchemia discolor* or *Zizyphus mucronata* on the performance of growing goats in Kenya. Journal of Animal Physiology and Animal Nutrition, *96(4),* 634-639.

Otte M.J & Chilonda P (2002). Cattle and Small Ruminant Production Systems in Sub-Saharan Africa: Systematic Review. Food and Agriculture Organization of the United Nations, Rome.

Peripolli V, Êr P, Barcellos J.O, Neto J.B (2010). Fecal nitrogen to estimate intake and digestibility in grazing ruminants. Animal Feed Science and Technology journal, *163,* 170–176.

Pickering N. K, Oddy V. H, Basarab J, Cammack K, Hayes B, Hegarty R. S, Lassen J, McEwan J. C, Miller S, Pinares-Patiño C & de Haas S.Y (2015). Animal board invited review: genetic possibilities to reduce enteric methane emissions from ruminants. Animal Journal, *9(9),* 1431–1440.

Pinares-patiño C.S, Ebrahimi S.H, McEwan J.C, Dodds K.G, Clark H & Luo D (2011). Is rumen retention time implicated in sheep differences in methane emission? Proceedings of the New Zealand Society of Animal Production, *71,* 219-222.

Pinares-Patiño C.S, Waghorn G.C, Machmüller A, Vlaming B, Molano G, Cavanagh A & Clark H (2007). Methane Emissions and Digestive Physiology of Non-Lactating Dairy Cows Fed Pasture Forage. Canadian Journal of Animal Science, *87*, 601-613.

Qingxiang M (2002). Composition, nutritive value and upgrading of crop residues. Food and Agriculture Organization of the United Nations (FAO).

Reynold C.K & Kristensen N.B (2008). Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. Journal of Animal Science, *86*, E293–E305.

Reynolds C.K & Kristensen N.B (2008a). Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. Journal of Animal Science, *86(E. Suppl.),* E293–E305.

Ribeiro L.G, Machado F.S, Campos M.M, Guimaraes R, Tomich T.R, Reis L.G & Coombs C (2015). Enteric methane mitigation strategies in ruminants: a review. Rev Colomb Cienc Pecu, *28,* 124-143.

Ricci P.1, Rooke J. A, Nevison I & Waterhouse A (2013). Methane emissions from beef and dairy cattle: quantifying the effect of physiological stage and diet characteristics. Journal of Animal Science, *91(11),* 5379-5389.

Rouviere P. E & Wolfe R. S (1988). Novel biochemistry of methanogenesis. Journal of Biological Chemistry, *263(7),* 7913–7916.

rumen methanogens. Vaccine Journal, *22 (29–30),* 3976-3985.

Rumpler W. V, Johnson D. E & Bates D. B (1986). The Effect of High Dietary Cation Concentration on Methanogenesis by Steers Fed Diets with and without Ionophores. Journal of Animal Science, *62(6),* 1737–1741.

Saggar S, Bolan N. S, Bhandral R, Hedley C. B & Luo J (2004). A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. New Zealand Journal of Agricultural Research, *47(4),* 513–544.

Sara C, Santoso B, Mwenya B, Gamo Y, Kobayashi T, Morikawa R, Kimura K, Mizukoshi H & Takahashi J (2004). Manipulation of rumen methanogenesis by the combination of nitrate with β1-4 galacto-oligosaccharides or nisin in sheep.

Journal of Animal Feed Science and Technology, *115(1–2),* 129-142.

Sara C, Santoso B, Mwenya B, Gamo Y, Kobayashi T, Morikawa R, Kimura K, Mizukoshi H & Takahashi J (2004). Effects of Combination of Nitrate with β1-4 Galacto-oligosaccharides and Yeast (Candida kefyr) on Methane Emission from Sheep. Journal of Animal Science, *17(1),* 73-79.

Sauer F.D, Fellner V, Kinsman R, Kramer J.K, Jackson H.A (1998). Methane output and lactation response in Holstein cattle with monensin or unsaturated fat added to the diet. Journal of Animal Science, *76,* 906–914.

Scott R.R & Hibberd C.A (1990). Incremental levels of supplemental ruminal degradable protein for beef cows fed low quality native grass hay. Animal Science Research Report, *129*, 57-63.

Shibata, M & Terada T (2010). Factors Affecting Methane Production and Mitigation in Ruminants. Journal of Animal Science, *81*, 2-10.

Singh G.P & Mohini M (1999). Effect of different levels on berseem on in vitro digestibility and methane production in crossbred cattle fed wheat straw-based diets. Indian J Dairy Biosci, *10,* 14–19.

Singh K & Singh G. P (1997). Effect of concentrate levels in diet of cattle on rumen microorganisms. Indian Journal of Animal Science*, 64,* 349–350.

Singh P, Verma A.K, Sahu D.S & Mehra U.R (2008). Utilization of nutrients as influenced by different restriction levels of feed intake under sub-tropical conditions in crossbred calves. Journal of Livestock Science, *117(2-3),* 308-314.

Sordi A, Dieckow J, Bayer C, Alburquerque M, Piva J, Zanatta J, Tomazi M, da Rosa C & de Moraes A (2014). Nitrous oxide emission factors for urine and dung patches in a subtropical Brazilian pastureland. Agriculture, Ecosystems & Environment, *190,* 94-103.

Sørensen P, Weisbjerg M. R & Lund P (2003). Dietary effects on the composition and plant utilization of nitrogen in dairy cattle manure. The Journal of Agricultural Science, *141(1),* 79-91.

Srivastava A.K & Garg M.R (2002). Use of Sulphur hexafluoride tracer technique for measurement of methane emission from ruminants. Indian Journal of Dairy Science, 55, 36–39.

Steinfeld H, Wassenaar T & Jutzi S (2006). Livestock production systems in developing countries: status, drivers, trends. Rev. sci. tech. Off. int. Epiz., *25 (2)*, 505-516.

Storm I.M, Hellwing A.L, Nielsen N.I & Jørgen M.J (2012). Methods for Measuring and Estimating Methane Emission from Ruminants. Animals Journal*, 2,* 160-183.

Sufyan A. B (2018). Partial replacement of forage fiber with non-forage fiber in ruminant ration: A review. Pakistan Veterinary Journal*, 6 (1),* 15-26.

Susmel P, Stefanon B, Plazzotta E & Spanghero M (1994). The effect of energy and protein intake on the excretion of purine derivatives. The Journal of Agricultural Science, *123(2),* 257-265.

Thauer R. K, Kaster A. K, Seedorf H, Buckel W & Hedderich R (2008). Methanogenic archaea: ecologically relevant differences in energy conservation. Nature Reviews Microbiology, *6(8),* 579–591.

Thornton P.K (2010). Livestock production: Recent trends, future prospects. Philosophical Transactions of the Royal Society B, *365,* 2853–2867.

Van Dorland H.A, Wettstein H.R, Leuenberger H & Kreuzer M (2007). Effect of supplementation of fresh and ensiled clovers to ryegrass on nitrogen loss and methane emission of dairy cows. Journal of Livestock Science, *11*, 57-69.

Van Nevel C. J & Demeyer D. I (1996). Control of rumen methanogenesis. Journal of Environmental Monitoring and Assessment, *42(1–2*), 73–97.

Van Soest P.J, Robertson J.B & Lewis B.A (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, *74*, 3583 – 3597.

Varga G.A & Kolver E.S (1997). Microbial and Animal Limitations to Fiber Digestion and Utilization. Journal of Nutrition, *127*, 819-823.

Verbic (1990). Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. The Journal of Agricultural Science, *114*, 243-248.

VSN International (2011). GenStat for Windows 14th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk.

Waghorn G (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. Journal of Animal Feed Science and Technology, *147,* 116-139.

Waghorn G.C, Clark H, Taufa V, Cavanagh A (2007). Monensin controlled release capsules for improved production and mitigating methane in dairy cows fed pasture. Proceedings of the New Zealand Society of Animal Production, *67,* 266–271.

Waghorn G.C, Tavendale M.H & Woodfield D.R (2002). Methanogenesis from forages fed to sheep. Proceedings of the New Zealand Grassland Association, *64*, 167–171.

Waghorn G.C, Tavendale M.H, Woodfield D.R (2002). Methanogenesis from forages fed to sheep. Proceedings of the New Zealand Grassland Association, *64,* 159–165.

Wickersham T. A, Titgemeyer E. C, Cochran R. C, Wickersham E. E & Moore E. S (2008b). Effect of frequency and amount of rumen-degradable intake protein

supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. Journal of Animal Science, *86*, 3089-3099.

Williams Y.J, Popovski S, Rea S.M, Skillman L.C, Toovey A.F, Northwood K.F & Wright A.G (2009). A Vaccine against Rumen Methanogens Can Alter the Composition of Archaeal Populations. Journal of Applied and Environmental Microbiology, *75(7)*, 1860–1866.

Wilman D & Moghaddam P (1998). In vitro digestibility and neutral detergent fibre and lignin contents of plant parts of nine forage species. Journal of Agricultural Science, *131 (1),* 51-58.

Wilson J.R & Kennedy P.M (1996). Plant and Animal Constraints to Voluntary Feed Intake Associated with Fibre Characteristics and Particle Breakdown and Passage in Ruminants. Australian Journal of Agricultural Research, *47*, 199-225.

Winders T. M, Boyd B. M, Hilscher F. H, Stowell R. R, Fernando S. C & Erickson G. E (2018). Evaluation of the Effect of Intake on Methane Production in Growing Cattle Fed Forage-Based Diets. Journal of Animal Science, *96 (Issue suppl 2),* 219.

Winders T. M, Boyd B. M, Hilscher F. H, Stowell R. R, Fernando S. C & Erickson G. E (2018). Evaluation of the Effect of Corn Oil on Methane Production in Finishing Cattle. Journal of Animal Science, *96 (Issue suppl 2),* 231–232.

Winrock International (1992). Assessment of Animal Agriculture in Sub-saharan Africa. Winrock International institute for Agricultural Development, Morrilton, Arkansas, USA.

Wright A.D, KennedyP, Neill C.J, Toovey A.F, Popovski S, Rea S.M, Pimm C.L & Klein L (2004). Reducing methane emissions in sheep by immunization against rumen methanogens.

APPENDICES

Appendix 1: Analysis of Variance table of CH_4 gas production (g/d/100kg LW)

Source of variation	DfSum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	3009.27	1003.09	18.45	<0.001
Residual	32	1739.88	54.37		
Total	35	4749.15			

SEM=2.458

Appendix 2: Analysis of Variance table of average weight gains (g/d/100kg LW)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	6896819	2298940	18.36	<0.001
Residual	8	1001681	125210		
Total	11	7898501			

SEM=204.3

Appendix 3: Analysis of Variance table of nitrogen intake (g N/100kgd-1)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	6275.137	2091.712	918.04	<0.001
Residual	68	154.935	2.278		
Total	71	6430.072			

SEM=1.226

Appendix 4: Analysis of Variance table of urine Nitrogen (g N/100kgd-1)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	36.523	12.174	6.7	<0.001
Residual	68	123.643	1.818		
Total	71	160.166			

SEM=0.318

Appendix 5: Analysis of Variance table of fecal Nitrogen (g N/100kgd-1)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	483.958	161.319	78	<0.001
Residual	68	140.637	2.068		
Total	71	624.595			

SEM=0.339

Appendix 6: Analysis of Variance table of Total Nitrogen output (g N/100kgd-1)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	751.698	250.566	58.48	<0.001
Residual	68	291.368	4.285		
Total	71	1043.066			

SEM=0.488

Appendix 7: Analysis of Variance table of DM Apparent digestibility

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	1596	532	0.25	0.864
Residual	68	146770	2158		
Total	71	148366			

SEM=10.95

Appendix 8: Analysis of Variance table of OM Apparent digestibility

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	4470	1490	1	0.397
Residual	68	100929	1484		
Total	71	105399			

SEM=9.08

Appendix 9: Analysis of Variance table of CP Apparent digestibility

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	45535	15178	4.81	0.004
Residual	68	214754	3158		
Total	71	260289			

SEM=13.25

Appendix 10: Analysis of Variance table of NDF Apparent digestibility

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	10119	3373	1.16	0.331
Residual	68	197509	2905		
Total	71	207627			

SEM=12.7

Appendix 11: Analysis of Variance table of ADF Apparent digestibility

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	30427	10142	2.15	0.102
Residual	68	320455	4713		
Total	71	350882			

SEM=16.18

Appendix 12: Analysis of Variance table of Intake as fed (g/d/100kg LW)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	24660737	8220246	288.18	<0.001
Residual	68	1939684	28525		
Total	71	26600421			

SEM=39.8

Appendix 13: Analysis of Variance table of DMI (g/d/100kg LW)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	16179909	5393303	288.18	<0.001
Residual	68	1272627	18715		
Total	71	17452536			

SEM=32.2

Appendix 14: Analysis of Variance table of OMI (g/d/100kg LW)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	11178272	3726091	268.77	<0.001
Residual	68	942708	13863		
Total	71	12120979			

SEM=27.8

Appendix 15: Analysis of Variance table of ADF Intake (g/d/100kg LW)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	2188957	729652	192.67	<0.001
Residual	68	257524	3787		
Total	71	2446481			

SEM=14.51

Appendix 16: Analysis of Variance table of NDF Intake (g/d/100kg LW)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	6275709	2091903	211.99	<0.001
Residual	68	671031	9868		
Total	71	6946740			

SEM=23.4

Appendix 17: Analysis of Variance table of CP Intake (g/d/100kg LW)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	245122.54	81707.51	918.04	<0.001
Residual	68	6052.13	89		
Total	71	251174.67			

SEM=3.14

Appendix 18: Analysis of Variance table of Nitrogen balance (g N/100kgd-1)

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	2693.56	897.853	167.89	<0.001
Residual	68	363.646	5.348		
Total	71	3057.206			

SEM=0.545

Appendix 19: Analysis of Variance table of % Nitrogen Efficiency

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	5675.68	1891.89	23.97	<0.001
Residual	68	5367.61	78.94		
Total	71	11043.30			

SEM=2.094

Appendix 20: Analysis of Variance table of Nitrogen balance as % of N intake

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Treatment	3	0.96120	0.32040	26.94	<0.001
Residual	68	0.80860	0.01189		
Total	71	1.76980			

SEM=0.026