EFFECTS OF FEEDING SYSTEMS ON ENVIRONMENTAL NITROGEN CONTAMINATION BY BORAN AND FRIESIAN CATTLE

BY

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A thesis submitted in partial fulfilment of the requirements for degree of Master of Science in Livestock Production Systems, University of Nairobi.

DECLARATION

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

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DEDICATION

This work is dedicated to my lovely mum Esther Sitienei, to my little nephews: Caleb and Dennis, and niece: Joy.

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LIST OF ABBREVIATIONS AND ACRONYMNS

AAs	Amino acids
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
AP	Absorbed purines
BW	Body weight
CCAFS	Climate Change, Agriculture and food security
CH_4	Methane
CO_2	Carbon IV oxide
CP	Crude protein
d	Day
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
FAO	Food and Agricultural Organization of the United Nations
FHP	Fasting heat production
g	Gramme
GIT	Gastrointestinal tract
GHG	Greenhouse gas
Gt	Gigatonne
H_2SO_4	Sulphuric acid
hr.	Hour

ID	Identification
ILRI	International Livestock Research Institute
IPCC	Intergovernmental panel on climate change
LSD	Least Significant Difference
LW	Live weight
MCP	Microbial crude protein
MDp	Mean degree of polymerization
mL	Millilitre
Mmol	Millimoles
MNF	Microbial Nitrogen Flow
N	Nitrogen
N_2O	Nitrous oxide
NO_3^-	Nitrate
NDF	Neutral detergent fibre
NH_3	Ammonia
NH_4	Ammonium
OM	Organic matter
PD	Purine derivatives
RAN	Ruminally available nitrogen
RDP	Ruminally degradable protein
SD	Standard deviation
SEM	Standard error mean

VFA	Volatile fatty acids
yr	Year

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ABSTRACT

Ruminants have long been known to be less efficient in utilizing nitrogen (N) when fed on high protein diets and decreasing these dietary levels supplied has been proposed as one way of significantly improving N economy in cattle. However ruminants in tropical, developing economies are typically fed diets that are generally low in protein especially during the dry season. A study was conducted using two breeds of yearling steers namely Boran and Friesian (n=12; LW: 161.8 ±10.89 kg) in a duplicated 3×3 Latin square to evaluate the effects of protein supplementation and supplementation frequency on DM intake, digestibility, nitrogen retention and microbial nitrogen supply in cattle consuming low protein basal diets. The steers were maintained on *ad libitum* chaffed wheat straw (DM: 877.3 ± 5.2 g/kg CP: 20.0±1.10 g/kg), with supplemental protein supplied as air dried *Calliandra calothyrsus* leaves (DM: 897.3 ± 3.06 g/kg, CP: 257.5± 4.04 g/kg on DM basis). The treatments were: basal diet alone, *Calliandra* supplemented every day at 0.2% LW kg and *Calliandra* supplemented every other day at 0.4% LW. Animals were fed once every day at 0930 hr with the supplement provided in separate buckets. Samples of basal diet, supplement, refusals, faecal matter and urine were collected and analyzed.

Supplementation increased DMI of the steers by 16.4 % and 20.0% for daily and alternate day supplementation respectively, but only crude protein apparent digestibility increased with supplementation Microbial nitrogen flow also increased with supplementation ($P<0.001$) but also daily supplementation had significantly higher ($P<0.001$) flow than alternate day supplementation (2.24 vs 0.18 g N/100kg.d). Steers lost body weight on all treatments but significantly ($P<0.05$) less when supplemented. Increased nitrogen retention ($P<0.001$) was observed with supplementation compared to control (-33.3% vs 15.7%, SEM 0.055). The increased N balance in animals receiving

supplemented diets indicates that N retention improves with increased protein supplementation in animals fed low-protein basal diets, implying that improving protein supply to animals fed sub-maintenance diets will not only ameriolate production losses in cattle, but also decrease environmental N losses.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Ruminants have long been established as being less efficient in utilizing high quality sources of nitrogen (N) compared to monogastrics (Leng and Nolan, 1984), making them a major cause of environmental N pollution. Low efficiency is associated with the pre-gastric losses during protein degradation and microbial protein synthesis in the rumen, resulting in low nutrient use efficiencies and increased exogenous nitrogen losses in their manure (Calsamiglia *et al.*, 2010). More than 60% of N ingested by cattle is excreted to the environment mainly through manure (both dung and urine) with this proportion increasing with increasing dietary N. Nitrogen metabolites (including nitrates, nitrites, nitrous oxide (N_2O) and ammonia (NH_3)) are sources of environmental contamination, and represent an important loss of nutrient from production systems.

Cattle, especially under intensive production systems, have been fed on diets with crude protein (CP) levels above 16% which are higher than their metabolic requirements (NRC, 2000) in bid to maximize production. This further increases the amount of N excreted from cattle production, as the amount of N in manure excreted by ruminants is mainly influenced by dietary N intake (Marini *et al.*, 2008). By comparison, cattle in developing countries are generally produced under extensive and semi-intensive systems, and are maintained on low protein basal diets (<5% CP on DM basis) such as crop residues, consequently experience low productivity because of low total DM intake (DMI) and digestibility (Koster *et al*., 1996). Such animals on a low protein diet have been shown to have improved nitrogen use efficiency; this being attributed to urea salvage mechanism in the gut (Reynolds and Kristensen, 2008b). Adoption of optimal protein feeding strategies in ruminants that would improve DM intake and digestibility while reducing environmental N contamination from

livestock production and its related activities will not only improve animal productivity in the tropics but also reduce environmental degradation from greenhouse gas (GHG) emissions.

Precision feeding, improving the efficiency of capture or recycled nitrogen (N) in microbial protein (MP) synthesis, and improving the efficiency of utilization of absorbed amino acids have also been explored in ruminant nutrition for their potential to reduce N losses to the environment and hence N_2O emissions (Marini *et al.*, 2004, Waghorn, 2008, Wickersham *et al.*, 2008a, Peripolli *et al.*, 2011).

Oscillating dietary protein level in ruminants' diets has been shown to improve DM intake and digestibility, and also enhance nitrogen retention (Beaty *et al.*, 1994, Currier *et al.*, 2004, Farmer *et al.*, 2004, Wickersham *et al.*, 2008a, McGuire *et al.*, 2013). This strategy thus carries the potential to reduce environmental N contamination while maintaining or even improving cattle productivity (Montes *et al.*, 2013).

This study was conducted to evaluate the effects of supplementation and supplementation frequency on nitrogen use efficiency in Friesian and Boran steers fed on a low protein basal diet. Development of feeding strategies that would lead to better utilisation of low quality tropical pastures and crop residues such as cereal straws and stovers by ruminants in Sub-Saharan Africa (SSA) would improve livestock production in the region as well as reduce associated N_2O and CH_4 emissions. In this study, we hypothesize that supplementation of cereal crop residues; feed resource often used to mediate pasture shortages by small and medium holder farmers in the tropics (Otte and Chilonda, 2002) with leguminous forage- *Calliandra calothyrsus* will improve the low quality basal diet utilization in cattle.

1.2 Objectives

Broad objective

To evaluate the effects of protein supplementation and supplementation frequency on nitrogen use efficiency in Boran and Friesian steers fed low quality basal diet.

Specific objectives

1. To determine the effects of daily and bi-diurnal protein supplementation on voluntary DM intake and digestibility in Boran and Friesian steers fed on a low protein diet.
2. To estimate the levels of nitrogen retention in Friesian and Boran steers under daily (Daily) and bi-diurnal (Bi-d) protein supplementation.
3. To determine the effect of protein supplementation and supplementation frequency on post ruminal microbial Nitrogen flow in Friesian and Boran steers.

1.3 Hypotheses

1. Daily or Bi-d protein supplementation does not have a significant effect on DM intake and DM digestibility in Friesian and Boran steers.

2. Supplementation and supplementation frequency has no significant effect on N retention on Friesian and Boran steers.

3. Microbial nitrogen outflow from the rumen is not affected by protein supplementation or supplementation frequency in Friesian and Boran steers fed a basal diet of cereal straws.

1.4. Problem statement

Cattle and ruminants in general are thought to have evolved to be efficient in assimilating dietary nitrogen when consuming low protein basal diets (Leng and Nolan, 1984). When put on low quality diet, increased recycling of urea back to the rumen and the higher proportion of ammonia (NH_3)-N capture by microbes result in net improved N use efficiency (Reynolds and Kristensen, 2008a). With rate of NH_3 capture and rate of carbohydrate fermentation in the rumen influencing backflow of urea back to the rumen, enhancing these two mechanisms could enhance recycling of urea hence improved N retention and reduced N excretion in ruminants. However, available information on digestibility of tropical pastures and response to protein supplementation in different breeds of cattle kept in the tropics especially during the dry season is limited. These animals during such dry seasons have access to limited amount of feed which is also of low quality (high NDF and low protein) and therefore digestion dynamics is expected to be altered as described in other regions. Additional information therefore gained on enhancing high fibre basal diet utilization and N recycling to the rumen will help animal nutritionists in improving nitrogen use as well as reducing nitrogen excretion from ruminants in the tropics.

Dietary factors are the main determinants of nutrient use efficiency in ruminants (Vercoe *et al.*, 1972) and studies into feeding strategies that will achieve optimal N retention are needed. This will lead to sustainable animal production moving into the future, with the anticipated rise in demand for protein of animal origin. Evaluation of nitrogen use efficiency also among cattle breeds used in different production systems (both *Bos indicus* and *Bos taurus)* in the tropics is equally important. This will better inform breeding programmes and decision making in selecting genotypes that would

be most efficient under the prevailing climatic conditions in the diverse agro-ecological zones in tropics.

CHAPTER 2: LITERATURE REVIEW

2.1 Ruminant GHG emissions

Livestock production is one of the major sources of anthropogenic greenhouse emissions, responsible for up to 18 % of the total global emissions when assessed on the basis of a life cycle analysis (LCA) and responsible mainly for CH_4 and N_2O emissions in the agricultural sector (Steinfeld *et al.*, 2006, Gerber *et al.*, 2013). The sector accounts for about 3.3 Gigatonne (Gt) CO_2-equivalents/yr of anthropogenic CH_4 and about 2.8 $GtCO_2$-equivalents/yr of anthropogenic N_2O, with the total emissions of these two representing about 30% of the agricultural sector GHG emissions (Smith *et al.*, 2007).

Of these emissions, Africa is the third largest source of enteric CH_4 emissions from livestock after Asia (~33%) and Latin America (~23%) with about 14.5% of the global methane emissions from livestock (O'Mara, 2011). These proportions notably correspond with the ruminant numbers in these regions with emissions being mainly linked to cattle populations because of their large numbers and large body size (Herrero *et al.*, 2011). Other species, such and pigs and poultry, also significantly contribute to N_2O emissions through manure deposition (Gerber *et al.*, 2013).

Up to 89% of CH_4 emissions from ruminants are produced in the rumen during fermentative digestion of feed and the remaining portion is produced in the hind gut fermentation and from residual microbial fermentation in faeces excreted (Hook *et al.*, 2010). The methane produced represent energy losses from the feed consumed by ruminants, and this varies between 2-12% of the gross energy intake depending on feed quality, feed intake, feed

composition and processing of feed (Johnson and Johnson, 1995). Higher enteric emissions per unit feed intake are observed in low quality diets with low digestibility (Mc Geough *et al.*, 2010). N_2O losses (discussed below) on the other hand are derived from N in the manure excreted by animals and from fertilizers applied in the crop fields (Oenema *et al.*, 1997).

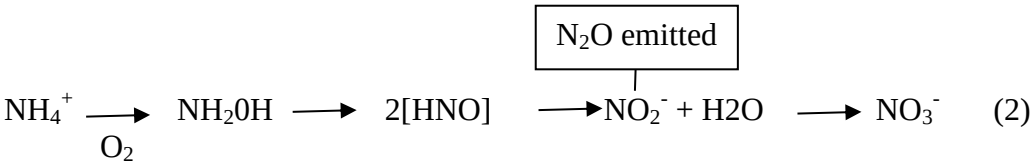
2.1.1 Nitrous oxide

N_2O is a highly potent GHG with a global warming potential of about 296 times that of CO_2 (compared to methane; 28), produced mainly from organic fertilizers used in agriculture and from animal manure deposited in grazing fields (Saggar *et al.*, 2004). Some of the N in these two sources is metabolized in a number of steps once excreted to yield N_2O amongst other metabolites, with urea in urine being the main form of N in ruminants' manure that is readily broken down.

Urea ($CO(NH_2)_2$) is formed in the liver from unutilized rumen ammonia absorbed into the portal circulation. Some of the urea formed is recycled through the saliva back to the rumen for microbial use; the level of recycling depending on dietary protein level and ruminal ammonia concentration (Leng and Nolan, 1984, Leng, 1990). Unrecycled portion is filtered out in the kidneys and excreted to the environment through urine. The excreted urea then undergoes ammonification process releasing ammonium (NH_4^+) ions (see equation 1) once in contact with faeces or with the soil. The process requires urease enzyme which is readily available in the environment from microbial and plant species present in soil and faeces (Saggar *et al.*, 2004).

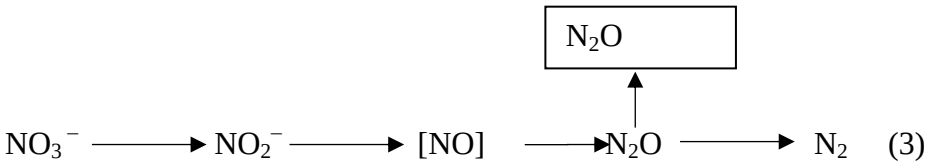
$$CO(NH_2)2 + H^+ + 2H_2O \rightarrow HCO_3^- + 2(NH_4^+) \quad (1)$$

Ammonium ions produced in the above process has two fates in the soil. First, in an alkaline environment created by hydroxyl ions also produced in the ammonification process, is dissociated further into gaseous NH_3, and this is lost to the atmosphere causing air pollution and acidification of the environment (Harrison and Webb, 2001). The rate of NH_3 formation is influenced by its concentration in the soil with high concentration, low temperature and low pH inhibiting formation (Oenema *et al.,* 1997). NH_3 concentration in the soil is a factor of rate of volatilization from the soil, with loose soil allowing higher volatilization rates. Up to 46% of urinary N may be lost in this form (ammonia) from the soil with high pH during hot seasons (Saggar *et al.,* 2004). NH_4^+ that is not converted to ammonia undergoes microbial nitrification and denitrification processes yielding N_2O. Nitrification is an aerobic process which involve oxidation of NH_4^+ to nitrate (NO_3^-), with N_2O as a by-product (see equation 2 below).



(2)

Equally, nitrate reduction (denitrification), an anaerobic process that reduces NO_3 to N_2, with N_2O as an obligatory intermediate (eqn. 3) (de Klein and Eckard, 2008) occur; when oxygen tension in the soil is reduced. This process, however, requires nitrification process (NO_3 production) to have taken place even though this actually represent the major process of N_2O production from manure (Saggar *et al.,* 2004).



(3)

Most of the N_2O formed in the above process escapes from the soil to the atmosphere.

N_2O emissions from cattle manure in the tropics and subtropical pastureland occurs on average, within 7 weeks after deposition and is seasonally affected by prevailing climatic factors; mainly temperature and rainfall- influencing soil moisture content (Saggar *et al.*, 2004, Luo *et al.*, 2008, Sordi *et al.*, 2014). The volume of urine voided per event also affects the level of nitrous oxide emitted, with emissions being lower with higher urine volumes (Sordi *et al.,* 2014). This is probably so because more urea is percolated to the deeper soils and less is available for nitrous oxide production in the top soil where conditions are conducive for formation for nitrification process (Sordi *et al.*, 2014).

Emissions are higher in urine than in the dung, and this has been related to the amount and form of nitrogen present in these excretory products (Marini and Van Amburgh, 2005, Sordi *et al.*, 2014). Urine N is largely in the form of urea which is readily broken down in the soil, unlike faecal N which is in organic form and is not readily available for hydrolysis (Sordi *et al.*, 2014). This also explains the delayed and prolonged N_2O and NH_3 emissions from dung with N_2O peak reported at 3-4 days after deposition, compared to urine with peaks reported within 24 hours of urination and up to 90% losses of N within 48 hours of excretion (Saggar *et al.*, 2004).

2.2 Mitigation options for GHG emissions from livestock

In absence of mitigation measures, agricultural GHG emissions are projected to rise from about 5.6 billion tonnes of CO_2 equivalents (in 2005) to about 8.2 billion tonnes by 2030 (Smith *et al.*, 2007), with most of this increase expected to occur in Africa and Asia. Ruminants in these regions are maintained on low quality feed resources resulting in higher methane emissions per unit of animal product produced (Hook *et al.*, 2010). Therefore, strategies geared towards improving utilization of such diets as well as improving animal productivity offer the greatest opportunity in reducing GHG emissions from livestock (Eckard *et al.*, 2010). Further, improved feeding strategies and better handling of manure offer potential avenues also in reducing N_2O emissions; with manure emissions representing an important source of N_2O (Saggar *et al.*, 2004, Gerber *et al.*, 2013).

Several technologies in mitigation of GHG emissions from livestock have been developed over time yielding varying outcomes. It has been suggested that full adoption and of these existing technologies on GHG emissions from livestock as well as enactment of incentives and regulations in the industry could substantially reduce projected emissions by 2050, from about 8.2 Gt. CO_2 equivalents/yr. to about 6.2 Gt/yr. despite projected increase in livestock numbers (Oenema *et al.*, 2007). Some of practical mitigation options in the tropical context, studied over time are discussed briefly below.

2.2.1 Methane inhibition

With up to 87% of methane emissions from ruminants occurring in the rumen during fermentation process, most of the mitigation technologies studied over time have revolved around reducing this source. Some of these options which hold some positive prospects in

reducing methane emissions from ruminants in the tropics are summarized below (Table 2-1).

Improved pasture management practices e.g. grazing pasture at optimal stage when N: energy ratio is close to animal requirements and also when fibre content is still low, have been suggested to reduce N losses compared to when animals are grazed on lush pastures when N is in excess of animal requirements hence reduction in N excreted to the environment (Eckard *et al*., 2010, Montes *et al*., 2013). Reduced cell wall content at this stage in pastures reduces CH_4 production during digestion in the rumen (Eckard *et al.*, 2010). Inclusion of tannin rich forage in ruminants' diets have been suggested to reduce CH_4 emissions compared to when animal fed predominantly in grass (McCaughey *et al.*, 1999; Waghorn *et al.*, 2002) by reducing fibre digestion in the rumen, even though this has not always been observed (Van Dorland *et al.*, 2007). This may, however, reduce whole tract digestibility of diet by ruminants because rumen is the main site of fibre digestion with limited activity in the lower digestive tract. Also, CT tend to bind enzymes in the digestive tract reducing their efficacy (Van Dorland *et al.,* 200

Table 2-1: Some of the tested possible interventions to reduce enteric methane production in ruminants

Method	Remarks
Dietary lipids[1, 2,3]	Reduce emissions by 20-50% but expensive especially to small scale farmers and has the potential reduce in fibre digestibility
Electron receptors- nitrates, fumaric acid, malic acids [4,5,6]	Desirable where diet CP is low. Nitrite toxicity and high cost may be prohibitive
Ionophores – monensin and antibiotics [7,8,9]	Moderate CH_4 mitigation through feed effiency improvement though the effect is short-lived. Banned in EU.
Plant phytochemicals e.g. tannins and saponins [10,11]	Inconsistent results with up to 20% increases recorded and risk of impairing rumen function and animal productivity.
Defaunation [12,13]	Variable results with negative impact on digestibility.
Halogenated methane analogues e.g. bromochloromethane and chloroform [14,15]	Potent CH_4 inhibitor but has transitory effectiveness and has gain public biasness.
Vaccination –against methanogen strains e.g. *Methanobrevibacter ruminantium* [16, 17]	Still under development with some positive results observed.
Animal selection for improved feed efficiency [18, 19, 20]	Greatest reductions achieved with low digestible diets.
Improving animal productivity [21]	CH_4 emissions go up slightly, but CH_4 emissions per unit of product decrease.

[1.] Dong et al., (1997a); [2.] Machmuller and Kreuzer (1999); [3.] Dohme et al., (2000); [4.] Sar et al., (2004); [5.] Nolan et al., (2010); [6.] Hushof et al., (2012); [7.] Benz and Johnson, (1982); [8] Garrett, 1982; [9.] Rumpler et al., (1986) [10.] Waghorn et al., (2002), [11.] Carulla et al., (2005) [12.] ; Van Nevel and Demeyer, (1996); [13.] Bird et al, (2008). [14.] Bauchopt, (1967), [15]. McCrabb et al, (1997). [16.] Wright et al., (2004); [17.] Williams et al., (2009). [18.] Okine et al., (2001); [19.] Hegarty et al., (2007); [20.] Johnson et al., (1996).

In the SSA where most ruminants in the region generally have low productivity and the diets fed are low digestibility mainly due to high fibre in them, methane emissions can be significant reduced by widely promoting animal selection for improved efficiency and

generally improving animal productivity at farm level. Use of anaerobic digesters in Kenya has been promoted but its adoption has been low.

2.2.2 Manure management

Mitigation practises in reducing GHG emissions from manure have mainly been applied in intensive systems where manure is concentrated within a small area unlike in extensive systems where it is spread in the grazing fields (Guarino *et al.*, 2006). Application of these practises in extensive systems which is the case in most tropical countries may not be practical. Some of the interventions which have been used include; use of semi permeable covers to reduce CH_4 emissions from heaped manure (Guarino *et al.*, 2006) and impermeable covers e.g. oil layers or sealed plastic covers. These methods have however, been found to increase N_2O emissions (Hansen *et al.*, 2009) by creating anaerobic conditions suitable for N_2O production. Composting (Amon *et al.*, 2001) and continuous or intermittent aeration of manure equally has been shown to reduce CH_4 emission although these have been reported to increase CO_2 emissions (Loyon *et al.*, 2007, Peterson and Sommer, 2011). Use of anaerobic digesters also, through harvesting of CH_4 from manure and utilizing it as a source of renewable energy in the form of biogas has reduced emissions and has shown potential to reduce GHG emissions associated with livestock production (Clemens *et al.*, 2006). Proper mechanisms however have to be put in place to ensure that the surplus biogas generated is stored rather than being released to the environment.

Conclusion

Livestock and livestock related activities contribute significantly to GHG emission in the agricultural sector. Mitigation measures including improving nutrient utilization by ruminants and manure management can significantly reduce CH_4 and N_2O emissions from livestock production systems without compromising productivity.

2.3 Feeds and feeding in the tropics

Most ruminants under extensive systems in the tropics, are maintained mainly on low-quality feed (< 7% CP, <55% digestibility and <100g/kg soluble sugars) for the better part of the production cycle as this is the only feed available (Preston and Leng, 1987). This diet consists of dry tropical C4 grasses and crop residues that are characterized by low protein content, high in cell wall content (>75%), low digestibility and often is insufficient in quantity (Paterson *et al*., 1998). During the dry season especially, in the arid and semi-arid zones in African tropics, CP is often the first limiting factor in ruminants' productivity because of the low quality diet (Poppi and McLennan, 1995). Due to the low digestibility (and hence longer retention time), such animals have low DMI and therefore cannot meet their maintenance nutrient requirements, resulting in weight loss during the dry season (Allen, 1996).

Offsetting this N deficiency in the diet through protein N supplementation to ruminants consuming low quality diet has been shown to increase digestibility and DMI (Del Curto *et al.*, 1990; Scott and Hibberd, 1990; Bohnert *et al.*, 2002). This improved utilization

offers an opportunity to improve animal productivity and reduce nutrient losses in the manure excreted to the environment. Protein N supplementation provides ruminally available nitrogen that it is broken down in rumen, yielding NH_3 required for microbial metabolism and multiplication therefore enhancing their growth and activity. The increased microbial population hastens fibre fermentation process in the rumen, reducing feed retention time and increasing DMI (Leng, 1990). As a result, VFA and microbial crude protein (MCP) supply to the animal is also improved, enhancing animal performance.

Conventional protein concentrates such as fish meal provide a good source of protein supplement for livestock but their high cost limits their use especially, by small scale farmers in developing countries (Paterson *et al.*, 1998). Alternative protein sources, including agro-industrial by-products from human food processing, and leguminous trees and shrubs provide a cheaper protein sources for animals.

2.3.1 Agro- industrial by products

Agro-industrial by products form an important source of protein for ruminant because of their relatively low cost compared to commercial concentrate (Sindhu *et al.*, 2002). Their use as animal feed, however, is limited by anti-nutritive compounds present in some of these products, negatively affecting their nutritional value. Ruminants however, have the inherent capacity to tolerate some of these compounds at higher concentrations than non- ruminants due to their ability to digest or inactivate them in the rumen (Du Toit *et al.*, 1991) with little or no negative effect in their productivity. Some of the agro- industrial by-products commonly used as protein sources in livestock feeds are presented in (Table 2-2) together with their approximate CP content, anti-nutritive factors and their treatment.

Table 2-2: Commonly used agro-industrial and other selected protein sources in livestock, their CP content, anti-nutritive factors and possible mitigation measures

Agro industrial By-products	% CP	Negative factor	Treatment
Cotton seed cake[1]	26- 48	Gossypol	Add iron salts
Palm kernel meal[2]	20.7	AA imbalance	AA supplementation
Groundnut meal[1]	24.3	Aflatoxin	Treat with NH_3 or NH_4OH
Urea- molasses diet and biuret[3]	29.2	Urea toxicity	Include sulphur in the formulation and gradually introduce the animals to them
Fish meal[1]	55- 76	Thiaminase in raw fish	Heat
Bone meal[4]	7	Pathogens, BSE	Heat treatment
Blood meal[1]	Up to 80	Pathogens, BSE	Heat treatment
Poultry manure[5]	3.4- 21.5	Pathogens and low palatability	
Soybean meal[6]	43.8	Trypsin inhibitor	Moist heating
Brewery by products[7]	15-30	Aflatoxin	Treat with NH_3 or NH_4OH

[1]Verma, (1997), [2] Onwudike, (1986)[3] Toppo et al., (1997), [4] Loerch et al., (1983), [5], Okanović et al., (2009), [6] Carlen and lansfors, (2002), [7] Nguyen, (2005).
BSE: Bovine spongiform encephalopathy.

Urea which had been widely used as a fertilizer has also been used in animal nutrition to improve crop residue utilisation by ruminants (Sarnklong *et al*., 2010). Urea can be obtained easily in many developing countries making it a suitable component in this. In addition, urea is considerably cheaper than NaOH or NH_3. Vadiveloo (2003) reported that rice varieties with a low degradability responded better to urea treatments than higher quality straw, increasing the in vitro dry matter degradability from 45 to 55-62%. Urea treatment may therefore be most suitable for small-scale farmers to improve the quality

of overgrown crop residues as it also increase N content in the diet which can be utilized by the microbes in the rumen.

2.3.2 Fodder Trees and Shrubs

Fodder trees and shrubs form an important source of livestock feed especially in small and medium holder systems (Devendra, 1989), providing a cheaper protein source in animals' diet. In the tropics, these feed resources also form an effective insurance against seasonal feed shortages because of their tolerance to dry seasons, attributed to their deep rooting system and longer life spans compared to grasses (Abel *et al.*, 1997).

Fodder trees, majority being perennial plants, are not susceptible to sudden climatic changes and continue to produce high quality fodder even during drought years when grasses and other annual forages are dry degrading their nutritive value (Giller, 2001). Their relatively fast growth rate and sustained productivity in the dry spell, enable them to produce large quantities of biomass, which can be used not only for animal feeding but also as mulch in cropping systems and control soil erosion through their rooting system (Devendra *et al.*, 2001). Leguminous species have been used to improve the productivity of rangelands by increasing the amount of nitrogen available for uptake by associated grasses (Giller, 2001).

Because of the above attributes, fodder trees and shrubs play an important role in sustaining livelihoods for small and medium scale farmers in the tropics, contributing to economic and environmental sustainability (Peters and Lascano, 2003). Their use holds the prospect of improving ruminant productivity in small and medium scale farms in the tropics that cannot viably use commercial concentrates in feeding their animals. Nutritive value of some of the

common fodder trees and shrubs that have been used in the tropics are summarized below (Table 2-3).

Table 2-3: Chemical constituents of some common fodder trees and shrub species in the tropics

Fodder trees/shrubs	Dry matter (% DM)	Crude protein (% DM)	Crude fibre (% DM)	Metabolizable energy (Kcal/kg DM)
Acacia tortilis	29.0	15.1	22.6	8.4
Manihot esculenta Crantz(cassava)	21.1	24.2	15.6	14.4
Calliandra calothyrsus	26.4	24.0	21.7	12.6
Erythrina fusca	32.0	25.8	17.4	14.3
Opuntia Ficus	17.0	14.0	22.4	12.0
Gliricidia sepium	25.0	14.7	19.9	12.8
Leucaena leucocephala	30.0	22.2	19.6	12.1
Pigeon peas (Cajanus cajan)	25.2	22.8	20.1	13.4
Prosopis spps	23.4	14.0	17.8	11.2
Sesbania sesban	18.0	22.6	18.4	13.6
Tamarind indica	28.0	14.0	21.0	14.4

Source: Devendra, (1992).

2.3.2.1 *Calliandra calothyrsus*

Calliandra calothyrsus is a leguminous shrub, producing fodder high in CP (20-30% DM), native to South America but has spread worldwide (Paterson *et al.*, 1999). The fodder tree has been widely promoted in the East African region and, consequently, is currently the most

preferred and highly adopted fodder tree among small scale farmers in the sub-Saharan countries (Wambugu *et al.,* 2011).

Calliandra is rich in tannins, mainly deposited in the leaves with concentrations and activity varying widely amongst different provenances (Ahn *et al.,* 1997) and this has been shown to affect the nutritive value of the fodder produced. DMI and protein digestibility are influenced by condensed tannins (CT) and other phenolic compounds present in the leaves (Rakhmani *et al.*, 2005). Moderate concentrations (2-4%) are thought to have nutritional advantages while high (> 5%) is thought to have deleterious effects (Hervás *et al.*, 2003). Nutritional advantages associated with CT reported in ruminants include increased bypass protein, reduce methane produced per feed intake, bloat suppression and anthelmintic effect (Barry and McNabb, 1999, Makkar, 2003, Min *et al.*, 2003). On-farm experiments in Kenya using calliandra fodder (Paterson *et al.*, 1999, Tuwei *et al.*, 2003) have indicated great potential for the tree as a supplement for dairy cattle, with field animal trials showing it as a potential substitute for commercial dairy concentrates. The authors reported increased milk production of up to 0.6 kg for every 3 kg of fresh Calliandra fed as a supplement.

Many small holder farmers in the tropics using *Calliandra Calothyrsus* practice the cut and carry system, where plant leaf material is cut and offered to penned livestock often in mixture with other forages such as crop residues (Tuwei *et al.*, 2003). Varying outcomes have been observed on its nutritive value as animal feed. Palmer and Schlink, (1992), feeding Calliandra as a sole feedstuff to merino wethers, observed a decreased in voluntary DMI of from 59 to 37 g/kg $LW^{0.75}$.d and also a decline in *in sacco* protein digestibility ($P<0.05$) when fresh Calliandra was fed compared to wilted.

The decrease in intake could be associated with the observed decline in in-sacco digestibility which could prolong retention time in the rumen (Ahn *et al.*, 1990, Palmer and Schlink, 1992) exerting rumen fill in the animals. Reduction in palatability caused by both astringency (bitter sensation) and tannin interaction with salivary muco-protein with increased tannin concentrations during wilting could also have caused the observed drop in intake when provided to ruminants as a sole diet (McLeod, 1974, Frutos *et al.*, 2004).

For protein, tannin binding to soluble proteins during wilting and chewing in the mouth could have made them unavailable for rumen digestion but released in the abomasum because of the low pH (~3) and in the small intestine; above 8 which is favourable for protein-CT complex dissociation. The apparent digestibility of Calliandra could be higher than that reported for in-sacco (60 and 30%) as this method captures only digestion in the rumen. Experimental findings have shown a decrease in ruminal protein degradability and increased total protein availability for absorption in the lower tract of ruminants (Barry and McNabb, 1999; Min *et al*., 2003).

When fed as a supplement on the other hand, drying actually appears to increase dry matter intake, increase DM digestibility and improves nitrogen retention in the animal. Ahn *et al.*, (1997) supplementing both dried and fresh (frozen) Calliandra to sheep fed on barley straw *ad libitum*, observed a significant increase in DMI and DM digestibility (DMD) digestibility (from 40 to 60%) when dried (at 60^0C) *Calliandra calothyrsus* leaves were fed. The concentrations of CT in the fresh and oven-dried Calliandra used in the experiment were 11.7 and 8.22% respectively, indicating that some CT might have been lost from the forage during drying. Tuwei *et al.*, (2003) though did not observe increased DMI when wilted

Calliandra was supplemented to lambs fed on maize stovers, but observed significant live weight gain suggesting improved digestibility with wilting. The destruction of tannins and possibly other toxic factors during drying of Calliandra may explain the significant improvement in the post-ruminal digestibility of protein following drying (Ahn *et al.*, 1997).

Conclusion

Most ruminants in the tropics are maintained on low quality diets (<7% CP) consisting mainly of dry crop residues and tropical grasses. These feed resources are characterized by low digestibility and are nutrient deficient especially, protein to sustain optimal productivity. Use of fodder trees to improve quality of such diets and their utilization by ruminants has been practised widely. *Calliandra calothyrsus* is the most popular fodder tree amongst farmers in East Africa (Paterson *et al.*, 1996) and its use has shown improved animal productivity when used as a supplement. Adverse effects associated with high tannin concentration in the plant have however been reported especially when fed fresh as a sole diet in some instances but measures to ameliorate the situation such as drying are also available.

2.4 Protein metabolism in ruminant animals

2.4.1 Requirements for protein in ruminants

Ruminants maintained on pastures alone meet their metabolic amino acid requirements to a large extent from microbial protein synthesis in the rumen, following breakdown of dietary protein to ammonia (NRC, 2000). The degradable portion of dietary crude protein (RDP) consumed by the animal is broken down in the rumen by microbial enzymatic activity

releasing NH_3. Some of the NH_3 released is used for microbial protein synthesis and the remaining portion is absorbed into the portal circulation from which virtually all that is absorbed is removed by the liver producing urea through the ornithine cycle (Reynolds, 1995). The absorption into the portal circulation is concentration dependent and this increases as dietary N increases (Reynolds and Kristen, 2008b).

Some of the urea produced by the liver is recycled back to the gut through either direct transfer from blood across the epithelial tissue or via saliva while the remainder is excreted in urine (Marini *et al*., 2008). Once in the rumen, the urea is degraded to NH_3 through the action of microbial urease, and this NH_3 can be recycled for microbial protein synthesis or absorbed back to portal circulation (Leng and Nolan, 1984). This recycling of N via NH_3 and urea transfers provides a source of N for microbial protein synthesis when N from the diet is insufficient especially in ruminants subsisting on crop residues and standing hay (Reynolds and Kristensen, 2008b).

Microbial protein source alone even when ruminal conditions are optimal however, may not supply adequate amounts of amino acids to meet metabolic and production requirements in growing and lactating animals (Clark *et al.*, 1992). Additional true protein source reaching the abomasum, in the form of bypass protein has to be supplied in the diet of these animals, so as to achieve desired levels of production (NRC, 2000). Mature, non-lactating animals on the other hand, can rely wholly for their amino acid requirements from microbial supply for maintenance (Clark *et al.*, 1992).

Several factors affect individual animal dietary CP requirements. Variations in body weight gain composition which is of a factor of breed, age and sex classes affect protein requirements in growing and finishing animals (Leng and Nolan, 1984, Paulino *et al.*, 2009). Non-castrated males tend to deposit more lean tissue in their body than castrated or female animals of the same age, therefore have higher dietary protein requirements (Paulino *et al.*, 2009).

When ruminants are maintained on low CP diet (below 7%) that cannot supply ammonia in concentrations required in the rumen for optimal microbial activity, animals tend to mobilize their tissue protein into circulation and transported to the liver in form of AA where they are broken down to urea then released in to portal circulation (Leng and Nolan, 1984). This is then absorbed across the ruminal wall or secreted in the saliva and swallowed to the rumen where it is broken down to NH_3 which can then be utilized by microbes for their metabolism (Wickersham *et al.*, 2004a). If this low dietary CP level persists for an extended period of time, animals lose weight (due to adipose and muscle tissues catabolism) and eventually succumb when they can no longer mobilize any more tissues (Grimaud *et al.*, 1999).

Cattle in most of the tropical countries are maintained on dry pastures and crop residues, especially during the dry season, that are generally low in protein content (<5% CP) and high in cell wall content (Lukuyu *et al.*, 2009, Cecchi *et al.*, 2010). This CP level is generally too low to induce optimal microbial activity in the rumen. Consequently, animals experience sub maintenance DM intake and animals mobilize their body tissues to meet their metabolic requirements (Grimaud *et al.*, 1999, Mathis *et al.*, 1999). This results in weight loss during the dry season and growing animals only experience weight gain during the wet part of the

year when the pastures are adequate and still nutritious enough (Steinfeld *et al.*, 2006, Kosgey *et al.*, 2008).

2.4.2 Nitrogen use efficiency in cattle

Ruminants generally have been known to have low N use efficiency with an average factor of about 0.25 (expressed as g N in product/g N intake) (Kohn *et al.*, 2005). Efficiency factors ranging between 0.15-0.4 have however been observed among different feeds and feeding practices (Calsamiglia *et al.*, 2010) indicating that feeds and feeding practises have a major influence on N use efficiency in ruminants. Lowest N efficiencies in this range have been observed in grazing beef animals with highest efficiencies reported in lactating dairy cows because dietary N is more efficiently used in the body in the synthesis of milk protein than muscle tissues (Mathis *et al*., 1999, Metcalf *et al*., 2008).

These ineffiencies in ruminants have mainly been attributed to NH_3 losses from the rumen into portal circulation during microbial synthesis with up to 60% of dietary N lost through this route (Reynolds and Kristensen, 2008b) with higher losses as dietary N increases and also with non-protein N sources such as urea. Other losses occur in form of microbial nucleic acid N which is not available for host animal use; this accounting for 15-20% of the total microbial N (Chen *et al.*, 1990). Other source of inefficiencies has been described post ruminally resulting from energy and amino acids imbalances in the tissues, with the excess amino acids 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 quality diet (Calsamiglia *et al.*, 2010); with this being associated with

improved urea recycling to the rumen and ammonia capture when ruminants in general are fed on a low protein diet. Animal experiments carried out support this hypothesis where cattle consuming low quality diet have lower N losses as a proportion of N intake compared to losses in animals fed higher dietary protein concentrations (Bunting *et al.*, 1989, Scott and Hibberd, 1990, Lintzenich *et al.*, 1995, Koster *et al.*, 1996, Marini *et al.*, 2004, Atkinson *et al.*, 2007).

Wickersham *et al.*, (2008b) evaluating the effect of Rumen Degradable Proteins (RDP) in urea kinetics in steers fed low quality prairie grass (4.9% CP) observed that 32.3% of microbial N flowing to the abomasum was derived from recycled urea (into the rumen) in the unsupplemented group of animals. This N proportion however, declined to 24.8% when 177g/d of RDP was added in the diet. Similarly, Bailey (2010) feeding hay (5.2% CP) and supplementing with casein (95.3% CP) to Angus steers also observed a decrease in the proportions of N recycled back to the rumen from 20.5 to 17% when the casein supplement was increased from 120 to 240 g/d. The above observations therefore suggest that the proportion of recycled N captured in the rumen decreases as dietary protein was increased, and this could be as a result of reduced recycling capacity of urea back to the rumen as dietary CP is increased.

This hypothesis is supported by Wickersham *et al.*, (2008b) who observed that as the level of supplemented RDP was increased from zero to 177 g/kg, the proportion of recycled N captured for anabolic use reduced from 70.1 to 46.3%. This then indicates that, as dietary protein level is increased, the amount of recycled urea captured decreases and N losses increases. It has been observed that increasing dietary protein level increases NH_3

concentrations in the rumen (Meijer *et al.*, 1990) and this could inhibit reabsorption of urea from the portal blood. This then increases the amount of urea filtered out in the kidneys and excreted in urine hence reduction in N use efficiency (Huntington *et al.*, 1996).

2.4.3 Use of Purine Derivatives (PDs) in determining microbial protein

As with other products of microbial digestion in ruminants, purine derivatives (PD); end-products of microbial nucleic acid digestion (including hypoxanthine, xanthine, uric acid and allantoin), are absorbed into circulation in the small intestine. These are then transported in blood to the liver where they are filtered and excreted from the body, mainly through urine (Chen and Gomes, 1995). Estimation of the total PDs excreted through urine has been used to quantify microbial protein contribution to the host animal. With the knowledge of the ratio of purine N: total N in a mixed population of microbes, this has been calculated with the assumption that all the plant nucleic material are destroyed during fermentative digestion in the rumen (Chen *et al.*, 1990). A mean digestibility of 0.85 in a mixed population of rumen microbes has been generally agreed and is used widely in literature in estimating microbial contribution to true protein reaching the abomasum in ruminants (Chen and Gomes 1995). Total microbial N absorbed is estimated based on the calculated absorbed purine (AP) N, where the proportion of purine N to the total microbial N is estimated at 0.116 (Chen and Gomes, 1995) in a mixed ruminal microbial population.

Species differences exist among domestic ruminants on the concentration of each purine derivative component found in urine. Unlike sheep, cattle have high xanthine oxidase activity in the intestinal mucosa converting nearly all absorbed purines to uric acid therefore making them unavailable for utilization by the host animal in synthesis of nucleic acid (Chen

and Gomes, 1995). Cattle cannot therefore recycle some of the endogenous and exogenous purine in their nucleic acid synthesis in the liver; a capacity which sheep have. This also explains why only uric acid and allantoin would be recovered from cattle urine while sheep will have all the four metabolites.

An estimate of microbial protein contribution in the intestinal protein flow especially on animals fed on low protein diet gives a better measure of its nutritive value as this forms the major source of protein to ruminants fed on such diets (NRC, 2000).

2.5 Improving protein use efficiency in ruminants

Improving ruminants' protein retention can significantly reduce N excretion in the manure (Rotz, 2004). With the ruminants' N use efficiency being directly related to dietary N level, total OM intake and animals' metabolic protein requirements, feed and feeding systems manipulation can significantly improve efficiencies in ruminants (Bunting *et al.*, 1989, Mathis *et al.*, 1999). Two strategies discussed below have the potential to improve N use efficiency in ruminants.

2.5.1 Reducing dietary protein level

The goal in feeding ruminants is to supply the right amount of protein with the proper balance of degradation rates in the rumen so as to provide the appropriate amino acids reaching the lower digestive tract. Most forage and concentrate protein is highly degradable, so problem of meeting the RDP requirement is minimal (Rotz, 2004). Excess protein then is normally fed to meet the minimum RUP requirement, but this leads to the excretion of considerable excess N. Protein requirements in cattle, particularly in lactating cows, can

optimally be met by reducing the proportion of RDP in the diet while increasing RUP (NRC, 2000).

Reynal and Broderick (2005) reduced dietary RDP from 13.2 to 10.6% fed to lactating cows and observed reduced N excretion with no effect on DMI and milk yield. This was probably because of reduction in concentration of ammonia produced in the rumen. In an earlier simulation study, Rotz, (1999b) demonstrated the potential of a whole-farm benefit of improved protein feeding. Compared to using soybean meal as the sole protein source, the use of a feed mix with lower RDP reduced N excretion by 39 kg/cow per year indicating possible improvement in nitrogen use efficiency in cattle by manipulating protein feeding.

2.5.2 Improving Production Efficiency

Steps taken to improve the productivity (growth rate or milk production) of ruminants normally improve feed efficiency (Herd and Arthur, 2009). This has been achieved through genetic selection over time for lower residual feed intake in beef cattle (Herd *et al*., 2004). Consequently, growing animals reach market weights faster, hence more products are obtained per unit of feed spend on maintenance. Although improved production often requires increased feed intake, the net result is lower N intake and excretion per unit of animal product produced (Arthur and Herd, 2008). A simulation that increased milk production by 25% was found to reduce N excretion per unit of milk by 8% (St-Pierre and Thraen, 1999). Reductions in N excretion achieved through improved production efficiency however are generally small, and therefore should be applied hand in hand with other methods so as to achieve significant reduction (Rotz, 2004).

2.5.3 Other strategies

Feed processing (commonly through grinding and pelleting) can equally influence feed intake, digestibility, and the excretion of N (Sarnklong *et al.*, 2010). Grinding to obtain the proper particle size is particularly important in maximizing feed efficiency in livestock by enhancing its digestibility in the rumen, with this shown to reduce DM and N excretion in the faeces by up to 22%. Chemical methods e.g. urea and ammonia treatment has been used in crop residues to enhance fibre digestibility with these also yielding positive results (Vu *et al*., 1999).

Other options that have been shown to improve N use efficiencies in cattle include hormones and various feed additives. Hormones e.g. Bovine somatotropin increased milk production per unit time and thus milking frequency because of increased dietary protein diverted to mammary glands, with increased total milk yield per lactation in well-fed dairy cows (Bauman, 1999). Other forms of steroids including oestrogen and progesterone treatment have been used to enhance growth rate, nitrogen retention and feed conversion efficiency during the 5-6 week period before slaughter in beef cattle (Van der Wal, 1976). This is thought to increase physiological requirements for amino acids for milk production in the mammary glands, therefore increasing partitioning of amino acids to the mammary glands where it is more efficiently utilized.

Conclusion

Dietary protein requirements in cattle vary with the physiological state of the animal which dictates metabolic and production requirements. Their efficiency in using protein however is generally low mainly because of the losses in the rumen in form of ammonia. N use

efficiency tend to improve when dietary level is low, with this associated with improved recycling of urea to the rumen and net increase in NH_3 capture by microbes.

Improved protein use efficiency will therefore reduce N losses from ruminant production as a larger proportion of what is consumed in the diet is captured in the body for production purposes. This then will not only result in reduced environmental N contamination by livestock but also improve ruminants, productivity.

2.6 Effects of protein supplementation on digestibility and feed intake

Cattle consuming low quality forage usually do not obtain adequate RDP in their basal diet to stimulate optimal passage rate, DM intake, and digestion (Hannah *et al.*, 1991). Rumen degradable nitrogen source in ruminants is required for the supply ruminal NH_3-N for microbial growth and multiplication (Satter and Slyter, 1974), therefore enhancing fibre degradation as well as increase the flow of microbial protein to the duodenum. At low dietary levels of protein, microbial activity in the rumen may therefore be diminished because of insufficient supply of NH_3 for microbial growth. Hence, protein or non-protein nitrogen (NPN) supplementation can offset this NH_3 deficiency in the rumen and improve microbial metabolism and fibre digestion (Poppi and McLennan, 1995).

2.6.1 Effects on feed intake

Many animal experiments that have been conducted using several temperate and tropical grasses have shown that DMI increase quadratically as CP provided in the diet is increased (Koster *et al.*, 1996; Mathis *et al.*, 1999, 2000; Wickersham *et al.*, 2008b; Lazzarini *et al.*, 2010). Wickersham *et al.*, (2008b) feeding low quality praire hay (4.9% CP) to steers observed a 29.1% increase in DMI when 59 mg/kg RDP was dosed

ruminally while Lazzarini *et al.*, (2010) observed a 15.2% increase when dietary CP of steers fed on *Brachiaria decumbens* (5.08% CP) was increased to 8.08%. The highest response in intake normally is observed with the first level of supplementation then declines or plateaus with further increase.

The plateauing or slight decrease indicates that the quadratic effect of protein supplementation on intake in ruminants is observed up to an optimal level, beyond which the stimulatory effect on intake starts to decline (Del Curto *et al.*, 1990, Mathis *et al.*, 2000). This restriction to further increase could be explained by animal consuming their optimal intake at this level and therefore ruminal fill and rate of ingesta fermentation and passage from the rumen become limiting factors (Koster *et al.*, 1996).

2.6.2 Effects on apparent digestibility

Protein supplementation also increase total tract apparent digestibility in cattle consuming low protein diets (Koster *et al.*, 1996, Mathis *et al.*, 1999, Archibeque *et al*., 2007b). OM digestibility has been observed to increase quadratically as CP supplement level was increased; as earlier described for intake. In some studies however, significant increase was only observed with the first supplementation level (above the controls) with no further increase on digestion as level CP supplement was increased (Koster *et al.*, 1996, Mathis *et al.*, 1999). This then suggest that protein supplementation levels required to stimulate optimal digestion is lower than the amount required in achieving optimal intake in cattle.

2.7 Effects of sub maintenance intake on DM digestibility in cattle

Above maintenance, as intake is decreased, DM and OM digestibility increases (Galyean and Owens, 1991). This relationship between digestibility and intake is a consequence of

modifications of the extent in ruminal digestion (Michalet-Doreau *et al*., 1997). A decrease in feed intake results in a more efficient mastication, by a longer time spent eating and ruminating per kg feed ingested (Aitchison *et al*., 1986).

As a consequence, ruminal particle size decreases and this increase the surface of attack by microbes resulting in increased fibre digestibility. Studies carried out in ruminants with intake restricted below maintenance however have shown either no variation in digestibility with intake (Grimaud 1995, Atti *et al*., 2002, Singh *et al.,* 2008) or a decrease in cattle (Grimaud *et al*., 1998). No modifications of turnover rates of particles in the fore stomachs were observed or any significant change in microbial activity in the rumen in the above mentioned studies. At very low intake the proportion of endogenous materials in faeces has been shown to increase, especially with low-quality forages which could have an abrasive effect on gut mucosa (Doreau *et al*., 2003). This increases OM in faeces, decreasing in OM digestibility but not fibre digestibility.

Unlike cattle fed above maintenance where decreased intake show increase in digestibility, when fed below maintenance decreased DM intake has been shown not to have an effect on DM digestibility despite observed increase in particle retention time (Grimaud *et al*., 1998, 1999). Similar observation has also been reported in studies with sheep (Atti *et al*., 2002). It is then possible that, in contrast to results obtained above maintenance, particle retention time in the rumen is not involved in modifications of digestive efficiency when ruminants' intake is below maintenance. It is likely that an increase in ruminal particle retention time does improve DM digestibility probably because at maintenance this retention time is already long enough to optimize microbial cellulolytic activity. Other ruminal and dietary

factors under these conditions other than microbial-fibre contact time are affecting digestibility (Atti *et al*., 2002).

Knowledge on the origin of a disturbance in fibre digestive processes in underfed animals is lacking but disturbance in microbial activity is postulated to cause this limitation (Grimaud *et al*., 1999). Nutritional factors and changes in microbial environment should be considered in further experiments to determine how bacterial activity may be altered by a change in intake.

2.8 Effects of protein supplementation frequency on DMI, digestibility and N balance

2.8.1 Effect on DMI and digestibility

Oscillating dietary protein supplementation in cattle has been shown to equally increase both intake and digestibility as continuous protein supplementation (Beaty *et al.*, 1994, Farmer *et al.*, 2001, Farmer *et al.*, 2004, Wickersham *et al.*, 2008a). Farmer *et al*., (2001) suggested that infrequent protein supplementation, with as low as 2 times in a week, do not significantly ($P>0.05$) affected forage DM intake or digestibility, provided the animals consumed the same level of protein per unit time.

Farmer *et al*., (2004a), supplementing urea at two levels (0.41% LW daily and 0.83% BW on alternate days) to steers fed on prairie hay (5.6% CP) did not observe significant difference on either OM intake, feed utilization or on animal's performance between daily or on alternate day supplementation. Wickersham *et al*., (2008b) equally feeding cattle prairie grass (4.7% CP) and supplementing with casein daily and every third day so as to provide low (61 g RDP/d) and high (183 g DIP/d) observed no effect of frequency on voluntary

DMI. The above observations supports the hypothesis that protein supplementation frequency in ruminants can be reduced without affecting their productivity (Farmer *et al.*, 2001).

2.8.2 Effect on N balance

Animal experiments conducted in both sheep and cattle have indicated higher nitrogen retention with infrequent high CP levels in the diet, compared to continuous supplementation as presented in Table 2-4.

Table 2-4: Effect of oscillating CP supplementation in ruminants on N balance

Author	Species used	Protein* levels oscillated	N balance with oscillated CP g/d	N balance with continuous CP supplementation	P value
Cole, (1999)	Sheep	10 and 15% CP	1.82	0.94	<0.01
Cole *et al.*, (2003)	Sheep	10 and 14 % CP	156.2	139.5	<0.05
Archibeque *et al.*, (2007b)	Cattle	9.1 and 13.9% CP	55.0	34.8 and 40.2	<0.05
Wickersham *et al.*, (2008)	Cattle	61 mg of N/kg/d and 183mg/3d	26.7%	25.9%	0.23

** CP levels indicated were alternated in the diet fed to the animals on 48 hr periods.*

Several hypotheses have been postulated to explain the increase in N retention with dietary CP oscillation. Increased N recycling to the rumen, improved quality of protein entering the small intestine, increased metabolic use of absorbed AA or by combination

of these factors and others (Cole, 1999, Marini and Van Amburgh, 2003, Calsamiglia *et al.*, 2010).

Cole (1999) suggested that the increased urea reabsorption to the rumen when high protein diet is fed infrequently was responsible for the increased N retention. He argues that, CP oscillation created a low NH_3 concentration in the rumen after the first 24 hours of feeding and this increased N reabsorption inform of urea back to the rumen from portal circulation. This is then broken down to ammonia which ruminal microbes can capture for their metabolism. A reduced urea loss in urine was as a result to the reduced urea filtration by the kidneys due preferential absorption in the kidneys, favoured by low ruminal ammonia concentration. This hypothesis is supported by Egan *et al.*, (1986) earlier observations in sheep, where urea reabsorption to the rumen from portal circulation was negatively related to the ammonia concentration in the ruminal content.

Conclusion

Low quality basal diet digestibility and utilization in ruminants can be enhanced by protein supplementation. Further, oscillation of dietary high CP concentration in ruminants could increase nitrogen retention and hence reduce N excretion to the environment. Archibeque *et al.*, (2007b) concluded that, though there is a slight increase in nitrogen retention, the reduction in manure N content is large enough to reduce overall NH_3 and NO_3^- content in manure - these being precursors for N_2O production. However, more experimental studies still need to be carried out to study this phenomenon using tropical feed resources. Most of the studies so far use CP levels above 10%, which does not represent the situation in most parts of the tropical Africa, where ruminants for the better part of the year have access only to dry pastures with little or no supplementation (< 5%). The objective of the current study therefore is to determine the effects of protein

supplementation; either daily or on alternate day on DMI, N retention, and microbial protein supply in Friesian and Boran steers fed on a basal diet of wheat straw.

CHAPTER 3: MATERIAL AND METHODS

3.1 Experimental site

The experiment was carried out at ILRI campus, Nairobi, Kenya. Experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) (no. IACUC-RC2014-05) and animals cared for in accordance with acceptable code of practice for animals in research (Institute of Laboratory Animal Resources (US), 1985).

3.2 Animals and Experimental Design

Friesian (n=6; LW 178.5 ± 24.4 kg) and Boran (n=6; LW 128.4 ± 12.5 kg) yearling steers housed in individual pens (0.7 m×1.4 m) in an enclosed animal unit, with continuous lighting and clean water supplied *ad libitum*, were used in this study. The animals were treated with an anthelmintic and acaricide wash for internal and external parasites control respectively, and ear tagged as part of the standard induction procedure one week before the commencement of the trial.

The experimental design used was a 3×3 Latin square in duplicate, with three feeding regimes (CON, Daily & Bi-d), three periods (Per) and two breeds (Br). Steers were stratified by LW and randomly allocated to three treatment groups; balanced for breed. The groups were maintained throughout the experimental period and were randomly allocated to one of the three experimental diets in the first period. The groups were then rotated in subsequent periods such that by the end of the experimental period the three animal groups had gone through all the dietary treatments. Details of the dietary treatments and period rotations are shown in Table 3-1 below.

Table 3-1: Animal groups (A, B and C)* allocation to dietary treatments (CON, Daily and Bi-d) over the three experimental periods

	Dietary treatments		
	CON	Daily	Bi-d
Period 1	A	B	C
Period 2	C	A	B
Period 2	B	C	A

**A, B, C*- *Animal groups each with 2 Friesian and 2 Boran steers.*

3.3 Diets, feeding and sampling

Experimental diets were as follows: Treatment 1 (CON): chaffed wheat straw hay (DM: 92% CP: 20.0 g/kg CP) provided ad *libitum*, Treatment 2 (Daily): CON plus 20 g/kg LW of air dried *Calliandra calothyrsus* leaves (DM: 897.3± 3.06 g/kg, CP: 257.5± 4.04 g/kg on DM basis) supplied daily and Treatment 3 (Bi-d): CON plus air dried *Calliandra calothyrsus* leaves supplied every other day at 40 g/kg LW.

Animals were weighed using a digital weigh scale (Gallagher Weigh Scale W210, Australia) at trial commencement and every 7d thereafter. The basal diet was offered at 120% of the previous 7d intake and the amount of Calliandra fed was calculated on previous week's LW. Animals were fed once daily at 0930 hr after the removal and weighing of refusals. Chaffed wheat straw was supplied in feeding trough, and the supplement provided in a separate bucket. Treatment periods were 21d with an adaption period of 14d prior to each measurement period. During the last seven days of each period, total urine and faecal collection was undertaken.

After weighing, the refusals were bulked by treatment, mixed by hand and a sub-sample of about 200 g taken and stored in zipped polythene bags at -20°C. Samples of the basal diet and *Calliandra calothyrsus* fed were also taken daily and stored as described for the refusals. At the

end of each period, the daily basal diet, refusals and *Calliandra calothyrsus* samples taken were again bulked by treatment, homogenized and sub-sampled for subsequent processing and analysis.

Total faecal excretion was determined by scooping all the faeces from the floor of the pen of each animal separately at 24 hr interval over 7d periods. Total collection from each animal was then weighed, homogenated and a sub-sample of approximately 500 g (actual weight recorded) transferred into weighed 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 aluminium lids then packed in zipped polythene bags and stored at room temperature waiting further processing and analysis.

Total urine voided over 24-hr periods was also collected using external catheters, attached to the animals using adhesive straps and channelled to a 5-Litre barrel containing 200 mL of 10% HCl with rubber tubing, over a 6d period. Sample of the total urine collected was then taken after total volume was determined volumetrically and diluted to 100 mL with distilled water. Forty (40) ml of the diluted portion was then dispensed in labelled plastic sample bottles and frozen at -20°C awaiting analysis.

3.4 Laboratory analyses

Dried faecal, diet and refusals were ground through 1-mm sieve using a hammer mill (MF10 basic, IKA, Germany).

3.4 Chemical Analysis

3.4.1 DM, OM and ash determination

DM was then determined by drying 2 g of samples in a forced air oven (Genlab Oven, Genlab Ltd, UK.) at 105°C for 24 hr (Association of Official Analytical Chemists- (AOAC), 1990). 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°C for 8 hr and the OM, obtained by subtracting ash weight from DM content.

3.4.2 Total nitrogen

The total N content in feed, faecal and urine samples was determined by micro Kjeldhal procedure (AOAC, 1990) as follows. Approximately 0.3 g of dried samples (the actual weight taken recorded) or 1ml of urine was weighed into digestion tubes in duplicate. Selenium catalyst tablets were then added to each digestion tube and 5 mL of concentrated sulphuric acid was added. Each batch was prepared with two blanks containing only the catalyst and the acid, and two control tubes with plant material of known protein content (Sesbania, 22% CP). The preparations were digested (Tecator 2000 digestion block, Foss. ltd., Australia) at 350 °C for 1.5 hr in a fume hood. Urine was digested for 30 minutes. The samples were then cooled for about 10 minutes, and then 30 ml of distilled water was then added to the tubes. Distillation was then done in automatic distillation units (Kjeltec system 1026 distilling unit, Tecator, Denmark) for 3 minutes.

The distiller was configured to dispense 30 mL of NaOH (35% weight /1ooml (w/v) and an indicator solution (2.5% w/v bromocresol green +2.5% w/v methyl red) into the sample tubes. Ammonia produced was then received in 4% boric acid with the color of the solution changing from pink to green. The receiver solution was then titrated against standardized HCl (0.01N for feed and faecal samples, and 0.001 N for urine) using a burette, and the volume of the acid used

to reach the end point (solution turning back to pink) was recorded. The percentages N in the samples were calculated using the formula:

$$\%N = \frac{1.401(V-B)N}{w \times DM\%}$$

Where,
V= volume of HCL used, B= Blank titration, N= Normality of HCl, W= Weight of sample taken, DM%= Dry matter of sample.

3.4.3 NDF determination

Feed and refusal samples were analysed for NDF by the procedure of Van Soest and Robertson (1985).

3.4.4 Condensed tannin determination

Calliandra samples were analyzed for CT at the Australian Wine Research Institute, Adelaide, Australia using direct phloroglucolysis as described below.

250 mg of ground Calliandra leaf was extracted with 5 mL of 70% acetone-water containing 0.1% ascorbic acid, three times and centrifuged at 10,000 revolutions per minute for 15 minutes at 4°C. The supernatants were combined and the acetone was removed by rotary evaporation at 40°C. The aqueous fraction was washed with 5 mL of dichloromethane, separated by centrifugation, rotary evaporated at 40°C to remove remaining traces of dichloromethane. The solution was then made up to 10 mL with water and then loaded into SepPak® reverse phase C 18 cartridges. The degree of polymerisation was calculated from the ratio of phoroglucinol adduct: monomer after acid degradation in the presence of phoroglucinol.

10 mg of calliandra CT was degraded using Acid- phloroglucinol procedure as described by Koupai-Abyazani *et al.*, (1992), in 200 µl of a solution comprising of 2% (v/v) HCl in ethanol, and 4% pholoroglucinol. Samples were heated at 80ºC for 16 hr then the preparations were dried

under vacuum for 30 minutes and the residues were suspended in 100 µl of water. The mixtures were then extracted three times with 200 µL of ethyl acetate, ethyl acetate fractions combined and dried. The residues were then dissolved in 70% methanol and analysed by HPLC. The concentration of monomeric flavan-3-ols were determined and the net concentrations of CT in the samples were determined by the sum of monomers detected, the average size of the tannins (MDp- mean degree of polymerization) obtained by the molar ratio of terminal subunits to extension subunits.

3.5 Estimation of apparent digestibility and N balance

Total tract DM, OM and CP digestibility were calculated from the average nutrient concentrations in diets fed and faeces excreted over a 7d period. Nitrogen balance calculations were carried out according to Maynard and Loosli, (1969) based on the equation: N animal products = N feed - N excreted.

Modified in this study as;

$$\text{N balance (g/d)} = \text{Intake N (g/d)} - (\text{faecal N (g/d)} + \text{urine N (g/d)}).$$

3.6 Purine derivatives determination

Urine samples were removed from the freezer and thawed overnight. The four PDs; allantoin, uric acid, xanthine and hypoxanthine in the samples were determined by colorimetric method, according to the procedures of Chen and Gomes (1995). Each assay was conducted as described below.

3.6.1 Allantoin analysis

Thawed urine samples were placed on a sonicator for 20 minutes to dissolve any crystals then 100µL was fetched using Gilson pipette and diluted in the ratio 1:40 with distilled water (by adding 3.9 mL). 0.5 mL of diluted samples was then dispensed in duplicates into 10 mL test

tubes. 1.25 mL of distilled water and 0.25 mL of 0.5 M NaOH were added into the tubes and mixed well by vortexing. The tubes were then incubated in a boiling water bath for seven minutes. The samples were then cooled in an ice bath for about five minutes.

0.3 mL of cold 0.5 M HCl and 0.25 mL phenylhydrazine HCl were added into the tubes once the samples had cooled down and vortexed again before incubating in boiling water bath for another seven minutes. Cooled samples were transferred into fume cupboard and 0.84 mL cold concentrated HCl was added then vortexed. Samples were then removed from the fume cupboard.

To the first tube, 0.25 mL potassium ferricyanide was then added and the timer set at 40 minutes, started. The tube was vortexed and placed back to the rack. The reagent was then added to the next tube and vortexed at intervals of 12 second, one after the other, until all the tubes were done. The tubes were then kept on the bench for color development. After 18 minutes, the tubes were again vortexed thoroughly and reading for optical density (OD) started after 20 minutes using spectrophotometer, (UV-150-02, Shimadzu corporation, Japan) at 522 nm at 12 second intervals.

Allantoin concentration in the samples was estimated by plotting a standard curve (concentration (mg/L) against optical density) using allantoin solution with concentrations ranging from 5 to 40 mg/L. The equation of the curve was only for the samples analysed on the same day when it was developed and new standard curve was developed on each day of analysis.

3.6.2 Uric acid

Preparation of urine samples was done as for allantoin by sonicating for 20 minutes then diluted in the ratio 1:40 with distilled water.

1ml of diluted samples, standard solution and distilled water (blank) were dispensed in duplicates into 10 ml test tubes using Gilson pipette. 2.5 mL phosphate buffer was then added to all test

tubes and vortexed. To one set, 150 µL of buffer was added and to the other, 150 µL of 0.12 units/mL uricase enzyme from *Bacillus fastidiosus* (Sigma Aldrich, UK) was added. The two sets were vortexed then incubated in a water bath at 37ºC for 90 minutes. The samples were vortexed again after incubation before reading for OD at 293 nm.

Standard curve was plotted using natural log. For concentrations of the standard solutions against natural log for OD. The equation of the curve derived was then used to estimate the amount of uric acid in the urine samples.

3.6.3 Xanthine and hypoxanthine

Urine samples were prepared and diluted as described for allantoin above (procedure 3.5.2). Two sets of the test tubes were prepared and in to each pair, 1ml of the standard solution (for standard curve) and diluted samples were dispensed. 2.5 mL phosphate buffer was added to all the tubes and vortexed. 0.35 mL L-Histidine was then added and vortexed again.

To one set, 150 µL of buffer was added and to the other, 150 µl of the xanthine oxidase was added and vortexed. The setup was then incubated in water bath at 37ºC for 60 minutes, before reading for absorbance in a spectrophotometer at 293 nm. Standard curve was also plotted using Ln (conc.) against Ln (OD) and the equation of the slope used to estimate the net concentration of xanthine and hypoxanthine in the urine samples. Absorbance of xanthine in the samples was corrected by adding a factor derived from multiplying xanthine standard curve gradient with sample xanthine/hypoxanthine concentration.

3.6.4 Total purine derivatives determination

Total purine derivatives excreted were determined as the sum of allantoin, uric acid, xanthine and hypoxanthine in mmol/day. The amount of purines absorbed by cattle is related to the urinary PDs excreted and were estimated using the equation of Chen and Gomes, (1995).

3.6.5 Microbial nitrogen flow

Equation developed by Verbic *et al.*, (1990) was used to calculate absorbed purines from total excreted purines, by as illustrated below.

$$\mathrm{AP} = \frac{\mathrm{PD} - (0.385 \times \mathrm{LW}^{0.75})}{0.85}$$

Where: AP is the Absorbed Purines (mmol.d); PD the total Purine Derivatives excreted (mmol .d); 0.85 the recovery of absorbed purines as purine derivatives in the urine (mmol $mmol^{-1}$); and 0.385 the endogenous purine derivatives excretion in the urine per unit of metabolic size (mmol).

Intestinal flow of microbial N (g N/d) was calculated from microbial purines absorbed as shown below.

$$\text{Microbial N (gN/d)} = \frac{\mathrm{X(mmol/\,d)} \times 70}{(0.116 \times\ 0.83 \times 1000)}$$

Where; digestibility of microbial purines was assumed to be 0.83, N content in purines is 70mg N/mmol. And the ratio of purine-N: total N in mixed rumen microbes was taken as 11.6:100.

3.7 Statistical analysis

Treatment effects on intake, digestibility, N use efficiency and microbial protein production were analyzed using R 3.0.3 (R core team, 2015). The treatment effects were compared using Analysis of Variance (ANOVA) type 3 using linear mixed model fitted by REML t-tests with Satterthwaite approximations of degree of freedom (LmerTest) (Kuznetsova *et al*., 2014) because the model was not fully orthogonal. A mixed model with breed, period and dietary treatments as

fixed effects and animal ID (identification) as a random variable was fitted on the data; as shown below.

$$\mathrm{Y} = \mathrm{Breed} + \mathrm{period} + \mathrm{dietary\ treatment} + \frac{1}{\mathrm{Animal\ ID}} + e$$

Where; ‘e’ is residual error.

The differences between means were compared by the least square means method and level of significance was determined at $P< 0.05$.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Chemical composition of diet ingredients

The chemical composition of wheat straw and air dried *Calliandra calothyrsus* leaves used in the current experiment are presented in Table 4-1.

Table 4-1: Chemical composition of wheat straw and air dried Calliandra calothyrsus leaves (g/kg DM ±SD) used as the basal diet and supplement respectively

Attributes	Wheat straw (g/kg DM)	Calliandra (g/kg DM)
DM	877.3 ± 68.7	897.3 ± 80.2
OM	936.4 ± 96.4	935.8 ± 103.5
NDF	807.2 ± 72.5	405.4 ± 73.8
CP	20.0 ± 3.9	257.5 ± 36.9
Condensed tannins	N/A	32.0 ± 7.4

SD- Standard deviation; DM- Dry Matter; OM- Organic Matter; NDF- Neutral detergent fibre; CP-Crude protein.

Crude protein content of the wheat straw in the current experiment (20.0±1.1 g/kg on DM basis) was substantially lower than values reported in literature. NRC (2000), report CP content at 3.8% while reports from animal feeding trials have wide ranging values between 1.8- 5.9% CP (Blümmel *et al*., 1997 Arora *et al*., 2011). The low CP in wheat straw hay used in the present trial than reported, could have been attributed to differences in crop and fodder production practices between developed and developing economies. The low protein levels could have been as a result of lack or low fertilizer application rates in crop production, poor soil fertility or poor post-harvest management e.g. exposure to weather elements- rain and sun; all of which are reported to lower CP content in crop (Ahn *et al.*, 1997); all these commonly observed in SSA.

While NRC values are derived from western economies cropping systems, lower values in the straws used in the present experiment could reflect poor crop and pasture management practices

in the sub-Saharan farming systems. The CP levels of *Calliandra calothyrsus* on the other hand were within range reported in literature (25.0- 26.4% on DM basis) (Perez Maldonado and Norton, 1996, Paterson *et al*., 1999, Tuwei *et al*., 2003). The slight variation in *Calliandra Calothyrsus* in CP levels from what has been reported could be due to the inherent ability of the crop to fix nitrogen, hence it is be less affected by soil level of N (Pengelly and Conway, 2000). Whatever the cause of the unexpectedly low CP content of the wheat straw fed, the result was that even the supplemented diets had less than the 7% CP per DMI, generally considered the minimum necessary for fully effective rumen digestion of plant material (Koster *et al.*, 1996).

The NDF in wheat straw at 807 g/kg was within range of the values reported in earlier studies ranging from 760- 887 g/kg (Acock *et al.*, 1979, Brand *et al.*, 1991, Haddad *et al.*, 1994, George *et al.*, 2006). NDF content in *Calliandra calothyrsus* (40.5%) was also within expectation with values in literature ranging between 386-428 g/kg (Norton and Ahn, 1997, Tuwei *et al*., 2003).

4.2 Feed intake

The effect of *Calliandra calothyrsus* supplementation on DM, OM and CP intake is shown in Table 4-2. Both Daily and Bi-d supplementation significantly increased ($P<0.05$) DM and OM intakes compared to the CON treatment but there was no difference between the two supplementation frequencies ($P=0.610$). Due to the variation on initial live weight of experimental animals, variables were expressed per 100 kg LW to eliminate its effect.

Table 4-2: Intake as fed, DM, OM and CP daily intakes ±SD (g/100 kg LW.d) and DM, OM and CP apparent digestibility (g/kg) in steers fed on chaffed wheat straw hay and supplemented with air dried *Calliandra calothyrsus* leaves

	Diet		
Item	CON	Daily	Bi-D
Total intake (g/100kg.d)			
Intake as fed	1711±215.3[a]	2029±392.8[b]	1993±324.2[b]
DM	1509±196.9[a]	1756±345.1[b]	1810±288.0[b]
OM	1401±197.2[a]	1633±325.7[b]	1683±278.1[b]
CP	32±3.5[a]	75±11.2[b]	78±8.6[b]
Digestibility (g/kg)			
DM	584±51.7[a]	584±40.5[a]	570±45.4[a]
OM	614±46.1[a]	616±34.0[a]	601±40.3[a]
CP	91±53.2[a]	290±92.3[b]	257±74.4[b]

DM- Dry matter; OM- organic matter; CP-Crude protein; CON- Control; Bi-d - Bi- diurnal; SD- Standard deviation. [a, b] Means within the same row(a, b) without a common superscript differ (P<0.05).

Increased DM intake with protein supplementation observed in this study was in agreement with previous experiments when low protein diets fed to ruminants were supplemented with a protein source (Del Curto *et al.*, 1990, Koster *et al.*, 1996, McGuire *et al.*, 2013). Increased voluntary intake in supplemented cattle has been associated with increased microbial activity and increased rumen turnover (Allen, 1996, Koster *et al.*, 1996, Herd and Arthur, 2009). The increased intake observed in the present study when steers were receiving Calliandra supplement suggest that low CP in the diet was limiting intake in the basal (CON) treatment, receiving the basal diet alone. Although supplementation increased total DM intake, the steers however, still may not have attained their maintenance energy and protein requirements; probably because the CP level in the supplemented diets were about 4.96% which were not only too low but also below 7% that is considered minimal for optimal intake in cattle on a high forage diet (Koster *et al.*, 1996). Intake by Boran steers did not increase with supplementation (Table 4-3) which was unexpected.

Supplementation frequency i.e. Daily and Bi-d did not affect intakes by the steers which was in agreement with Currier *et al.* (2004) and Archibeque *et al.* (2007b). Farmer *et al.* (2004) also observed no difference in DMI with infrequently N supplementation as low as once every three days. Sustained higher intake with infrequent supplementation could be explained by improved N utilization by the animals on the days when no supplement was fed (Archibeque *et al.*, 2007b). Archibeque *et al.* (2007b) observed that lower concentrations of rumen ammonia increased reabsorption of urea back to the rumen from the portal blood and lower N losses in urine on the low protein days, when alternating high and low dietary CP levels fed to finishing cattle. The possible improved N recycling with infrequent supplementation could have increased ammonia concentration in the rumen on the day when no supplement was provided. This possibly enhanced rumen activity turnover hence increased intake observed.

Table 4-3: Average DMI (g/100 kg LW.d) of steers fed on wheat straw and supplemented with air dried Calliandra leaves

	Breed	
Treatment	Boran	Friesian
CON	1506.6±47^{a,x}	1548.8±106^{a,x}
Daily	1566.0±65^{a,x}	1946.7±134^{b,y}
Bi-d	1605.1±70^{a,x}	1954.3±153^{b,y}

$^{a, b}$ *Means within the same row(a, b) and column(x, y) without a common superscript differ (P<0.05)*

4.3 Digestibility

Dry matter digestibility (DMD) of basal wheat straw was 584g/kg which was within the range of the values reported in literature (Acock *et al.* 1979; Wiedmeier *et al.* 1983; Brand *et al.* 1991; Haddad *et al.* 1994; George *et al.* 2006). *Calliandra calothyrsus* supplementation did not affect apparent digestibility of DM or OM and this could be explained by the sub-maintenance intakes

in all treatments- evidenced by weight loss by the animals. At levels below maintenance intake, digestibility has been shown to remain unchanged (Doreau *et al.* 2003) and even in some instances decreased with decreasing DM intake (Atti *et al.* 2002). This is normally not the case above maintenance where decreased intake increase ruminal retention time resulting in increased fibre digestibility (Frisch and Vercoe 1977). It has been postulated that at maintenance intake, retention time is long enough to optimise microbial degradation (Grimaud *et al.* 1999). Any further increase therefore would not alter digestibility because fibre contact time with microbes is no longer the limiting factor and this could explain the current observation. No change in digestibility in the present study was observed even in the supplemented treatments which had increased DM and OM intake probably because total intake in all dietary treatments were below maintenance requirements, and hence would remain unchanged. The positive effect of CP supplementation on digestibility of a low protein diet in this study was not observed probably because the effect may have been masked by the sub maintenance intake effect on the supplemented animals. The still low N intake and probably other dietary limiting factors e.g. energy may have inhibited any increase in microbial activity.

Apparent CP digestibility increased with supplementation (Table 4-2), in agreement with Currier *et al*., (2004a) and McGuire *et al*., (2013). Supplementation frequency however had no (P=0.48) effect on CP digestibility probably because the supplementation interval was narrow (48 hr) and the treatments were receiving the same level of Calliandra per unit time. Protein supplementation frequency as low as once in every 6 days has been shown to have similar effect as daily supplementation in cattle (Farmer *et al.*, 2001). The apparent low CP digestibility observed in the CON (9.1%) was most likely a result of a high level of metabolic N in the excreta (Ferrell *et al.*, 2001) which is not of dietary origin and therefore falsely reducing digestibility coefficients. Generally, metabolic N of about 5.35 g N/kg DMI is reported in ruminants' faeces when

consuming low-quality forages (Currier *et al.*, 2004). This significantly reduces the apparent digestibility coefficient when total N intake is low explaining the low digestibility on in the present study. Also, Cereal straws have highly lignified protein that is not acid- soluble and this would likely contributed to the low digestibility of CP in the wheat straw used (Mgheni *et al.*, 1994).

The higher CP digestibility coefficients reported with both supplementation regimes compared to the CON was likely as a result of increased amount of dietary CP consumed by the steers with minimal increase in N excreted in faeces (Currier *et al.*, 2004; Wickersham *et al.* 2008a). Supplemental Calliandra also could have provided readily digestible protein improving overall total tract CP digestibility. There were no breed differences observed in DM, OM and CP digestibility between Friesian and Boran steers ($P>0.34$). It has been suggested that digestibility is influenced more by feed factors rather than the genetic differences between breeds (Vercoe and Frisch 1992) hence the feed characteristics must have overridden the breed effect between the two breeds in the present study.

4.4 Total N intake, excretion and balance

As expected, the nitrogen intakes (g N/100kg.d) were higher ($P<0.001$) for the supplemented treatments (both Daily and Bi-d) compared to the CON in this experiment (Table 4-4). This is because the *Calliandra calothyrsus* supplemented to the steers was higher in CP (257.5± 4.0) compared to the basal diet.

Table 4-4: N intake, faecal N, urinary N, total N excretion (g /100kg LW.d) and N balance ± SD (%) of steers fed on wheat straw hay and supplemented with air dried *Calliandra calothyrsus* leaves.

	Diet		
Item	CON	Daily	Bi-d
N intake (g N/100kg.d)	5.0±0.71[a]	12.0±1.70[b]	12.7±1.63[b]
Faecal N (g N/100kg.d)	5.4±0.61[a]	8.5±1.05[b]	9.3±1.74[b]
Urinary N (g N/100kg.d)	1.1±0.13[a]	1.3±0.34[a]	1.4±0.30[a]
Total N excretion (g N/100kg.d)	6.5±0.67[a]	9.76±1.29[b]	10.7±1.94[b]
N balance %	-33.3±6.7[a]	17.7±8.9[b]	13.7±6.5[b]

[a, b] *Means within the same row(a, b) and column(x, y) without a common superscript differ (P<0.05)*

Feacal N excretion significantly (P>0.001) increased with supplementation, while urinary N was not different in the three dietary treatments (P>0.09) (Table 4-4). This was in agreement with the findings by Tiemann *et al.*, (2008). The response in faecal N to supplementation but not in urinary N suggest that the most of the supplemented protein may not have been available for microbial breakdown in the rumen maintaining low ammonia concentrations despite increased dietary protein level. This trend also was observed by Tiemann *et al*., (2008), when feeding CT-rich fodder to cattle. *Calliandra* supplement used in this study had moderate levels of CT (32.0±1.9 g/kg) and this could have bound the protein in the supplement forming CT-protein complexes rendering it unavailable for microbial breakdown in the rumen. The close proximity of CT and protein; the two being concentrated in the supplement could have enabled complexes formation during mastication in the mouth (Theodoridou, 2010). The then likely low ammonia concentration in the rumen resulted in low ammonia losses to the portal circulation, consequently low levels of urea produced in the liver and N in urine from the animals. Similar conditions have

been described by Tiemann *et al.*, (2008) while supplementing tannin-rich *Calliandra calothyrsus* and *Flemingia macrophylla* fodder to sheep.

Increased faecal N excretion with supplemetation may be due incomplete digestion of additional dietary protein reaching the abomasum and the duodenum probably because of incomplete dissocoation of CT-protein complexes hence bypassing lower tract digestion also. Increased hindgut fermentation as a result of high fibre in the ruminal outflow may have also increased post ruminal microbial growth and hence again enriching microbial N in faeces (Mlay *et al.*, 2003) . Low urinary N losses on the other hand could be explained by the fact that CP dietary levels were low and the animals could have been capturing it optimally (Reynolds and Kristensen, 2008) - both from the diet and recycling from the portal circulation.

The proportion of total N excreted by the steers in urine were 16.9%, 13.3% and 12.7% for CON, Daily and Bi-d respectively. In contrast to the CON, the supplemented treatments had a higher proportion of their N excreted in faeces (Figure 4.1) suggesting that most of the N excreted by the steers was not readily volatilized in the environment. Unsupplemented steers however had the lowest ($P<0.001$) total N excretion amongst the three treatments. N excretion in the two breeds significantly increased with supplementation more so when the supplement was fed on alternate days. Excretions within breed however was not different ($P>0.05$) between the two supplementation frequencies (Figure 4.1). The higher N excretion with supplementation is attributable to the increased dietary CP levels and the presence of CT making some of this additional protein escape breakdown in the gut of the animals Tiemann *et al.*, (2008).

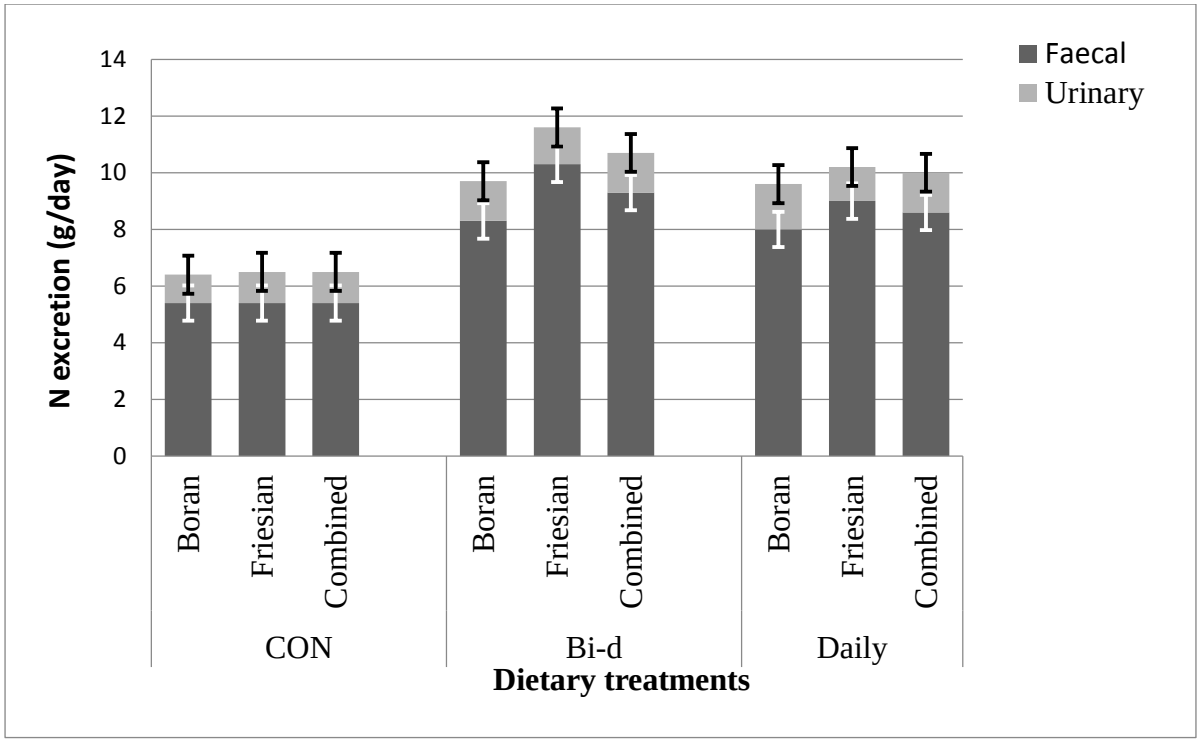


Figure 1: Faecal and urinary N excretion in Friesian and Boran steers fed *ad libitum* on wheat straw alone (CON) and supplemented either daily (Daily) or bi-diurnal (Bi-d) with air dried *Calliandra calothyrsus* leaves

Supplementation improved nitrogen retention (P<0.001) in the steers but supplementation frequency had no effect (P=0.6) (Table 4-4). The improved N balance with supplementation was consistent with Wickersham *et al*., (2008b) observation, while supplementing rumen RDP - casein at both low (61mg of N/kg LW.d and 183 mg every third day) and high (183 mg of N/kg of LW.d and 549 mg every third day) levels in cattle. As in the current experiment, the authors did not observe a significant difference in the supplementation frequencies, even though they observed a higher plasma urea level with infrequent high RDP supplementation. The authors also reported higher recycling of urea back to the rumen on days in between supplementation events and a higher capture of recycled N by microbes which could explain the improved N efficiency despite higher urea plasma levels with infrequent supplementation. These parameters were, however, not measured in the present study.

Improved N balance in both supplemented treatments (Daily and Bi-d) ($P<0.001$) in this study suggests that most of the supplemental protein in Calliandra fed to the steers was available for animal use. Most of the CT-protein complexes bypassing ruminal digestion must have been dissociated in the lower tract, intestinally dissected, with the protein being absorbed by the animals. Some of the protein however must have escaped enzymatic digestion in the abomasum and duodenum and excreted – as indicated by increased faecal N probably because of incomplete CT-protein complexes dissociation (Frutos *et al*., 2004).

Both Daily and Bi-d supplemented animals were in a positive N balance (17.70 and 13.74% respectively) whereas the unsupplemented steers were in a negative N- balance (-33.3%). The negative N balance could be explained by the low N in the diet consumed by the CON; lower than the metabolic N excreted by the animals.

4.5 Urinary purine derivatives and microbial protein synthesis

In all dietary treatments, urine concentrations of PDs were in the following order: allantoin (85-89%) followed by uric acid (9-13%) then xanthine and hypoxanthine (1-3%) which were in agreement with other findings in literature from cattle studies (Sampaio *et al*., 2010, Souza *et al*., 2010). All the three derivatives excreted in urine were higher in the supplemented steers than the CON treatment ($P<0.001$) (Table 4-5). Allantoin and total PDs excreted were significantly higher for Daily supplementation than Bi-d supplementation ($P<0.01$) indicating that PD excretion was highest when Calliandra supplement was provided daily. There was no breed effect on the total PD excreted ($P> 0.52$).

Microbial N supply was highest ($P<0.001$) with Daily supplementation and lowest in the CON- which had negative coefficient (-2.57 g N/100 kg LW.d) (Table 4-5). When efficiency of

microbial N supply was expressed on digestible organic matter intake (DOMR) basis, microbial N supply reduced slightly for in all the treatments (Table 4-5).

Table 4-5: Allantoin, uric acid, xanthine + hypoxanthine, total PD excretion, absorbed purines, microbial N supply (mmol/100 kg LW.d) and efficiency of microbial N supply in steers fed chaffed wheat straw hay and supplemented with air dried *Calliandra calothyrsus* leaves

	Diet		
Item	CON	Daily	Bi-d
Allantoin (mmol/100kg.d)	7.2±1.52[a]	12.3±1.73[b]	9.5±0.92[c]
Uric acid (mmol/100kg.d)	0.8±0.13[a]	1.4±0.26[b]	1.4±0.322[b]
Xan+ Hyp (mmol/100kg.d)	0.2±0.03[a]	0.3±0.05[b]	0.3±0.05[b]
PD excretion (mmol/100kg.d)	7.9±1.5[a]	13.6±1.3[b]	11.1±0.92[c]
AP	-3.5±1.59[a]	3.1±1.17[b]	0.3±0.07[c]
Microbial N supply (g N/100kg.d)	-2.6±1.5[a]	2.2±1.15[b]	0.2±0.53[c]
Efficiency of microbial N supply (g N/kg DOMR)	-3.0±1.84[a]	1.8±1.05[b]	0.3±0.41[c]

DOMR: 0.65×DOMI (ARC, 1984). DOMR- Apparently digested organic matter intake; DOMI- Digestible organic matter intake; PD- Purine derivatives; AP- Absorbed purines.

Microbial N supply and efficiency of microbial N supply were generally low in all the three treatments in this study compared to values reported in literature in cattle also fed on low quality diets. Sampaio *et al*., (2010) reported microbial N flow of 11.7 g/100 kg LW.d when feeding cattle with *Brachiaria decumbens* hay (CP, 4.86%), while Souza *et al*., (2010) feeding *Brachiaria decumbens* (CP, 5.16%) to crossbred heifers reported N flow of 17.6 g/100 kg LW.d. The low supply was because of inadequate protein levels in the diet to support optimum microbial growth in the rumen. The probable reduced availability of supplemental protein for

ruminal breakdown due of the presence of CT in the Calliandra could have moderated the increase in the supplemented treatments. Inadequate energy supply from the diet due to sub maintenance intake could have further hampered microbial growth in this study hence low microbial N supply and efficiency. The observed increase in microbial N supply with supplementation might have been as a result of increased recycling of urea back to the rumen as described by Wickersham *et al*., (2009) when feeding a low quality diet supplemented with ruminally unavailable protein rather than direct breakdown of supplemental protein in the rumen.

Unlike the present study where negative coefficients for microbial N supply were observed (Table 4-5), Sampaio *et al.*, (2010) reported positive supply 11.7 g /100 kg LW.d in the control treatment where no protein supplement was provided. This may be explained by the higher crude protein level in the basal diet (4.86%) compared to that in the present study (2.0%). Apparent negative microbial N supply in the present study could have been because the steers lost weight.

4.6 Live weight changes

Steers in all treatment groups lost weight ($P<0.05$) during the experiment. CON lost the most weight at an average of 1.24 kg per week, followed by Bi-d at 0.63 kg, then Daily supplementation at 0.32 kg per week. There was no effect of breed ($P=0.22$) on the model fixed on weight change.

Table 4-6: Weight changes (kg/week) in Friesian and Boran steers fed on chaffed wheat straw alone or supplemented with air dried *Calliandra calothyrsus* leaves

	Treatment		
Breed	CON	Daily	Bi-d
	(Kg/week)		
Boran	-1.07±0.32[a]	-0.23±0.14[b]	-0.55±0.12[b]
Friesian	-1.41±0.23[a]	-0.41±0.20[b]	-0.71±0.26[b]
Average	-1.24±0.25[a]	-0.32±0.19[b]	-0.63±21[b]

[a, b] *Means within the same row(a, b) and column(x, y) without a common superscript differ (P<0.05)*

Decrease in LW observed across all treatment groups in this study was directly attributable the high NDF (80.7%) in the basal diet restricting intake to sub maintenance levels. Low protein (2.0%) level also, may have compromised rumen microbial growth and fibrolytic activity (Grimaud *et al.*, 1998) leading in underutilization of the diet consumed by the steers. The resultant low energy and microbial N supply to the animals could have resulted in mobilization of body tissues by the animals so as to meet both their energy and protein metabolic requirements hence the weight loss. CON treatment lost the highest weight probably because they had the lowest DM and CP intakes (as a proportion of their body weight). Singh *et al*., (2008) feeding cattle on a rationed diet observed that cattle fed on up to 80% their *ad libitum* intake of a low quality diet (CP, 2.24%) lost weight of 250 g per day; agreeing with the present study. Dixon *et al*., (2011) also feeding *ad libitum* low protein basal diet (CP, 3.8%) observed weight loss in the animals. Overall CP content in the supplemented diets were also low (4.96%) and less than expected, restricting rumen microbial efficiency and thus intake.

CHAPTER 5: CONCLUSIONS AND RECOMENDATIONS

5.1 CONCLUSIONS

1. Daily or alternate day Calliandra supplementation improved total DM intake in cattle but had no effect on apparent digestibility. Friesian steers had higher intake than the Boran but there was no difference in digestibility between the two breeds.
2. Nitrogen retention in both Friesian and Boran steers improved with *Calliandra calothyrsus* supplementation irrespective of whether the supplement was provided daily or on alternate days.
3. Microbial N flow and efficiency of microbial N supply improved with supplementation in Boran and Friesian steers with daily higher increase with Daily supplementation.

5.2 RECOMENDATIONS

1. Dry matter intake in cattle consuming low protein basal diets can be improved by supplementing their diets with *Calliandra calothyrsus* as a protein source either daily or on alternate days as long as the animals receive the same amount of supplement per unit time.
2. Supplementation improved microbial nitrogen flow but had no effect on total tract digestibility. More studies on minimal supplementation level are needed to establish optimal level required to meet microbial growth requirements in the rumen which will subsequently improve fibre digestibility in cattle and also increase microbial protein supply to the host animal.
3. *Calliandra calothyrsus* supplementation to cattle consuming low protein diet can reduce the amount of volatile N excreted to the environment therefore potentially reducing the amount of NH_3 and N_2O produced from cattle production systems. This potentially slows down rate of GHG emission to the environment.

CHAPTER 6: REFERENCES

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