NUTRITIVE VALUE AND MANURE QUALITY IN SUPPLEMENTED MAIZE STOVER AND GRASS HAY DIETS FOR RUMINANTS

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DECLARATION

The experimental work presented in this thesis was carried out at the University of KwaZulu-Natal, Pietermariztburg, from June 2004 to September 2006. The work was Supervised by Professor Ignatius V. Nsahlai and Co-supervised by Professor Albert T. Modi.

The studies reported here represent original work done by myself and have not been submitted in any form for any award of degree or diploma to any university, Where use was made of the work of others, it has been duly acknowledged in the text.

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I declare that the above statement is correct.

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SUMMARY

Production of ruminants is highly dependent on utilization of roughages. In tropical regions, particularly Sub-Saharan Africa, the roughages are predominantly of poor quality due to high fibre and low protein content. For these reasons supplementation with protein sources and other feeds with high degradability is essential. Maize is an important cereal in the world, and in Africa it produces the highest amount of crop residue. The stovers left after harvesting maize grain are potentially an important feed resource. The overall goal of this study was to identify nutritive attributes of diets comprised of maize stovers or grass hay and their mixtures with different protein supplements for ruminant production, and to determine the influence of the diets on manure quality.

There were three different roughages and three different protein supplements used in the study. The roughages were grass hay and maize stovers harvested when the grain was at milk stage or when the grain was dry (approximately 80% DM content).The roughages were characterized by high fibre and low protein content. The protein supplements included lucerne, Sericea lespedeza and sunflower oil cake. Although lucerne has been widely used as a model protein source for ruminant production there are agronomic and climatic limitations which constrain its widespread use, particularly in smallholder systems. Hence there is a need for alternative protein sources such as those included in the present evaluation.

There were three sets of investigations carried out. The first set involved laboratory chemical composition and *in vitro* rumen fermentation of feeds using automated gas production technique (IVGPT). Over forty feed rations were evaluated. Multivariate cluster analysis was performed to create clusters of diets exhibiting homogeneous nutritive characteristics from IVGPT measurements. The diets were grouped into three different clusters. Ten diets were selected, with each cluster represented, and tested in a second set of investigations. These investigations involved conducting feeding trials using Damara rams. Data collected included intake, digestibility, weight gain and manure (faeces) production. The third set of

investigations involved manure quality analyses. This was done by determining the mineral composition and nutrient release, particularly nitrogen mineralization. The relationships among chemical composition, IVGPT, *in vivo* and manure quality measurements were determined. Models to predict *in vivo* performance from chemical composition and IVGPT measurements were derived.

Maturity stage influenced the nutritive characteristics of the forages whereby both maize stovers and lespedeza depreciated in quality with age, but the effect was small in the stovers with regard to degradability. The stovers harvested at grain milk stage showed superior nutritive quality over stovers harvested at dry stage by having higher soluble carbohydrate, lower fibre content and shorter lag time (time taken for microbes to colonize and begin fermenting the substrate) under *in vitro* fermentation.

The positive effects of supplementation with lespedeza included enhancing fermentation of fibre fraction of and decreasing lag time. However, increasing the ratio of lespedeza caused a decrease in degradability, and this was attributed to the presence of tannins in lespedeza.

In vitro gas production technique (IVGPT) is currently one of the most widely used methods in ruminant feed evaluation. It measures the gas production arising from fermentation of feeds, and the degradability can be measured by weighing the residues left at the end of incubation. Gas production profile from IVGPT can be fitted in models to derive parameters of fermentation kinetics. Although IVGPT can give several nutritive measurements to help in understanding the fate of substrates in the rumen, this study obtained inconsistent and confounding results in some parameters. These included gas volume, microbial yield and degradability:gas production ratio or the partitioning factor-PF (mg of DM Degraded/ml of gas produced). On the other hand, it was found that time taken to produce half of the maximum gas ($T_{½}$) was consistent across rations. The degradation efficiency factor (DEF), calculated as the ratio of degraded material : gas produced x $T_{½}$, was also consistent across rations. These parameters therefore showed potential of being useful to include as indices for evaluating ruminant diets.

The study demonstrated that the measurements derived from gas production technique can be used in multivariate cluster analysis to logically separate feeds into distinct homogeneous groups of nutritive characteristics, and this was helpful in identifying diets for the *in vivo* trial. The directly measurable variables from *in vitro* gas production technique which significantly contributed to distinctiveness of the clusters included degradability, microbial yield, $T_{½}$, PF and DEF. Other significant distinguishing variables for the clusters were derived from kinetic of gas production. They included lag time, gas from the Fibre or soluble fraction of the feed and the rate of gas production from the fibre fraction.

Diets induced different performances by sheep, with the best (60:40 grass:sunflower cake) having an intake of 43 g DM/kg liveweight and weight gain of 172 g/d. The poorest performance was obtained in 60:40 milk stage maize stover:lespedeza diet where the intake was 25 g DM/kg liveweight and marginal weight loss of 11 g/d was recorded.

Evidence in the literature indicates that lespedeza has a great potential due to adaptation to poor climate and low inputs requirements. Though not determined in this study, the major limitation to lespedeza nutritive value is high tannin content. Reports in the literature indicate that presence of tannins can be of nutritional and health benefits, and negative effect of tannins can be moderated through use of chemical additives. Also, there are reports indicating that lespedeza can be invasive, hence its cultivation may require effective control. Further work is recommended to investigate means of optimizing lespedeza utilization in ruminant production systems, particularly where it can have climatic and economic advantages.

Ideally, evaluation of diets should be done by conducting *in vivo* trials. However, *in vivo* trials are widely considered to be impractical due to many reasons including high costs, animal welfare and inflexibility. *In vitro* methods are therefore widely used as alternative to mimic and predict what would happen *in vivo*. This study showed that *in vivo* performance of sheep was predictable with high precision (R^2 = 0.75-0.85 for intake, 0.70-0.89 for Digestibility and 0.77-0.82 for weight gain) by use of models combining chemical composition and IVGPT measurements. The important chemical composition measurements included fibre (NDF or ADF) and protein contents, while important IVGPT measurements included degradability (true

or apparent), $T_{½}$ and DEF. There were confounding factors affecting the validity of gas volume, microbial yield and PF measurements as predictors of nutritive value. Consequently, these measurements lacked consistency and generally had poor relationships with *in vivo* measurements.

Drying fresh manure caused 25 to 89 % loss of ammonium (NH_4^+), emphasising the need to avoid drying manure in order to maximise the quality. Mineralization results showed that manure from diets with crude protein content above 170 g/kg can be similar to limestone ammonium nitrate (LAN) fertilizer in supplying N. Notwithstanding, the manures would have additional advantage of improving soil humus and supply more nutrients as decomposition continues.

In the overall, the results demonstrated that maize stover or grass hay supplemented with sunflower cake or lucerne can support high ruminant (sheep) production while at the same time yield good quality manure. For practical purposes, diets containing 20-40% of DM contributed by these supplements showed high potential in this dual purpose role. The choice between these two supplements would therefore depend on socio-economic considerations. As for lespedeza, high tannin content is stipulated to be responsible for depression of microbial activity, hence nutritive value. However, given that lespedeza may have agronomic and climatic advantages, plus the fact that the impact of tannin can be ameliorated, it is viewed that lespedeza has potential to boost productivity in specific situations. Further evaluation of lespedeza should therefore focus on detailed chemical composition analyses at its different stages of maturity, *in vivo* trials using different ruminants and measures to moderate tannin effects.

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Glossary of abbreviations and symbols

ADF	Acid detergent fibre
ADFn	Nitrogen content of acid detergent fibre
AIBs	Feeds originating from Agro-industrial by-products
Al	Aluminium
ANOVA	Analysis of variance
ApDeg	Apparent degradability
ATP	Adenosine triphosphate
C	Carbon
°C	Degrees centigrade of temperature
Ca	Calcium
$CaCl_2$	Calcium chloride
CHO_{sol}	Soluble carbohydrates
CO_2	Carbon dioxide
CP	Crude protein
Cu	Copper
cv	Coefficient of variation
DEF	Degradability efficiency factor (Deg mg/(Half GasVol ml x $T_{½}$)) arising from in vitro fermentation
Deg	Degradability (refers to degradation by rumen microbes)
DM	Dry matter (oven dried if not stated otherwise)
DMD	Dry matter digestibility (refers to whole tract digestion)
DMDIG	Total dry matter digested
DMI	Dry matter intake
DMI/LW	Dry matter intake per liveweight
EE	Ether extract
Fe	Iron
FR	Feed residue with solid attached bacteria extracted
GasVol	Gas volume arising from in vitro fermentation
GE	Gross energy

GELF	Ratio of gross energy intake lost in faeces
GELU	Ratio of gross energy intake lost in urine
GH	Grass hay
H_2O	Water molecule(s)
IVGPT	In vitro gas production technique
K	Potassium
KARI	Kenya Agricultural Research Institute
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen orthophosphate
LH	Lucerne hay
LPZ	Sericea lespedeza
LSD	Least significant difference between means
MD	Maize stover harvested at grain dry stage
$MgCl_2.6H_2O$	Hydrated magnesium chloride
$MgSO_4.7H_2O$	Hydrated magnesium sulphate
MIC	Rumen microbial yield estimated from in vitro fermentation
MM	Maize stover harvested at grain milk stage
Mg	Magnesium
Mn	Manganese
MPTs	Multipurpose trees and shrubs
MS	Maize stover
N	Nitrogen
Na	Sodium
NH_3	Ammonia
NH_4^+ /NH_4	Ammonium
Na_2.HPO4.12H_2O	Hydrated disodium hydrogen orthophosphate
$NaHCO_3$	Sodium hydrogen carbonate
NCFs	Non-conventional feeds
NDF	Neutral detergent fibre
NDS	Neutral detergent solution
NO_3^+/NO_3	Nitrate

ns	Not significant ($p>0.05$)
OM	Organic matter
P	Phosphorus
p	Statistical significance level
PF	Partitioning factor (Deg /GasVol) arising from in vitro fermentation
PS	Protein supplement
r	Correlation coefficient
R^2	Coefficient of determination
RForm	Roughage physical form (coarseness)
S	Sulphur
SCFA	Short chain fatty acids, also commonly known as volatile fatty acids (VFA)
SFC	Sunflower cake
SSA	Sub-Saharan Africa
$T_{½}$	Time taken to produce half of maximum gas volume from in vitro fermentation
TruDeg	True degradability
WtGain	Daily weight gain by sheep
Zn	Zinc

Chapter 1

GENERAL INTRODUCTION

1.1 Background

Agriculture is the most widespread human land use and forms the mainstay of many developing countries, with mixed farming systems covering about 2.5 billion hectares of land (FAO, 2001). In Sub-Saharan Africa (SSA), crop-ruminant systems predominate. Important crops include maize, pearl millet, beans, sorghum and wheat, while cash crops include sunflower, cotton, sugar cane and groundnuts (Bedingar and Degefa, 1990). Livestock holdings range from a few to hundreds of head per household with varying ratios of cattle, sheep, and goats (Swinton, 1988). Large amounts of crop residues are produced, for which appropriate utilization can greatly enhance livestock production. For instance, maize stovers yield has been estimated to be 340 million tonnes annually in Africa (Reddy *et al.*, 2003). This implies that maize stovers can play an important role as feed resource for ruminant livestock in Africa.

Ruminant diets are basically comprised of poor quality roughages that require supplementation, especially with protein and readily available energy sources, to attain reasonable production. Popular supplements include forage legumes and agro-industrial by-products such as oil-crops seed cakes. Among the legumes, lucerne (*Medicago sativa*) is perhaps the most popular worldwide for ruminants, but requires good soils, high moisture availability and high agronomic input. For these reasons, lucerne is generally out of reach to most resource constrained smallholder farmers in Africa. Alternative forage legumes are therefore necessary, particularly for low potential areas. In this regard, Sericea lespedeza (*Lespedeza cuneata* G. Don) has shown high potential due to hardiness (Powell *et al.*, 2003) and improvement of livestock performance (Silanikove *et al.*, 1996).

A principal challenge facing agriculture in many parts of SSA is how to achieve sustainable increases in crop and livestock production. Low rural incomes and the high cost of inputs (fertilizer and feed supplements), among other factors, prevent the widespread use of external nutrient sources (Powell *et al.*, 2003). Farmers continue to rely principally on organic matter recycling in order to maintain soil fertility whereby animal manures are generally the most

important. Productivity of the system is dependent on the quantity and quality of the inputs, and the efficiency of their flows as depicted in Figure 1.1

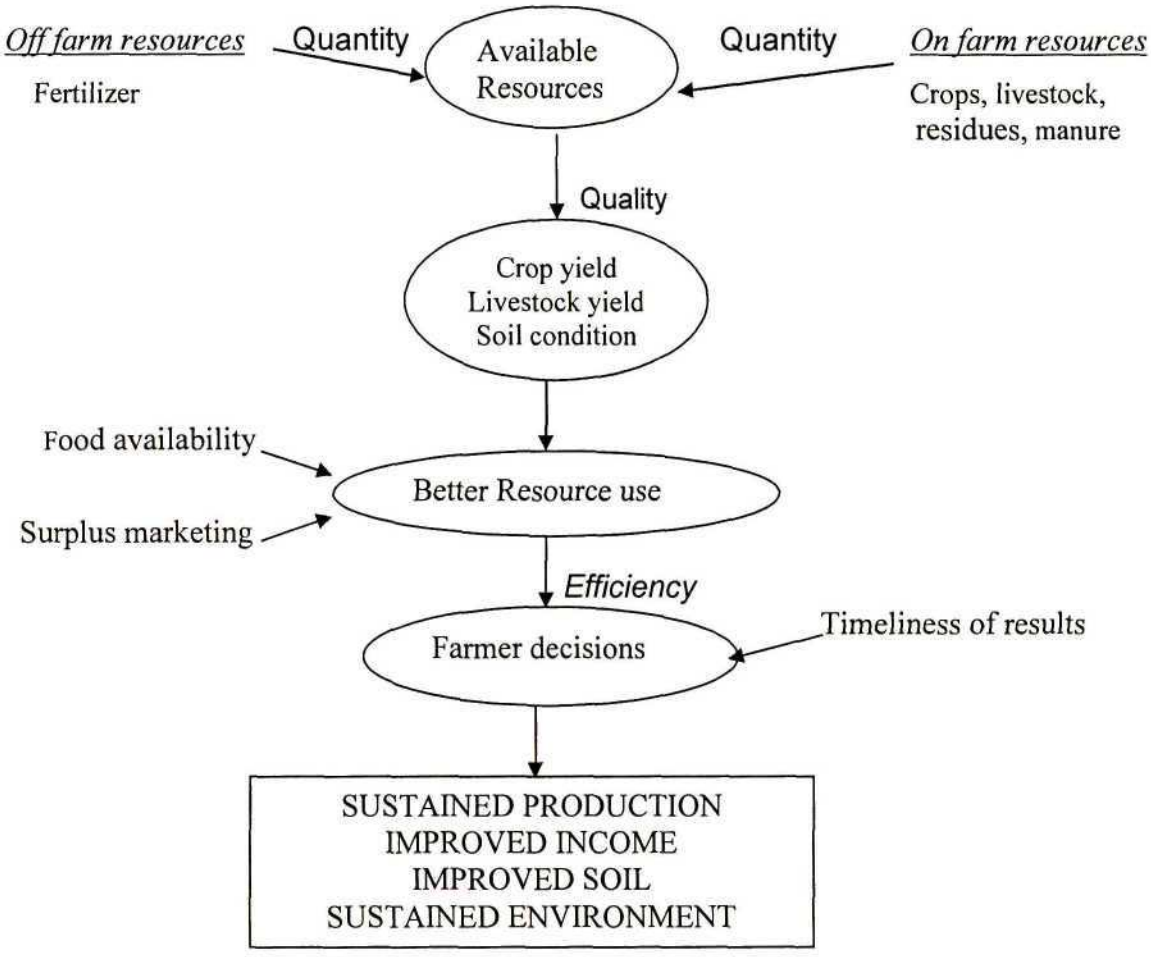


Figure 1.1 Management considerations in livestock-crop system (Adapted from Beets, 1990)

Recent studies (Palm *et al.*, 1997; Delve *et al.*, 2001; Lekasi *et. al.,* 2003) have provided technological information on use of manure to improve soil fertility and consequently crop production. However, as pointed out by Powell *et al.* (1995) systematic linkage of the nutritive characteristics of the ruminant diets to the quality of by-products such as the effects on manure quality, the maintenance of soil fertility and optimisation of crops-ruminant production through nutrient recycling are lacking.

The diets fed to the animals have implications on overall farm productivity with regard to quality of manure. As feeds are carefully balanced for optimal nutrient use by the animals,

the nutrients excreted in manure are liable to change. Thus, it is essential to determine the influence of diets on manure quality in terms of both chemical composition and nutrients release, and to determine the relationship with animal production measuements.

1.2 Study Goal and Objectives

The overall goal of this study was to identify nutritive attributes and opportunities for improvement of ruminant production using diets comprised of maize stovers or grass hay mixed with different protein supplements. The influence of the diets on manure quality in terms of chemical composition and nitrogen mineralization was also evaluated. The specific objectives were to determine:

(i) the chemical composition and *in vitro* nutritive characteristics of different feeds and diets resulting from their mixtures,

(ii) the influence of diets on animal production performance and nutrient partitioning in excreta,

(iii) the relationships among the diets parameters derived from chemical composition, *in vitro* gas production analysis and *in vivo* performance and

(iv) the influence of diets on manure quality in terms of chemical composition and nitrogen release.

Chapter 2

LITERATURE REVIEW

2.1 The role of livestock, a global perspective

Farm animals are producers of meat, milk and eggs, which are part of the modern food chain providing high value protein. In addition, farm animals also provide non-food functions. For instance, to millions of smallholder farmers, animal draught power and nutrient recycling through manure compensate for lack of access to modern inputs such as tractors and fertilizer, and help to maintain the viability and environmentally sustainable production. Also, livestock constitute the main, if not the only, capital reserve of farming households and adds stability to the overall farming system. As such, livestock can satisfy a large variety of human needs. Yet, in many places, livestock production is growing out of balance with the environment. Bouwman *et al.* (2005) stated that there is a major concern about the potential of the global agricultural systems to expand production and the environmental consequences arising from such increases. Seré and Steinfeld (1996) and Delgado *et al.* (1999) reported that a significant part of grazing land used for ruminants consists of marginal unproductive grassland with low carrying capacity and high risk of land degradation due to overgrazing, especially in the arid and semi-arid tropics and subtropics.

The driving force behind the surge in demand for livestock products is a combination of population growth, rising incomes and urbanization. Projections indicate that the world population may increase from about 6 billion presently to 8.2–9.3 billion in 2030 (Nakicenovic *et al.*, 2000). The world urban population reached 45% in 1995 and was projected to reach 60% by 2025 (UNFPA, 1995). Food production will have to increase to meet the increasing demand for the growing population. With increasing prosperity, dietary patterns may shift towards a higher share of meat and milk. This is because as the income and urbanization increase, there is a tendency to increase consumption of high quality protein of animal origin.

In recognition of the need for intensification of agricultural production, FAO (2001) stated that world crop and livestock production must expand by more than 3% annually to keep pace with the increasing food demand. FAO (2001) further pointed out that despite a general tendency towards specialised farming over the past few decades, it appears that there is again an increasing interest in the advantages of mixed farming systems.

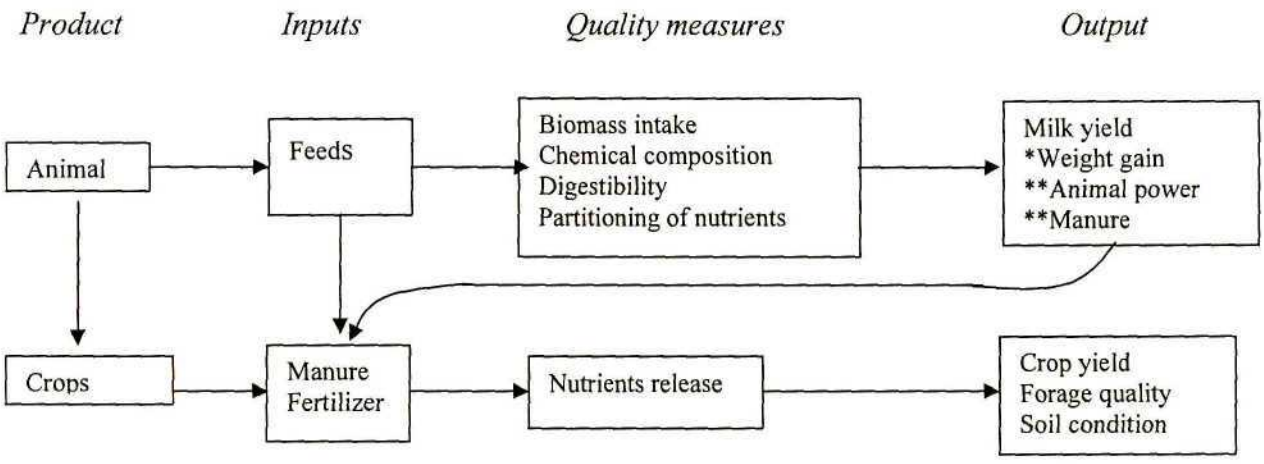
2.2 Characteristics, challenges and opportunities in tropical farming systems

The term 'Farming System' has no universal single definition. In the current work, Farming System is conceived to be '*a unit consisting of human group (usually households) and resources it manages in its environment, involving the direct production of plant and/or animal products*' (Beets, 1990). It is an ecosystem in which all components: land operators, hired labour, crops and cropping systems, animals and machinery are considered together to produce goods to meet the requirements and/or basic needs or to exchange for goods and services. The determinants of farming systems are: physical (climate, soil etc), biological (crop, livestock, etc), endogenous (family, food preferences etc) and exogenous (population, off farm opportunities, markets, prices, etc).

There are about 500 million smallholder farmers in the tropics worldwide (FAO, 2001) practising different farming systems. In SSA, mixed farming systems are predominated by small land holding (less than 10 ha in most cases) and low resources. Good examples are in high potential areas and give way to nomadism and bush fallow in drier areas (Beets, 1990). Biomass production is a major factor since crops and crop residues are meant to feed animals and maintain soil fertility. Water availability and soil quality are important considerations since they determine biomass production. The plants and crops are grown typically as staples, vegetables, fodder/feeds, trees/timber and for cash. The livestock are mostly kept for multi-purpose use including provision of milk, meat, skins, blood and for socio- cultural reasons.

The presence of productive livestock enterprises on farms is one strategy that can offer a more profitable route for nutrient return to the soil (Jama *et al.,* 1997). Crops and their residues feed the animals and in turn the animals' manure fertilizes the soil. By keeping livestock, arable farmers are able to add value or produce surplus food, use labour more

efficiently and diversify risk (Jama *et al.*, 1997). By adding manure to the herds value, not only are nutrients recycled but the improved soil structure helps water to infiltrate rather than run off and this in turn reduces soil erosion. Components and linkages in crop-livestock systems are summarized in Figure 2.1.



*Includes increase in herd size and individual body weight plus associated products such as skins and socio-cultural values.

**Outputs from animals utilizable in crop production

Figure 2.1 Schematic presentation of proposed important crop-livestock system components and linkages

2.3 Feed resources in tropical farming systems

Inadequate quantity and poor quality of feeds available year round is a major constraint to ruminant productivity in tropical farming systems. There are four main categories of feeds potentially available for use in mixed farming systems. These are pastures (native and improved grasses, herbaceous legumes, and multi-purpose trees), crop residues, agro-industrial by-products (AIBs) and non-conventional feeds (NCFs). Good examples of AIBs include oil seed cakes, molasses and cereal brans. NCFs are diverse and are not widely used traditionally e.g. cocoa pod husks, rubber seed meal, distillers/brewers wastes, shrimp waste, leather shavings and poultry litter (Devendra, 1992). Ruminants mostly survive on low-

quality roughage basal diets such as standing hay or crop residues. The high content of cell wall component (fibre) in these feeds often leads to digestibility below 50% due to lack of sufficient nutrients for the fibre-degrading microbes (Leng, 1990). This also decreases rumen passage rate and intake, thus resulting in insufficient nutrient supply, low productivity and even weight loss. In SSA, maize stover is perhaps the most important crop residue, since maize grain is staple in many communities.

2.3.1 Maize stovers

Maize is the third most produced grain after wheat and rice but leads in crop fodder production both globally and in Africa. Annual maize fodder yields have been estimated to be 1816 and 340 million tonnes in the world and Africa, respectively (Reddy *et al.*, 2003). Maize is largely grown and left to dry to less than 20% grain moisture content before harvesting. The dry grain is a widely used staple in tropical regions. In Africa, many communities differently prepare 'porridge' and 'cakes' from maize meal e.g. 'Ugali', 'Fofo', 'Kita' and 'Pap' which are popular in East, West, North and South Africa, respectively. Harvesting maize at grain milk stage for human food (roasting and boiling) is also popular. Stovers harvested at grain milk are greener (Appendix 1) and more appealing to ruminants, hence generally have high palatability. More importantly, they have been shown to have higher nutritive quality due to lower fibre content as compared to stovers harvested at later maturity stage (Tolera *et al.*, 1999). Yield and nutritional quality of maize vary considerably with varieties (Tolera and Sundstøl, 1999) and harvest stage (Flachowsky *et al.*, 1993, Tolera *et al.*, 1999), climatic conditions and agronomic factors. Given that maize stover is potentially an important source of roughage for ruminant production, improvement in their utilization is expected to result into considerable positive impact on the overall productivity. This can explain the continued interests by scientists in tropical and sub-tropical regions to explore means of improving maize stover utilization by ruminants (Akbar *et al.*, 2002; Nguyen *et al.*, 2003; Hindrichsen *et al.*, 2004). It is widely recognized that a major limitation to maize stover utilization by ruminants is the high fibre and low protein contents, and this has formed the focus of many studies (Ibrahim *et al.*, 1995; Hindrichsen *et al.*, 2004).

2.3.2 Role of protein supplements

Nitrogen, which is mostly available in proteins, is a limiting nutrient for the utilisation of poor quality roughages by ruminants (Leng, 1990; Kaitho et al., 1993; Hindrichsen *et al.*, 2004). The adverse effects of protein under-nutrition can be alleviated by judicious feeding of ruminants with nitrogen-rich supplements. Commercial concentrate sources of proteins such as fish meal and blood meal are not only scarce in some SSA regions, but are also generally unaffordable by smallholders. For these reasons, opportunities in improving ruminant productivity in SSA will mostly depend on production of protein sources at farm level or utilization of cheap industrial by-products. Consequently, nutritive evaluation of alternative protein sources has continued to attract research interests. Several workers (Umunna *et al.*, 1995; Mpairwe *et al.*, 2003; Ngwa *et al.* 2003) have shown that forage legumes and foliage of multipurpose trees and shrubs (MPTs) are suitable protein supplements for ruminant diets.

Lucerne

Lucerne is a forage legume reputed to be the first forage to receive wide cultivation (Suttie, 2000). It was grown in Iran in 700 BC, spread throughout southern Europe, North Africa and Asia, and was taken to the Americas by the Spaniards, and later spread to the USA in the mid-nineteenth century. It reached China in the second century BC, when Iranian horses were acquired for military use. It only came into use in northern Europe and Australasia during the past two centuries. Lucerne (also known as Alfalfa in USA) is an upright, deep-rooted perennial with many, usually erect, stems which arise from crown buds (Appendix 2). It is the world's most important forage crop and a high-quality feed for all types of livestock (Suttie, 2000). It has high protein content, e.g. ranging from 19.5 to 25.4% reported by Martens (2002) and 18.7% reported by Turner *et al* (2005). Besides high CP content, lucerne also has high yields under suitable condition. For example, Slarke and Mason (1987) reported yields ranging from 15.7 to 18.7 t/ha from four lucerne cultivars..

Lucerne is a crop which requires low humidity and neutral-to-alkaline, well-drained soils, but can be grown on moderately acid ones. It does not, however, tolerate humid climates at high temperatures, and performs poorly in humid, tropical and subtropical sites on acid soils. It requires good, well-drained land which can enable deep rooting, since its rooting depth can be 3 to 5 m under favourable conditions. Leaf diseases and pests are common problems with

Lucerne under some climatic conditions. Thus although a valued forage, the climatic and high agronomic input requirements limit lucerne cultivation under resource constrained smallholder systems. Hence there has been continued exploration of alternative adaptable forage legumes, especially in the SSA mixed farming systems.

Lespedeza

Characteristics and potential

Lespedeza (*Lespedeza cuneata* (Dum.-Cours.) G. Don) is known by different common names including Sericea lespedeza, Chinese lespedeza, Chinese bushclover and Himalayan bushclover. It was also nicknamed 'Poorman's lucerne' by farmers in USA. History, botanical and phenological features of lespedeza have been described by various authors (Guernsey, 1970; McGraw *et al.*, 1995; Mosjidis, 2001; Powell *et al.*, 2003). In summary, lespedeza is a member of the Pea or Fabaceae family. It is widely planted in the southern USA as a grazing, hay, soil restoration and conservation crop (Powell *et al.*, 2003). Morphologically, Lespedeza has numerous branching stems and may grow up to two meters. Leaves are compound in groups of three. Wedge shaped leaflets are oblong, green with silver highlights. Leaflets are covered with densely flattened hairs, giving a grayish-green or silvery appearance. Flowers are predominantly white with violet colour along the veins. Flowers occur in clusters or solitary and emerge close to the stem in the leaf axils on the upper third of the plant (Appendix 3). The seeds of lespedeza are borne in a legume that is flat and oval shaped. The seeds are tan or greenish in colour measuring from 2 to 7.5 mm in size. Scarification is necessary for the germination of lespedeza seeds. Mature seeds of this genus remain viable for up to twenty years; one study found a germination rate of 60% after cold storage for 55 years (McGraw *et al.*, 1995). Seedlings may represent only 1% of the seeds actually available in the soil (Ball *et al.*, 1995)

Lespedeza is native to Eastern China, Korea and Japan. It was first imported to USA (North Carolina) about 1896 and by 1940's was planted on large scale by poor farmers who could not afford lime and phosphorus fertilizer, hence the name – Poorman's Lucerne. A number of Sericea lespedeza cultivars have been developed, such as Arlington, Serala, AU Lotan, AU Donnelly, AU Grazer and Interstate, for low-input cattle forage and other uses (Ball *et al.*,

1995; McGraw *et al.*, 1995; Mosjidis, 2001). Stems of these cultivars are generally shorter, finer, leafier, and more numerous (Guernsey, 1970; McGraw *et al.*, 1995). Many of these cultivars were developed with the intent to create a more palatable forage, with lower levels of undesirable tannins. Early cultivars (1920s-1940s) were generally high in tannins, making them relatively unpalatable to livestock (Ball *et al.*, 1995). Low-tannin cultivars subsequently have been developed that can produce acceptable forage for livestock (Stubbendiek, *et al.*, 1989) but they are relatively more sensitive to overgrazing (Ball *et al.*, 1995). About twenty years ago, the first Interstate cultivar was introduced to South Africa from USA (2005, H. Botha, pers. Comm., Harmonie Trust, P.O. Box 27, Matatiele 4730, South Africa). This cultivar was highly unpalatable. Later AU Lotan, a more palatable cultivar was imported.

Lespedeza has been recommended as a pasture species in the southeastern U.S.A. in particular. Besides potentially having high CP content (e.g.16.1 and 18.2% reported by Powell *et al.*, 2003), lespedeza's deep rooting habit improves vigor and survival during summer drought (Guernsey, 1970; Schimidt *et al.*, 1982). According to Joost and Hoveland (1986) root growth in acid soils is less inhibited than that of lucerne. Hay from cultivated lespedeza often cures more rapidly than many other common hay species (Guernsey, 1970, 2005; H. Botha, pers. Comm., Harmonie Trust, P.O. Box 27, Matatiele 4730, South Africa). In addition to utilization by cattle (Guernsey, 1970; Griffith, 1996; Ohlenbusch, *et al.*, 2001), goats also make good gains grazing lespedeza (Silanikove, *et al.*, 1996; Escobar, 1998).

Invasion and control

Lespedeza can escape cultivation and become self-establishing and potentially invasive. This has happened in many areas in eastern U.S.A. (Ball and Mosjidis, 1995; Diggs *et al.*, 1999). Invasion into areas where plant species of higher nutritive quality are adapted can degrade forage quality (Vermeire *et al.*, 1998) as well as diminish native plant density. Negative impacts of lespedeza generally derive from its ability to out-compete, displace , or inhibit establishment of native plants (Vogel, 1981). The tall, upright growth habit, multiple branches, and dense foliage typical of established lespedeza plants confer considerable competitive advantage for light in grassland habitats (Vermeire *et al.*, 1998; Ohlenbusch *et al.*, 2001). Lespedeza is thought to be allelopathic, although mechanisms for this are largely

unstudied. There is speculation that tannins leached from foliage may have negative effects on associated plants, root exudates may negatively impact germination and establishment of grasses (Kalburtji and Mosjidis, 1993; Ohlenbusch *et al.*, 2001). In a greenhouse experiment, Kalburtji and Mosjidis (1993) found that in the presence of lespedeza root exudates: (i) radicle and coleoptile length and aboveground biomass of bahiagrass and some cultivars of tall fescue were significantly ($p<0.05$) reduced and, (ii) percent germination, percent emergence, and radicle and coleoptile length of Bermuda grass were significantly ($p<0.05$) reduced. However, the mechanisms and biological significance of lespedeza allelopathy has not been fully understood.

Despite reports of invasive potential, escaped populations of lespedeza may not always be strongly competitive. Wright *et al.* (1978) characterized lespedeza as "non-aggressive", when comparing seedling emergence of various grasses and legumes commonly used for erosion control. According to Hoveland *et al.* (1971), lespedeza seedlings "are weak and compete poorly with spring and summer weeds." Indeed, slow germination and poor seedling establishment have been long known (Logan *et al.*, 1969) to be reasons for diminished popularity of lespedeza as a crop species. Even once established, lespedeza may not necessarily become invasive. It can establish at low population levels in such areas as "fence rows, brushy and grassy areas, and where fire and grazing have been excluded for years", although its long-term competitiveness on such sites is unclear (Logan *et al.*, 1969). Lespedeza has been observed growing in ditches, fences rows, or pastures without invading adjacent "well-managed" rangeland and pastures (Ohlenbusch *et al.*, 2001).

Controlling populations of invasive lespedeza will likely require multiple treatments, perhaps over several seasons. Established plants may sprout in response to mechanical damage of aboveground tissue. Also, a seed bank may be present, with the potential for establishment of new seedlings for many subsequent years. Jordan *et al.* (2002) showed that, despite seemingly successful control of preexisting populations using several different methods, lespedeza can quickly reestablish and increase to levels equaling or exceeding initial abundance. In this instance, it is not clear if reestablishment was due to sprouting and recovery of plants that had been established prior to treatments, or due to establishment of new plants from the seed bank. Nevertheless, subsequent monitoring and follow-up treatments will likely be required

for long-term control. Some methods which have been effectively used to control lespedeza invasion include: (i) Suppression of seed germination and seedling survival and establishment by maintaining a substantial amount of plant residue or ground cover (Griffith, 1996). (ii) where a substantial seed bank is known or suspected, removing residual plant material in spring and encouraging a large flush of lespedeza seed germination may be useful. The goal of this strategy is to rapidly deplete the seed bank so that restoration of a desirable native plant community may proceed. Again, activities such as grazing or burning that remove substantial amounts of existing plant cover, especially when conducted early in the growing season, can result in conditions favorable to lespedeza seedling establishment (Ohlenbusch *et al.*, 2001).(iii) Integrated management includes not only killing the target plant, but also establishing desirable species and discouraging nonnative, invasive species over the long term(Ohlenbusch *et al.*, 2001).(iv) Physical/mechanical control such as mowing or cutting plants, especially early in the growing season, may result in vigorous re-growth. Frequent mowing can reduce plant vigor. Plants should be mowed when they reach a height of 12-18 inches, and should be cut as close to the ground as possible (Ohlenbusch *et al.*, 2001; Vermeire *et al.*, 2003). Frequent cutting on droughty or infertile sites may be particularly effective (Guernsey and Walter, 1970). (v) Ohlenbusch *et al.* (2001) indicated that hand digging can be effective for controlling small, scattered lespedeza populations. (vi) Chemical control using herbicides may provide initial control of a new invasion (of small size) or a severe infestation, but are rarely a complete or long-term solution to invasive species management (Bussan et al., 1999). Herbicides are more effective on large infestations when incorporated into long-term management plans that include replacement of weeds with desirable species, careful land use management, and prevention of new infestations. Successful control of lespedeza invasion using different herbicides has been reported (Alton *et al.*, 1992; Yonce, 1989). However, as cautioned by Jordan and Jacobs (2003), care need to be observed when herbicides are to be used to prevent the killing of desirable plant species in the neighbourhood.

2.3.3 Agro-industry by-products feed resources

Agro-industrial by-products (AIBs) are important high-energy or high-protein feedstuffs. They can therefore make substantial contribution to feed supplies, particularly during the dry season when the feeds are not only scarce, but also the available ones are poor in quality. The major AIBs in SSA include molasses, groundnut cake, cottonseed cake, sunflower seed cake, palm kernel cake, and fishmeal (Table 2.1). Their use in animal feeding in SSA has been limited owing to export policies aimed at earning foreign exchange, poor internal transport infrastructure coupled with the great distances involved, the lack of convenient and reliable suppliers, and unfavourable price ratios (Bedingar and Degefa, 1990). Furthermore, there is generally scarce technical information on the nutritive value of these AIBs, particularly in mixed diets with locally available roughages for ruminants. The estimates (Table 2.1) reveal that in 1984, Southern Africa was the major producer of molasses, followed by Eastern Africa, West Africa, and Central Africa in that order. West Africa dominated in groundnut cake, palm kernel cake, and fishmeal production while Eastern Africa and Southern Africa dominated in cottonseed cake and sunflower seed cake production. The nutritive value of protein sources AIBs are shown on Table 2.2.

Sunflower oil cake

Sunflower (*Helianthus annuus*) seed cake is a source of high-quality protein (Table 2.2) and can be used freely in balanced diets for poultry and pigs owing to the absence of toxic compounds. While it has been used extensively in ruminant feeding in temperate countries, there is scarce information on its utilization in ruminant feeding in SSA. Although no information on aggregate feed use of sunflower cake by species of livestock was accessed in this review, Bedingar and Degefa (1990) reported that where sunflower has been used in animal production, much of it has probably been fed to monogastrics rather than to ruminants. Exploring the potential of sunflower seed cake in mixtures with typical roughages_for ruminant is therefore essential.

Table 2.1 Production, utilization and real export values of by-products in Sub-Saharan Africa, 1984[a].

Region		Molasses	G. nut* cake	C. seed* cake	SF. seed* cake	Palmk.* cake	Fish meal
West Africa	Production	160	270	93	NA	211	26
	Feed use	31	190	43	NA	173	6
	Exports	43	102	53	NA	43	22
East Africa	Production	415	84	268	21	NA	0
	Feed use	235	50	210	21	NA	2
	Exports	101	101	34	0	NA	0
Central Africa	Production	77	53	48	8	57	3
	Feed use	57	53	40	8	33	1
	Exports	13	0	7	NA	23	2
Southern Africa	Production	603	36	87	34	NA	1
	Feed use	118	33	87	34	NA	2
	Exports	283	7	4	0	NA	_b
SSA	Production	1255	442	497	63	271	29
	Feed use	441	327	381	63	210	11
	Exports	441	210	98	0	67	25
	Export value	18098	20840	13140	0	6761	NA

Source: Bedingar and Degefa,1990

a Quantities are in 1000 metric tons and Export value is in 1000 US dollars.
b Less than half of the unit.
NA: not available
* G. nut - Groundnut; C. seed = Cotton seed; SF = Sunflower; Palmk.= Palm kernel

Table 2.2 Feed values of major protein source agro-by products in Sub-Saharan Africa (Data expressed on an As-Fed and Dry Matter Basis for cattle)

Byproduct	Production ('000MT, 1984)	Dry Matter (%)	ME (Mcal/kg) (%)	CP (%)	DP (%)	TDN (%)
Groundnut cake	442	AF 94	3.27	46.4	41.7	83
		DM 100	3.50	49.6	44.6	89
Cottonseed cake	497	AF 92	2.63	36.5	23.1	68
		DM 100	2.87	39.8	25.1	74
Sunflower seed cake	63	AF 93	2.49	41.5	36.9	69
		DM 100	2.68	44.6	39.6	74
Palm kernel cake	271	AF 92	3.05	18.8	15.9	84
		DM 100	3.31	20.4	17.3	91
Fish meal	29	AF 92	3.38	60.9	54.2	85
		DM 100	3.69	66.2	59.2	95

Source: Bedingar and Degefa,1990

Note:

ME = metabolizable energy; CP = crude protein

DP = digestible protein;

TDN = total digestible nutrients.

AF = As fed

DM = Dry matter

2.4 Ruminant Feed evaluation methods

Productivity of ruminants depends on adequate nutrition with respect to composition and quality of feedstuffs, which is mainly reflected in voluntary intake and digestibility. The digestion is achieved through essential but complex symbiotic association with micro-organisms living in the gut of ruminants. Although feed quality assessment has been primarily viewed in terms of nutrient composition and digestibility, the accumulated

knowledge of gastro-intestinal physiology, and its influence on nutrient utilization, have contributed to orient development of feed evaluation techniques towards those that mimic the fate of feed nutrients in the gut. Such approaches have reduced dependence on animals and created greater flexibility in feed evaluation. The digestibility of feeds can be estimated by *in vitro* biological methods which simulate the digestion process. Biological methods are more meaningful since microorganisms and enzymes involved are more sensitive to factors influencing the rate and extent of digestion than are chemical methods (Van Soest, 1994). Three major biological digestion techniques used to evaluate the nutritive value of ruminant feeds are: (1) digestion with rumen microorganisms as in the work of Tilley and Terry (1963) or gas method (Menke *et al.*, 1979), (2) Enzymatic digestibility assays e,g. ell-free fungal cellulase (De Boever *et al.*, 1986), and (3) *in situ* incubations of samples in nylon bags in the rumen (Mehrez and Ørskov, 1977). *In vitro* methods have the advantage of not only being less expensive and less time-consuming, but they also allow one to control experimental conditions more precisely than do *in vivo* trials.

The technique of Tilley and Terry (1963) became an important tool for the evaluation of ruminant feeds and is used widely because of its convenience, particularly when large-scale testing of feedstuffs is required. This method is employed in many forage evaluation laboratories and involves two stages in which forages are subjected to 48 h fermentation in a buffer solution containing rumen fluid, followed by 48 h of digestion with pepsin in an acid solution. The method was modified by Goering and Van Soest (1970), in that the residue after 48 h incubation was treated with neutral detergent solution (NDS) to estimate true dry matter digestibility. Although the method of Tilley and Terry (1963) has been extensively validated with *in vivo* values (Van Soest, 1994), the method appears to have several disadvantages. For instance, the fact that it gives end-point measurement of single samples implies that unless lengthy and labour-intensive time-course studies are made, the technique does not provide information on the kinetics of forage digestion. Furthermore, the net residue determination through digestion with pepsin followed by treatment with NDS renders the method to be unsuitable for determination of fermentation end product fractions such as microbial matter.

Enzymatic digestibility assays (Jones and Hayward, 1975; Dowman and Collins, 1982; De Boever *et al.*, 1986) which use enzymes instead of micro-organisms have appeared largely as

a result of the increased availability of commercially produced enzymes. Enzymatic methods of evaluation are routinely used as end-point digestibility procedures and therefore suffer from similar disadvantages as the Tilley and Terry (1963) technique. The enzymes may be insensitive to factors such as associative effects and toxins which can affect microbial degradation. However, the main advantage of the enzymatic method over the rumen fluid methods is that it does not require a fistulated animal as inoculum donor. Unlike the method of Tilley and Terry (1963), results from the enzymatic method have not been extensively validated with *in vivo* values.

In situ nylon bag technique (NBT) has been used for many years to provide estimates of both the rate and extent of disappearance of feed constituents (Mehrez and Ørskov, 1977). However, several criticisms have emerged concerning the suitability of this technique as highlighted. Only a small number of samples can be assessed at any one time, and it also requires at least three fistulated animals to account for variations due to animal. It is therefore of limited value in laboratories undertaking routine screening of a large number of samples. In addition, it is laborious and requires large quantities of samples. Tiny feed particles may disappear through the nylon bag pores and this can cause error in estimation. For instance, Dewhurst *et al.* (1995) compared NBT with *in vitro* Tilley and Terry (1963) method and found that the former overestimated the fermentation. The extent of overestimation was strongly related to the carbohydrate composition of feeds, particularly at short incubation times, suggesting that it was caused mainly by loss of the rapidly fermentable fraction from bags before it was fermented. Suspending nylon bag samples in the voluminous rumen may also result in overestimation of digestibility for feeds with anti-nutritive compounds such as tannins. This is because tannins can be solubilised but might be indigestible (Makkar *et al.*, 1998). On the other hand, Ørskov and Ryle (1990) indicated the possible underestimation of dry matter loss from the nylon bag at early periods of incubation due to adherence of microbes. For poor quality roughages, adherence of microbes at early stages can even lead to higher weights and thus distortion of results (Getachew *et al.*, 1998). Other discrepancies of NBT include variations in qualities of nylon bags from different manufactures and laboratories, size of the bags, the animals used and amounts of samples incubated.

2.4.1 *In vitro* gas production technique

The close association between rumen fermentation and gas production has long been recognised (Tappeiner, 1884, cited by Marston, 1948), but the history of the rumen fermentative gas measuring technique started in the early 1940s (Quin, 1943). The gas measuring technique was considered to be a routine method of feed evaluation after the work of Menke *et al.* (1979), where a high correlation between gas production *in vitro* and *in vivo* apparent digestibility was reported. *In vitro* rumen gas techniques (IVGPT) has gained popularity as a research tool, as compared to other *in vitro* techniques, due to their ability to generate fermentation kinetics information using small amounts of single or combinations of feed samples, thereby allowing large number of samples to be evaluated simultaneously. Researchers have used IVGPT to predict digestibility of feeds (Blümmel *et al.* 2005), study effects of secondary compounds on rumen microbial activity (Makkar, 2005), assess kinetics of fermentation (Campos *et al.*, 2004; Calabrò *et al.*, 2005), study associative effects of various types of feeds (Rymer *et al.*, 2001; Cone and Van Gelder, 1999), examine influences of feed additives on rumen fermentation (Carro *et al.*, 2005) and study partitioning of fermented substrates (Moss and Newbold, 2000; Rymer and Givens, 1999).

Feed degraded in the rumen may be partitioned either to microbial biomass production, or to fermentation. The end products of fermentation process are short chain fatty acids (SCFA) and gases (Figure 2.2). The microbes ferment the degraded substrate in order to obtain energy (ATP) for their own growth. In the process microbes multiply and are passed to the lower gut where they get digested to become the major source of protein for the host. The most important SCFA are acetate, propionate and butyrate. The SCFA are absorbed through the gut wall and metabolized to become the source of energy for the host ruminant. Energy concentrations of the SCFA are 14.6, 20.8 and 24.9 MJ kg^{-1} for acetate, propionate and Butyrate, respectively (Moss, 1993). Production of SCFA is accompanied with production of the gases, principally methane (CH_4) and carbon dioxide (CO_2). Methane has an energy concentration of 55.6 MJ kg^{-1} (Moss, 1993) and its production represents feed energy loss besides its being a greenhouse gas.

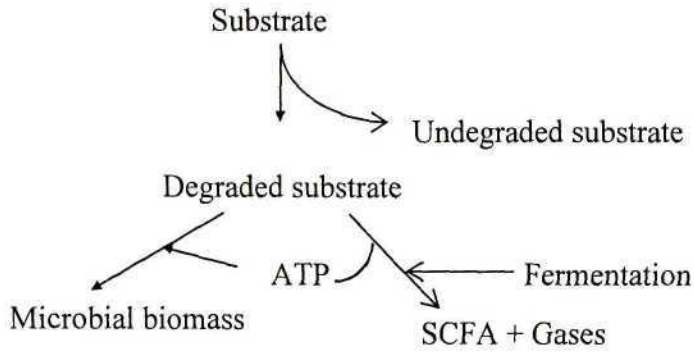


Figure 2.2 The partitioning of degraded substrate between different products

Rumen gas production is basically the result of fermentation of carbohydrates to SCFA (Wolin, 1960; Beuvink and Spoelstra, 1992; Blümmel and Ørskov, 1993). Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation (Wolin, 1960). The contribution of fat to gas production is negligible. When 200 mg of coconut oil, palm kernel oil and/or soybean oil were incubated, only 2.0 to 2.8 ml of gas were produced while a similar amount of casein and cellulose produced about 23.4 ml and 80 ml gas (Menke and Steingass, 1988). The gas produced in the IVGPT is the direct gas produced as a result of fermentation (CO_2 and CH_4) and the indirect gas produced from the buffering of SCFA (CO_2 released from the bicarbonate buffer). For roughages, about 50% of the total gas is generated from buffering of the SCFAs and the rest is evolved directly from substrate fermentation (Blümmel and Ørskov, 1993). Gas is produced mainly when substrate is fermented to acetate and butyrate. Substrate fermentation to propionate yields indirect gas only, therefore, relatively lower gas production is associated with propionate production (Wolin, 1960; Hungate, 1966; Van Soest, 1994).

Since the utilisation of roughages is largely dependent upon microbial degradation within the rumen, description of roughages in terms of their degradation characteristics, including the kinetics, would provide a useful basis for their evaluation (Hovell *et al.*, 1986). Kinetics of fermentation of feedstuffs can be determined from fermentative gas and the gas released from buffering of the SCFAs (Makkar, 2004). Since the gas production technique is not subject to the errors associated with the loss of fine particles, as is the case with the *in situ* technique, it

has more potential to provide reliable data on the kinetics of rumen digestion (Rymer, 1999).The kinetics of gas production is dependent on the relative proportions of soluble, insoluble but degradable, and undegradable particles of the feed (Getachew *et al.*, 1998). The relative contribution of these fractions in a given feed influences the outcome of fermentation (rate, extent and stoichimetery), hence the nutritive value. It is expected that individual feeds and their combinations will produce different fermentation kinetics, and this can be beneficially exploited or the limiting factors identified.

Various mathematical models have been used to describe gas production profiles. The exponential model (Ørskov and McDonald, 1979) was originally used to describe degradation kinetics as measured with the nylon bag method, but the model has also been used to describe kinetics of gas production data (Blümmel *et al.*, 1990; Khazaal *et al.*, 1993; Siaw *et al.*, 1993). This model is based on first-order kinetics, assuming a constant fractional rate of fermentation (Groot *et al.*, 1996). Since some feed particles ferment at different rates, this assumption in the exponential model is not universally valid. Beuvink and Kogut (1993) evaluated various curve fitting models and reported that the exponential model resulted in larger residual mean squares as compared to sigmoidal models. Groot *et al.* (1996) introduced a three-phasic model which differentiates soluble, insoluble but fermentable, and microbial turnover. Conceptually, this model should provide useful data, however, it requires sophisticated equipment to record gas production at different times of incubation. Furthermore, the model performed poorly when recently used in the prediction of voluntary feed intake of 24 roughages from Ethiopia (Blümmel *et al.*, 1998). A simpler two-compartmental mathematical model was applied by Campos *et al.* (2004) and performed well in distinguishing gas production (amount and rate) from soluble and insoluble substrate fractions as well as lag time.

2.4.2 Prediction of animal response using *in vitro* gas production parameters

Although several workers have found good relationships between *in vitro* and *in vivo* measures, poor relationships among *in vitro* parameters and between *in vitro* and *in vivo* measures have been also recorded. Good relationships were reported by Blümmel and Ørskov (1993) who observed a high correlation between the total volume of gas produced and

digestible dry matter intake as well as growth rate of steers fed cereal straws. Blümmel and Becker (1997) observed good relationships between the voluntary intake of roughages and the volume of gas produced. Romney *et al.* (1998), studying a range of feeds, observed that the total volume of gas produced was well related to the digestibility of hays and straws, but was only well related to the digestibility of other foods if a medium rich in N was used. Murray *et al.* (1998) found that the intake of straw based diets was well related to the underlying rate of gas production, while apparent DM digestibility was related to the time-dependent rate and the volume of gas produced at 48 h. They suggested that the intake of roughages, and perhaps more importantly supplemented roughage diets, could be predicted using the gas production technique. Rymer and Givens (1999) reported good relationship between the amount of starch apparently disappearing in the rumen and the total volume of gas produced when data were fitted to the model of Groot *et al.* (1996). The measurement of the partitioning factor (Degraded substrate:gas volume ratio mg/ml), described by Blümmel *et al.* (1997a), was compared with measures of rumen microbial protein synthesis *in vivo* (Blümmel *et al.*, 2003). Rumen microbial protein synthesis was estimated using urinary purine derivatives in 13 goats fed two varieties of maize stover. Partitioning factor was positively correlated with the efficiency of microbial protein synthesis of the maize varieties. This suggested that substrate dependent factors which affect the efficiency of microbial protein synthesis may be detected by IVGPT. When applied to lactating cows, Nataraja *et al.* (1998) observed that the IVGPT proved to be a better predictor of the metabolisable energy (ME) value of mixed diets than either chemical analyses (based on the detergent system) or digestibility trials.

On the contrary, several studies have found poor prediction of *in vivo* response by measurements derived from IVGPT. For instance, Mills *et al.* (1998) who reported a poor relationship between the gas production profile and both dry matter intake and digestibility of Mediterranean forages. Poor prediction of organic matter degradability of legume grains (Abreu and Bruno-Soares, 1998) and whole wheat crop (Adesogen *et al.* 1998) have also been reported. The digestible organic matter content of Mediterranean forages was only moderately predicted (R^2=0.609) by IVGPT (Susmel *et al.*, 1998). A poor relationship was also observed by Khazaal *et al.* (1993) when comparing the volume of gas produced from the incubation of hays and the intake and apparent digestibility of the hays.

The use of various models for intake prediction was investigated and it currently appears that combination of gas volume measurements (4–8 h) with concomitant determination of the amount of substrate degraded (> 24 h) is superior to the models based on kinetics of gas production only (Blümmel *et al.*, 1997a, Blümmel *et al.*, 1997b; Blümmel and Becker, 1997). The *in vitro* gas production from NDF explained more (82% vs. 75%) of the variation in dry matter intake than gas production from whole roughage (Blümmel and Becker, 1997).

2.5 Role of animal diet in manure quality

In recent years, the rising costs of inorganic fertilizers, the high concerns and interests in environmental conservation and increasing rural poverty in developing countries has led to a lot of scientific efforts being geared towards the development of technologies to utilize organic soil amendment based on locally available resources including crop residues, animal manures and green manures. Such efforts have been reported by by Myers *et al.* (1994), Lekasi *et al.* (2003) and Nhano *et al.* (2004). In the past, particularly in SSA livestock were predominantly grazed extensively on natural pastures, kralled or kept to sleep on the farms and manure collected for cropland soil fertility amendments (Powell *et al.*, 2004). Such practices are rapidly diminishing due to population pressure on land, which has led to livestock getting confined to small production units. Considering the importance of sustainable agricultural production, a holistic understanding of nutrient recycling coupled with determination of efficiency factors at different stages is important. Supply of nitrogen is the most commonly recognized value of manure. The fate of manure nitrogen is shown Figure 2.3.

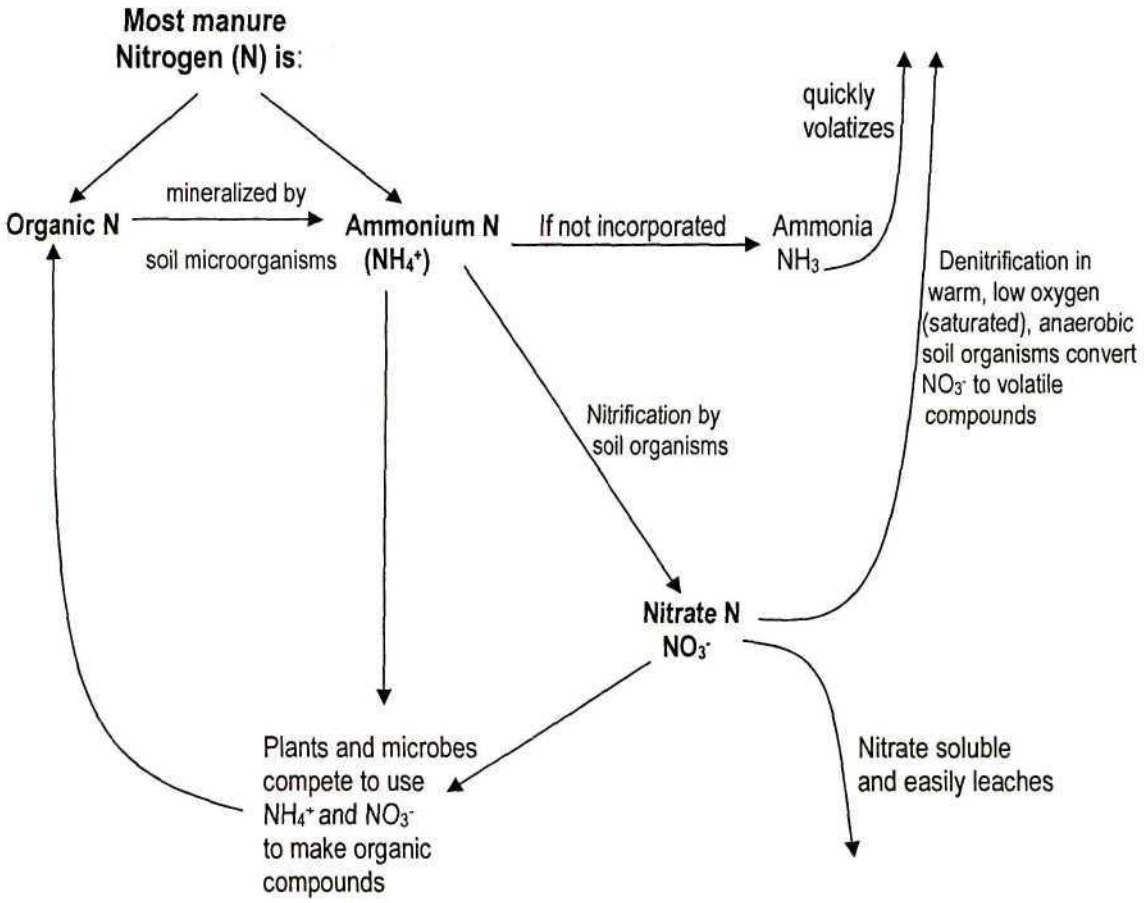


Figure 2.3 Pathways of nitrogen flow from manure (Adapted from Follett, 2004)

It is unequivocal that the quality of manure is influenced by the diets fed to the animal concerned (Mugwira and Murwira, 1997; Delve *et al.* 2001; Lekasi *et al.* 2003). Delve *et al.* (2001) suggested that poor quality diets were better fed to animals, instead of composting, and the manure produced should be utilized for soil fertility amendment. In western Africa, manure contracts between sedentary farmers and pastoralists are practised whereby the manure is exchanged for grain, crop residues or water (Powell *et al.*, 2004). On the other hand, application of manure can be counter productive due to slow release of nutrients and N immobilization (Nhano *et al.* 2004; Delve *et al.*, 2001. These findings emphasize the need for systematic linkage of the nutritive characteristics of the diets to the animal performance, quantity and quality of the manures and their influence on the soil fertility so that optimisation of crops/plants and animals production through nutrient recycling can be attained. However, such linkages have been lacking in past studies, a concern which was also raised by Powell *et al.* (2003), and partly addressed in this study.

2.6 Conclusions and justification

Mixed farming has been the basis of many sustainable farming systems in many societies, but has lost in focus because of the specialization on only crops or animals. Attention to mixed farming is gaining global importance due to the increasing need to optimize utilization of scarce resources, nutrient recycling, environmental conservation and sustainability. Thus modern technologies need to re-address ways of understanding when, where and how mixed farming approaches can be beneficial through complementary roles played by crops and animals.

Livestock make an important contribution to the sustainability of intensive smallholder farming in Sub-Saharan Africa. Apart from the products directly obtained, livestock may also contribute to soil fertility and crop production. In addressing livestock-crop technologies, it is therefore important to consider inter-relationships and synergies obtainable from integration, rather than issues confined to either crops or livestock alone.

Production of ruminants in Sub-Saharan Africa is highly dependent on utilization of roughages. The roughages are predominantly of poor quality due to high fibre and low protein content. For these reasons supplementation with protein sources and other feeds with high degradability is essential in order to boost utilization. Commonly used supplements include forage legumes and agro-industrial by-products. Because of diversity of climatic conditions, identification of adapted forages (roughages and supplements) and factors influencing their quality is important. The factors may include the stage of maturity and interaction between the roughages and supplement type or supplement level. Basically, the quality is influenced by the feeds chemical composition and the impact of this on nutrients release. These can be investigated in a laboratory (*in vitro*) in order to identify the limitations and remedial interventions before testing the feeds *in vivo*.

Use of biological *in vitro* methods provide meaningful means to evaluate ruminant feeds since microorganisms and enzymes involved are sensitive to factors influencing digestion, which cannot be determined through chemical methods. Of the biological methods, the *in vitro* gas production technique has great potential as it is able to provide fermentation kinetics measures of many samples at the same time, hence enabling pre-screening of several feeds

and rations prior to *in vivo* verification. Although gas production technique is rapidly gaining popularity, there is scarcity of information directly linking nutritive measurements determined by gas production to *in vivo* performance. Thus, there is a need to perform simultaneous evaluation of selected diets using gas production technique and animal feeding trial, from which the linkage between *in vitro* and *in vivo* measurements can be drawn. This would help to establish the authenticity of gas production technique as a tool in feed evaluation. It would also help in identifying important parameters to include in routine evaluation using this method.

In this study, roughages and protein supplements typically available in SSA crop-ruminant systems were evaluated by determining their chemical composition, *in vitro* nutritive value measurements, *in vivo* performance by sheep and the relationships among the measurements. Manure production, quality and mineralization were also determined to assess the influence of diets on nutrients recycling.

Chapter 3

NUTRITIVE VALUE OF MAIZE STOVERS HARVESTED AT TWO STAGES OF MATURITY AND MIXED WITH DIFFERENT TYPES AND LEVELS OF PROTEIN SUPPLEMENTS[a]

ABSTRACT

Studies were conducted on two maize stovers types (ST) harvested at grain milk (MM) or dry (MD) stages and mixed with graded ratios (20, 40, 60, 80%) of protein supplements (PS) including Lucerne hay (LH), Lespedeza hay (LPZ) and Sunflower oil cake (SFC). Automated *in vitro* gas production technique (IVGPT) was used. The crude protein (CP) content of LH, LPZ and SFC were 199, 73 and 391 g/kg DM, respectively. The corresponding NDF values were 355, 525 and 324 g/kg DM. The degradability was high in both stovers with that of MM being overall higher ($p<0.001$) than MD (mean 775.9 vs 720 g/kg DM). MM rations had higher gas production (GP) than MD (range 95.4-173.3 vs 98.8-163.2 ml/g DM for MM and MD, respectively). Only ST x PS interaction had an effect ($p<0.001$) on the ratio of degradability to gas production i.e. the partitioning factor (PF), in which MD:LPZ rations had highest values apparently as a result of suppressed GP. The rate of GP from fibre fraction (b_1) was increased ($p<0.001$) by supplementation with LH or SFC although the margins were small across the rations. ST, PS and ST x PS interaction affected lag time (C) whereby MD and supplementation with LPZ caused increase of C. MM showed better nutritive value by having marginally higher degradability and shorter lag of fermentation. Among the PS, LH and SFC had generally similar but superior nutritive effects as compared to LPZ. It is suspected that presence of tannins in LPZ was responsible for the negative effects observed. The results suggest that supplementation of the stovers with at most 40% DM contributed by LH or SFC would optimize their utilization, since improvement in degradability or lag time were minimal beyond this ratio.

[a] J. O. Ouda and I.V. Nsahlai. Nutritive value of maize stovers harvested at two stages of maturity and mixed with different types and levels of protein supplements. *Journal of Applied Animal Research (Submitted)*

3.1 INTRODUCTION

Maize is the third most produced grain after wheat and rice but leads in crop fodder production both globally and in Africa. Annual maize fodder yields have been estimated to be 1816 and 340 million tonnes in the world and Africa, respectively (Reddy *et al.*, 2003). Maize is largely grown and left to dry to over 80% grain DM in the fields before harvesting. Harvesting at grain milk stage for human food is also popular. There are many maize varieties developed for different climatic conditions. Consequently, the nutritive characteristics of maize stovers as ruminant feed can be highly varied. Maize stovers have low crude protein (CP) content (Fadel, 1999, Tolera *et al.*, 1999, Hindrichsen *et al.*, 2004), which is a major constraint to their utilization by ruminants. The CP is essential in supplying nitrogen (N) required for rumen microbes growth and consequently enhanced degradation of consumed roughage. Supplementation of stover based rations with cheaply available feeds having high CP is therefore expected to improve the productivity of maize-ruminant systems.

It is essential to evaluate the nutritive potential of stover based rations with alternative CP supplements in order to establish their suitability. In most tropical regions where maize-ruminant systems are predominant, the potential protein supplements include browse legumes and agro-industrial by-products such as oil cakes. Browse legumes belong to a highly heterogeneous group of plants, with CP content ranging from 81 to 306 g/kg dry matter (DM), and have variable ruminal degradable and intestinally digestible fractions (Kaitho *et al.*, 1993). Lucerne (*Medicago sativa* L) is a globally popular protein supplement for ruminants. However, it has high climatic and agronomic input requirements, which limits its cultivation by the numerous resource-poor farmers. A forage legume such as Sericea lespedeza [*Lespedeza cuneata* (Dun-Cours) G. Don] may be a suitable alternative. Sericea lespedeza (lespedeza) is a tall growing, drought tolerant, coarse-stemmed perennial legume that is productive on wide range of soils including those with poor fertility (McGraw and Hoveland, 1995, Powell *et al.*, 2003).

Evaluation of the potential benefits arising from supplementation of stover based rations would require screening of numerous dietary combinations; ideally through *in vivo* trials. However, in agreement with Dijkstra *et al.* (2005), *in vivo* trials are expensive and impractical

to screen a large number of diets. *In vitro* methods are often used as an alternative, but the suitability of a chosen method is essential. The present study used *in vitro* gas production technique (IVGPT) whose merits include being relatively of low cost, ability to screen a large number of substrates concurrently and provision of fermentation kinetics information (Dijkstra *et al.*, 2005). The study objective was to evaluate the influence of different types and levels of protein supplements on nutritive value of maize stover-based rations.

3.2 MATERIALS AND METHODS

3.2.1 Feeds and chemical composition analysis

The study evaluated nutritive attributes of rations comprised of maize stovers (ST) and protein supplement (PS). Stovers were from white maize hybrid PAN6479 cultivated in Kwa-Zulu Natal Province, South Africa. The maize was either harvested at grain milk (MM) or grain dry (MD) stage. The PS feeds included sunflower seed cake (SFC), lucerne hay (LH) and lespedeza hay (LPZ). Sixty whole maize plants were randomly selected from the field, cut at the first node above ground and divided into two groups. One group had morphological components (husks, leaf-sheath and stem) individually separated while the other was left whole. The stovers or morphological components were chopped, placed in labelled containers and dried in fanned oven dried at 50°C overnight or until they were fit for milling through a 1-mm screen. Dry matter (DM) was determined by drying the samples in a fanned oven at 100°C overnight. Total ash was determined by igniting the samples in a muffle furnace at 500°C overnight. Nitrogen was determined by micro-Kjeldahl method and CP was calculated as N x 6.25 according to AOAC (1990). Methods described by Van Soest *et al.* (1991) were used to determine the neutral detergent fibre (NDF) and acid detergent fibre (ADF). Soluble ash was determined by extracting the total ash with hot water followed by filtration using a crucible with sintered glass filter, ignition at 500°C overnight and weighing the residue to determine insoluble ash. Soluble carbohydrates fraction (CHO_{sol}) was calculated by applying formula of Harris (1970) as follows:

$$CHO_{sol}\ (g/kg) = 1000 - (NDF + \text{Crude fat} + \text{Crude protein} + \text{soluble ash g/kg}).$$

3.2.2 Gas production analyses

In vitro automated gas production technique (IVGPT) described by Pell and Schofield (1993) was used (Appendix 8). A total of 1.0 ± 0.0010 g DM in proportions (MS:PS) of 0:100, 20:80, 40:60, 80:20 and 100:0 % were weighed into 250 ml Duran bottles for incubation. The incubation was done thrice with all the treatment rations represented each time. Buffer solution was prepared using ingredients of McDougall (1948) whereby solution A was prepared by dissolving 19.60, 7.40, 1.14 , 0.94 and 0.26 g of $NaHCO_3$, Na_2HPO_4, KCl and $MgCl.6H_2O$, respectively in 2 L distilled water. Solution B was separately made by dissolving 5.3 $CaCl2.2H_2O$ g in 100 ml distilled water. Immediately before starting the incubation, 2 ml of solution B was added drop-wise to a warming up solution A while continuously stirring, to form complete buffer solution. The buffer solution was cooled to 39°C after which 67 ml from it was added to sample bottles. The bottles, including three sample blanks, were transferred to the incubator maintained at 39°C where they soaked for at least one hour. In the meantime a mixture of rumen fluid was collected from three cannulated cows before morning feeding. The cows were maintained on a diet composed of 50:50 grass:lucerne hay. The rumen fluid was filtered through four layers of muslin cloth into a pre-warmed (39°C) vacuum flask flushed with carbon dioxide (CO_2). Inoculation was done by adding 33 ml of rumen fluid to the sample bottles under a stream of CO_2. The bottle lids were tightened and pressure sensors fitted. Settlement time of approximately 30 min was allowed to pass before starting pressure logging at 20 minutes interval in a 48-h incubation. The pressure data was converted to gas volumes (ml) using a predetermined calibration equation. Gas profiles were fitted into model described by Campos *et al.* (2004) to derive GP kinetics as follows:

$$y = \frac{A}{1+\exp[2+4a_1(C-t)]} + \frac{B}{1+\exp[2+4b_1(C-t)]}$$

where y is the total gas volume (ml) at time t, A and B the gas volume (ml) from fast (soluble sugars and starch) and slowly (cellulose and hemicellulose) degradable fractions, respectively, a_1 and b_1 are the degradation rates (h^{-1}) for fast and slowly degradable fractions, respectively, and C is the bacteria colonization or lag time (h).

3.2.3 Determination of degradability

At the end of incubation, terminal pH was taken and samples centrifuged at 18,000 G. The supernatant was discarded and the pellet residue (R) dried in a fanned oven at 100°C for 48 h until constant weight was attained. The difference in weight between R and incubated material was regarded as apparently degraded fraction (ApDeg). R was refluxed with neutral detergent solution (NDS) and the residue (NDF) dried. The weight of NDF was similarly subtracted from that of the incubated material and the difference regarded as truly degraded fraction (Degradability).

3.2.4 Statistical analysis

SAS (2002) software was used to perform statistical analysis and fitting the gas profile data to the model described by Campos *et al.* (2004). Analysis of variance (ANOVA) was performed to determine treatment effects whereby stover type, supplement type, supplement ratio and the interactions between and among them were used as sources of variation. Analysis of covariance (ANCOVA) was performed using CP content as covariate. The sources of variation that maintained significant effects in the ANCOVA were taken to cause effect independent of CP content. Means were compared by least significant difference (LSD). Coefficient of variation (cv) was calculated to assess consistency of the observed values.

3.3 RESULTS

3.3.1 Contribution of morphological components and chemical composition

The contributions to the total biomass by different morphological parts of the stover are shown on Table 3.1. The proportion (as % of total DM) of leaf-sheath fraction was lower (24.5 vs 36.3 %) and that of husk was higher (31.5 vs 16.6%) in MD as compared to MM. The proportion of stem remained stable. The chemical analyses of the stover (whole and components) and the PS are shown on Table 3.2. The fibre content (NDF) of the various components was higher in MD while CP was highest in the leaf-sheath and lowest in the stem

in both MM and MD. NDF content of MM husks was markedly lower than that of MD (546.9 vs 739.8 g/kg) but NDF:ADF ratios remained the same at approximately 2:1 in both. MM had higher CHO_{sol} content than MD. The PS showed high variation in CP content with SFC having the highest and LPZ the lowest.

3.3.2 Gas production kinetics

The kinetics of GP are shown on Table 3.3. The two stover types showed similarity in the fermentation of the soluble fraction depicted by close A values within each PS type. The PS types differed ($p<0.001$) in A values and there was interaction ($p<0.01$) of PS type x PS ratio. The highest A values were recorded in LH and the lowest in LPZ rations. The value of A increased and remained then stable when LH or SFC ratios were beyond 40% while LPZ rations had low A values and did not show a clear trend. Only PS type influenced the rate of fermentation of the soluble component depicted by a_l with rations of LPZ having highest values.

The B values were higher ($P<0.001$) in MM rations indicating a higher fermentation of its fibre fraction and there was generally a decrease in B as PS ratio increased. Both PS type and PS ratio influenced B ($p<0.001$) with LH having the highest and LPZ the lowest values. ST x PS type interactions also influenced B ($p<0.001$) whereby MD showed lower values with LPZ. Both stovers exhibited similar rate of fibre fermentation as depicted by their similarity in b_l. Although PS, ST x PS and PS x PS ratio interactions all had an effect on b_l the margins were small. A major nutritive superiority of MM was its rapid colonization by rumen microbes depicted by shorter lag (C). Other factors which affected C included PS type, PS ratio and ST x PS interaction. LPZ had longer C than LH and SFC, which had similar values. ST x PS interaction also had an effect on C ($p<0.001$) with MD:LPZ rations having the longest lag.

3.3.3 Degradability and partitioning factor

The results of degradability, GP and PF are shown on Table 3.4. Normal fermentation was maintained as indicated by near neutral (6.65-7.08) terminal pH values. Degradability was high in both stovers with that of MM being overall higher ($p<0.001$). Both PS and PS x PS ratio affected degradability. Rations of LPZ had lower values as compared to LH and SFC which were similar. Increasing the ratio of LPZ resulted in a decrease in degradability, but increasing ratios of either LH or SFC beyond 20% caused a marginal increase in degradability. The difference in degradability between LH and SFC was minimal across supplementation ratios. MM rations produced more ($p<0.001$) gas than MD and the ST x PS interaction was significant ($p<0.01$). Both stovers had similar GP when supplemented with LH or SFC but MD had lower GP when supplemented with LPZ. Increased ratios of LPZ or SFC resulted in a decrease in GP while the ratio of LH beyond 20% caused no change. The ratio of degradability to GP i.e. the PF was only affected by ST x PS interaction whereby stable PF was maintained in LH and SFC rations but MD:LPZ had higher values.

Table 3.1 Dry matter content and contributions to total biomass (DM basis) of different morphological components of maize stover harvested at different stages of grain maturity (%).

Morphological fraction	Maize stage of maturity			
	Grain milk stage stover (MM)		Grain dry stage stover (MD)	
	DM content	Biomass contribution	DM content	Biomass Contribution
Leaf (blade + sheath)	30.7(± 0.89)	36.3(± 0.02)	85.5(± 0.66)	24.5(± 0.02)
Husk	20.2 (± 0.68)	16.6 (± 0.01)	95.4 (± 1.31)	31.5(± 0.05)
Stem	19.4 (± 0.50)	47.7 (± 0.02)	91.7 (± 0.43)	44.0 (± 0.05)

Table 3.2 Feeds chemical composition.

Feed	CHOsol	CP	NDF	ADF	Ash
Milk stage stover - whole	347.7	47.6	594.4	361.8	75.5
leaf +sheath		77.3	609.3	377.1	110.8
Husk		47.5	597.7	300.8	34.2
stem		22.6	599.4	388.2	48.0
Dry stage stover -whole	232.5	36.2	724.6	440.5	49.5
leaf +sheath		65.7	746.1	460.7	64.5
Husks		61.6	795.5	419.9	30.2
stem		42.5	730.1	481.7	30.1
SFC	214.3	390.1	324.2	215.3	68.2
LH	369.2	198.5	355.0	270.0	85.8
LPZ	347.9	72.6	525.1	395.4	57.5

SFC sunflower cake, LH Lucerne hay, LPZ lespedeza hay, CP- crude protein, EE-ether extract, NDF-neutral detergent fibre, ADF-acid detergent fibre.

Table 3.3 Mean gas production kinetics of maize stover harvested at different stages and fermented using rumen fluid *in vitro*

Stover type (ST)	Supplement (PS)	Stover ST:PS	A	B	a_I	b_I	C
Milk stage (MM)		100:0	19.4	155.4	0.21	0.034	3.9
	Lucerne	80:20	24.9	139.4	0.16	0.037	3.6
		60:40	43.4	122.9	0.12	0.041	3.4
		40:60	45.1	113.2	0.12	0.042	3.5
		20:80	39.6	117.6	0.13	0.039	2.8
		0:100	44.7	109.1	0.13	0.038	1.50
	Lespedeza	80:20	15.3	151.7	0.18	0.036	4.4
		60:40	20.8	131.6	0.24	0.034	3.8
		40:60	17.3	136.4	0.27	0.030	3.3
		20:80	16.9	109.5	0.23	0.032	3.3
		0:100	22.6	81.7	0.18	0.037	3.1
	Sunflower cake	80:20	28.4	139.8	0.12	0.038	3.6
		60:40	33.0	128.5	0.16	0.036	2.9
		40:60	33.7	108.7	0.16	0.040	3.9
		20:80	33.4	95.7	0.16	0.040	4.0
		0:100	34.9	82.0	0.27	0.042	4.2
Dry stage (MD)		100:0	21.8	139.5	0.15	0.035	4.6
	Lucerne	80:20	32.5	132.2	0.17	0.037	4.8
		60:40	33.0	125.4	0.15	0.038	4.0
		40:60	36.4	120.6	0.14	0.038	3.8
		20:80	41.4	116.1	0.15	0.038	2.5
	Lespedeza	80:20	23.7	110.8	0.18	0.037	7.4
		60:40	23.2	103.8	0.17	0.034	6.9
		40:60	24.2	87.1	0.16	0.039	7.3
		20:80	14.9	84.3	0.26	0.032	7.4
	Sunflower cake	80:20	34.4	117.5	0.15	0.037	4.1
		60:40	29.9	116.7	0.09	0.035	3.5
		40:60	33.9	110.0	0.14	0.038	2.8
		20:80	38.5	94.4	0.17	0.041	2.9
		LSD	14.48	19.1	0.17	0.004	2.4
		p	0.001	0.001	ns	0.001	0.001
		cv	31.4	7.32	56.1	6.43	32.2
Sources of variation effects		ST	ns	0.001	ns	ns	0.001
		PS	0.001	0.001	0.001	0.001	0.01
		PS ratio	ns	0.001	ns	ns	ns
		ST x PS	ns	0.001	ns	0.001	0.001
		PS x PS ratio	ns	ns	ns	0.01	ns

A and B- gas volume (ml) from fast (cell content) and slowly (cell wall) degradable fractions, respectively, a_I and b_I - degradation rates (h^{-1}) for fast and slowly degradable fractions, respectively, and C - lag time (h).

Table 3.4 Degradability and gas production of maize stovers harvested at different stages and fermented using rumen fluid *in vitro*

Stover type (ST)	Supplement (PS)	ST:PS	pH	Degradability g/kg DM	Gas production ml/kg DM	Partitioning factor mg/ml
Milk stage (MM)		100:0	6.68	775.2	154.5	5.08
	Lucerne	80:20	6.73	817.9	165.4	5.00
		60:40	6.65	826.6	172.7	4.81
		40:60	6.79	821.5	165.0	5.02
		20:80	6.67	816.4	161.3	5.03
		0:100	7.08	807.8	139.2	5.93
	Lespedeza	80:20	6.79	759.0	173.3	4.39
		60:40	6.72	699.0	153.1	4.57
		40:60	6.87	649.3	144.7	4.50
		20:80	6.88	642.4	135.3	4.72
		0:100	7.00	517.6	95.4	5.54
	Sunflower cake	80:20	6.82	815.8	171.6	4.75
		60:40	6.87	815.7	164.0	4.94
		40:60	6.84	818.1	147.0	5.60
		20:80	6.90	791.7	134.0	6.00
		0:100	6.91	797.7	125.9	6.41
Dry stage (MD)		100:0	6.83	709.3	142.2	5.23
	Lucerne	80:20	6.90	759.0	148.4	5.33
		60:40	6.83	779.4	163.2	4.79
		40:60	6.88	789.3	162.2	4.88
		20:80	6.82	796.7	163.3	4.88
	Lespedeza	80:20	6.75	674.1	137.4	5.07
		60:40	6.82	630.3	110.5	5.82
		40:60	6.80	587.2	125.9	4.66
		20:80	6.88	551.1	98.8	5.70
	Sunflower cake	80:20	6.84	718.5	156.8	4.64
		60:40	7.02	744.4	150.2	5.01
		40:60	6.90	775.1	145.8	5.32
		20:80	6.90	798.2	138.8	5.77
		LSD	0.22	58.3	24.5	0.95
		p	0.12	0.001	0.001	0.01
		cv	2.2	5.0	9.5	10.9
	Sources of variation effects					
		ST	ns	0.001	0.001	ns
		PS	ns	0.001	0.001	ns
		PS ratio	ns	ns	0.001	ns
		ST x PS	ns	ns	0.001	0.001
		PS x PS ratio	ns	0.001	0.001	ns

ns not significant

3.4 DISCUSSION

3.4.1 Role of stover maturity stage and morphological components

Forage maturity stage is known to significantly influence the nutritive value whereby younger age is associated with high quality. In this regard, MM could be expected to have remarkable superiority over MD. The present results however, showed that both stovers had high degradability with that of MM being only overall higher than MD. Consequently, the higher degradability of MM led to its higher GP because under *in vitro* incubation significant proportion of hydrolyzed material would undergo fermentation and produce gas. The results showed that the superiority of MM was as a result of higher and more rapid availability the fibre fraction depicted by similarity in A values, but MM having higher B values and shorter lag (C) as compared to MD. Furthermore, the lower NDF and higher CHO_{sol} in MM further suggest its nutritional superiority over MD, and this is suspected to be responsible for the shorter C exhibited by MM. The close degradability values shown by the two stovers may be therefore an artefact of long *in vitro* incubation and only depict the potential. They also indicated that despite the substantial increase in fibre content (NDF) with maturity, there was minimal impact on the degradable fraction which was an important revelation. The high Deg of the stovers corroborate with the findings by Akbar *et al.* (2002), who reported Deg ranging from 694-768 g/kg DM in a 96 h in situ incubation using stovers obtained from six maize varieties. However, under *in vivo* situations much lower degradabilities have been reported. For instance, sheep fed maize stovers supplemented with various multipurpose trees had degradability ranging from 490 to 513 g/kg (Hindrichsen *et al.*, 2004). This emphasises the need to explore how whether the superior characteristics exhibited by MM, other than degradability, can be of practical value *in vivo*.

Although the Deg can be expected to be higher in the younger MM as compared to MD, the present results, showed that the difference between these stovers was not remarkable. The high Deg of the stovers corroborate with the findings by Akbar *et al.* (2002), who reported Deg ranging from 694-768 g/kg DM in a 96 h in situ incubation using stovers obtained from six maize varieties. However, under *in vivo* situations, much lower maize stover Deg ranging from 490 to 513 g/kg were reported by Hindrichsen *et al.* (2004).

Although chemical composition limitation of maize-stovers has been widely recognised, there has been little consideration of physical/textural limitations due to morphological components. The stem contributing highest fraction of the biomass, followed by husks and lastly leaves corroborated with other findings (Russel, 1986; Harika and Sharma, 1994; Tolera and Sundstøl, 1999). Stem coarseness poses a major limitation to stover utilization, which is more pronounced at the dry stage when the nutritious components (leaf, sheath and husks) become harder and hence more difficult to pluck and chew by a feeding ruminant. Moreover, more leaf material than stem is likely be lost during harvesting, thus the stovers offered to the animals may contain higher stem proportions than those of the present study. Therefore, besides changes in chemical composition at late maturity stage, textural changes can also cause serious limitation to nutritive value, particularly intake. Efforts towards improving productivity of maize-ruminant systems should therefore explore ways to moderate chemical, physical/textural and morphological limitations all together. In previous studies of maize-stovers, Harika and Sharma, (1994) reported varietal differences in quality. Tolera and Sundstøl (1999) reported varietal differences in both chemical composition and contribution of morphological fractions and Patil *et al.* (1977) found no correlation between stover and grain yields. These findings indicate that there are opportunities to improve maize-ruminant productivity through strategic cultivar selection coupled with storage and processing interventions. Processing should include determination of milling size to moderate physical/textural limitations.

As has been pointed out (Dijkstra *et al.*, 2005), the advantage of IVGPT is provision of many parameters to use in rations evaluation. However, caution needs to be undertaken in choosing which parameters to make inference from. Degradability and GP are routinely used as predictors of nutritive value. Considering the present degradability and or GP results, it can be erroneously deduced that stovers at the two maturity stages closely compare. This can be deceptive because under extensive *in vitro* incubation (beyond 24 h) degradable substrate is expected to be exhausted. In this regard, degradability or GP measures would have been good indicators of contrasting nutritive values if the differences were large. The same can be said about PF whose authenticity have been found (Rymer *et al.*, 2001, Blümmel *et al.*, 2005) to depend on, among other factors, the length of incubation. Reliable values were at short (less than 24 h) incubations. It can be therefore argued that extensive incubation durations are good

for establishing the potential degradability and GP. Where rations show close values under such incubations, other parameters should receive closer scrutiny in evaluating the nutritive value. In this respect, the present chemical composition and fermentation kinetics results provided clear evidence of a decline in nutritive value of MD. Specifically, MM showed superiority by having higher CHO_{sol} and lower fibre (NDF), which are stipulated to be responsible for its shorter lag time (C). Long lag time has been reported (Van Soest, 1988) to be an important factor limiting intake and utilization of cereal straws and stovers. Thus the results suggested that MM would support higher ruminant performance, most likely through having higher intake. Although the b_1 values were close, the significant ($P<0.001$) influence of ST x PS interaction whereby SFC had higher b_1 values with MM indicated an opportunity to select supplements and roughages for enhancing the rate of fibre degradation.

3.4.2 Role of protein supplements

Supplements used in this study were endowed, to varying degrees, in CP, soluble material and readily degradable fibre contents. They were expected to promote microbial growth and enhance intensity of hydrolysis and fermentation of hydrolysed material. It is expected that once the microbial requirements are met, further increase of supplements ratios may result in continued extra-cellular hydrolysis of degradable fibre without further change in the intensity of fermentation. The fact that there was a minimum ratio (40%) of LH or SFC beyond which A remained stable was therefore an interesting and important revelation. It could indicate existence of a threshold concentration of soluble fraction beyond which the surplus was assimilated for microbial growth. It could as well be due to the fact that at high concentration of proteins, fermentation stoichiometry was altered towards yielding less gas as has been reported by other workers (Cone and Van Gelder, 1999, Rymer *et al.*, 2001). The two possibilities could as well occur simultaneously with the obtained values representing a balance. As for LPZ, the decrease in degradability and GP as its ratio increased can be attributed to chemical composition, particularly the tannin content. Turner *et al.* (2005) observed high tannin content of 23.1 mg/g in lespedeza, and a much lower value of 0.24 mg/g in Lucerne. Having high tannin content limits the nutritive value, but does not necessarily hamper productivity. Ruminants like goats have been found to tolerate and perform well when consuming high tannin containing plants (Silanikove *et al.*, 1996). Caygill and Mueller-

Harvey (1999) pointed out that some tanniniferous feeds can produce beneficial effects in ruminants, e.g. improved amino acid absorption and anthelmintic effects. Douglass *et al.* (1995) reported greater live weight gain when lambs grazed birdsfoot trefoil versus alfalfa due to condensed tannins (CT) influences in the ruminant digestive tract. Medium concentrations of CT (45 to 55 g CT/kg DM) in forages can improve N-use efficiency in ruminants (Min *et al.*, 2003). Positive attributes of LPZ which have been pointed out include being a long term forage once established, is low cost pasture, provides good quality-early spring or autumn grazing, copes well under water logged conditions, easily makes good quality and 'cheap' hay, less wear-and-tear on mowing equipment and increases summer carrying capacity (2005, H. Botha, pers. Comm., Harmonie Trust, P.O. Box 27, Matatiele 4730, South Africa). Thus it can be said that LPZ use will largely depend on the perceived advantages. In any case, the anti-nutritive effects due to presence of tannins can be ameliorated through use of additives e.g. of polyethylene glycol (Getachew *et al.*, 2000). However, such intervention may be difficult or impractical under small scale operation. Hence there is need to explore other viable options such as the optimal maturity stage for minimal tannin content, planting of improved varieties with low tannins and keeping livestock which can perform well under high tannin diets.

3.5 CONCLUSIONS

The results of this study showed depreciation of nutritive attributes with maturity of stovers but the difference in degradability was minimal. In general, MM showed superior nutritive value by having higher soluble carbohydrate content, lower fibre content and shorter lag time. It can be therefore postulated that MM could support higher performance principally by positively influencing intake. Care needs to be undertaken in conservation of stovers since morphological components differ in nutritive value. In particular, retaining a high ratio of the leaf fraction would be advantageous. Protein supplements differently influenced the nutritive value of stover rations. While Lucerne or sunflower oil cake yielded positive results, supplementation with lespedeza led to a decline in degradability, which was more severe in its rations with dry stage stover. The results suggest that supplementation of the stovers with at most 40% DM contributed by lucerne or sunflower cake would optimize their utilization, since improvement in degradability or lag time were minimal beyond this ratio. The choice

between these two supplements would therefore depend on socio-economic considerations. High tannin content in lespedeza is stipulated to be responsible for depression of microbial activity as its ratio increased, which caused reduction in degradability and fermentation. However, given that lespedeza has agronomic and climatic advantages, plus the fact that the impact of tannin can be ameliorated, it is viewed that lespedeza has potential to boost productivity in specific situations. Further evaluation of lespedeza should therefore focus on detailed chemical composition analyses at its different stages of maturity, *in vivo* trials and measures to moderate tannin effects.

Chapter 4

IN VITRO DEGRADABILITY AND GAS PRODUCTION PARAMETERS OF SERICEA LESPEDEZA (*LESPEDEZA CUNEATA*) MIXED WITH VARYING TYPES AND LEVELS OF ROUGHAGES[b]

ABSTRACT

Sericea lespedeza (*Lespedeza cuneata*) re-growth was harvested at early (ELP) or late (LLP) flowering stages, mixed with varying types and levels of roughage and fermented for 72 h *in vitro*, using the gas production (GP) technique. The roughage : lespedeza rations were 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100. The roughages included maize stover harvested at grain milk (MM) or dry (MD) stages and veld grass hay (GH). The crude protein (CP) content of ELP, LLP, MM, MD and GH were 187, 97, 48, 29 and 34 g/kg, respectively. The corresponding NDF values were 283, 589, 696, 73.3 and 665 g/kg dry matter (DM). Degradability was slightly higher in MM as compared to MD and GH (mean 704.9 *vs.* 676.6 and 685.0 g/kg, respectively) in ELP rations. The roughages had similar but lower degradability in LLP rations (means 633.4, 632.6 and 631.1 g/kg for MM, MD and GH, respectively). Increased proportion of ELP and LLP resulted in decreased degradability in all the roughages. Microbial yield was similar among roughages in ELP rations, but the roughages differed in microbial yield among LLP rations whereby GH had the highest value and MD the lowest. Increased proportion of ELP elicited an increase in microbial yield but increasing LLP had no effect (range 135.0 – 264.8 g/kg in ELP and 143.4 – 295. 9 g/kg among LLP rations). Roughage type affected GP with MD and GH having the lowest and highest values, respectively. The values ranged from 167.4 – 209.8 and 160.4 – 221.0 ml in ELP and LLP rations, respectively. There was a decrease in GP as the proportion of ELP or LLP increased and roughage type x supplement level interaction had effect. The ratio of degradability to GP, i.e. the partitioning factor (PF) ranged from 3.43 – 4.74 and 3.13 – 4.23 in ELP and LLP rations, respectively whereby GH had highest and MD lowest values. The

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rate of GP from soluble fraction was not affected, but that of fibre fraction differed among the roughages in ELP rations whereby GH had lower rate than MM and MD (mean 0.023 *vs.* 0.026 and 0.025, respectively). The lag time (lag) tended to be reduced as ratio of ELP increased (range 1.83 to 6.59 h). In LLP rations, roughage type, supplementation level, roughage type x supplementation level interactions affected lag. The GH had the longest and MM had the shortest lag among the roughages (range 0.88 – 9.61 h), and likewise lag reduced as ratio of LLP increased. The results indicate that the various nutritive attributes considered are differentially influenced by lespedeza type and level, roughage type and the interactions among these aspects, hence the importance of their implications in formulating ruminant diets. The results further indicate that when using roughages with similar quality as those studied, lespedeza ratio of 40-60% of DM consumed can be beneficial.

4.1 INTRODUCTION

Feed resources are a major component of economic animal production. Their availability and efficiency of use in specific agro-ecological zones dictate to a very large extent the performance of the livestock production system. During the dry season ruminants in tropical and sub-tropical regions mostly survive on low quality roughages such as standing hay or crop residues, leading to poor performance. Maize is a widely grown cereal and is the staple food in most African smallholder systems. It is largely grown and left in the fields to dry to over 80% grain dry matter (DM) before harvesting. Alternatively, maize is popularly harvested at milk stage for roasting, boiling or cooking with vegetables. The stover left after harvesting is potentially a major feed resource to boost ruminant production if properly utilized. However, maize stovers are low in protein or nitrogen (N) concentration (Fadel, 1999) necessitating supplementation with N-rich feed resources. Lucerne (*Medicago sativa* L.) is the predominant pasture legume that has been used as N source for many classes of livestock (Van Keuren & Matches, 1988). However, due to ecological limitations and high agronomic requirements, alternatives to lucerne are sought, of which Sericea lespedeza (lespedeza) has shown potential (Terrill *et al.*, 1989). Lespedeza [*Lespedeza cuneata* (Dun-Cours) G. Don] is a tall growing, drought tolerant, coarse-stemmed, non-bloating, deep tap-rooted, self-seeding, perennial legume, adapted to a wide range of soils (Powell *et al.*, 2003). It is widely planted in southern USA for grazing, hay, soil restoration and conservation crop

(Powell *et al.*, 2003). Although common lespedeza are known to have a high tannin content which may limit the nutritive value (Turner *et al.*, 2005), ruminants such as goats have been reported to tolerate and perform well when consuming high tannin-containing plants (Silanikove *et al.*, 1996). Other advantageous features of lespedeza include being long term forage once established (Barkley, 1986), provision of good quality grazing (Schmidt *et al.*, 1982) and coping well under waterlogged conditions (Guernsey, 1970). In addition, South African farmers have described lespedeza as a low cost pasture, provides good quality early spring and autumn grazing, makes good quality and 'cheap' hay, hay cures quickly, less wear-and-tear on mowing equipment, increases summer carrying capacity, seldom needs lime and/or phosphorus corrections at planting and does not require use of pesticides. However, the farmers pointed out disadvantages of lespedeza as slow establishment and inability to be used as foggage ("standing hay") because it is frost sensitive (H. Botha, 2005, Personal communication, Harmonie Trust, P.O. Box 27, Matatiele 4730, South Africa). Lespedeza was imported to South Africa about 15 years ago and commercial seed is now available. Being a relatively new forage in African livestock systems, there is little information on the nutritive value of lespedeza rations with locally available roughages. To provide such information requires screening numerous dietary combinations; ideally through *in vivo* trials. However, *in vivo* trials are expensive and impractical to screen a large number of diets. *In vitro* methods are alternatively used, but the suitability of a chosen method is essential.

In a recent review of the *in vitro* gas production (GP) technique, Dijkstra *et al.* (2005) pointed out that many workers have observed the relationship between degradability predicted from GP using fixed time points and *in vivo* degradability to be only moderate, and that this relationship was improved substantially by including parameters related to the dynamism of GP (e.g. Chenost *et al.*, 2001; Carro *et al.*, 2002). Blümmel & Bullerdick (1997) suggested complementation of *in vitro* GP with residue determination in evaluation of the nutritive value of feeds. In this approach, the residue determination reveals how much substrate is degradable and the gas measurement reflects how much of the degraded portion is converted into fermentation acids and gases. The ratio of substrate truly degraded to gas volume produced, defined as 'partitioning factor' (PF) was found to be valuable in predicting voluntary feed intake. The present study used *in vitro* gas GP technique to investigate the

nutritive effects caused by supplementing locally available roughages in South Africa with lespedeza.

4.2 MATERIALS AND METHODS

This study used maize stovers (MS) and grass hay (GH) as roughages and lespedeza (*Lespedeza cuneata* (Dum-Cours.) G. Don) re-growths as protein supplements (PS). Lespedeza was harvested at early (ELP) or late (LLP) flowering stages. The MS were from a white maize hybrid PAN 6479 grown at Ukulinga Research Farm, University of Kwa-Zulu Natal. The GH was also harvested from Ukulinga farm. The MS were harvested at grain milk (MM) or grain dry (MD) stages. Both MS and GH were part of the experimental feed materials bulked for use in a feeding trial (Chapter 7). The MM was oven dried at 50°C overnight while the rest of the feeds were air dried. The dried feeds were milled through 1-mm screen using a Cyclotec mill (Perstorp Analytical Ltd, Bristol, UK). A total of 1.0 ± 0.0010 g DM in proportions (RG:PS) of 0:100, 20:80, 40:60, 80:100 and 100:0 % were weighed into 250 mL Duran bottles for *in vitro* incubation as described in section 3.2. Two sets of incubations lasting-72 h were carried out separately, one set with ELP and the other set with LLP rations. In each set, incubation was done thrice with all the treatment rations represented each time. Analogous procedures as described in section 3.2 were followed in sample preparation and chemical composition analyses.

SAS (2002) software was used to perform statistical analysis and fitting the model described by Campos *et al.* (2004) to the gas production data. Analysis of variance (ANOVA) was carried out to determine treatment effects whereby stover type, supplement type, supplement level and the interactions among them were used as sources of variation. Means were compared by least significant difference (LSD).

4.3 RESULTS

The chemical composition of the feeds is shown in Table 4.1. The roughages CP content were 48.1, 28.6 and 34.3 g/kg for MM, MD and GH, respectively. ELP had markedly higher CP, lower NDF and lower ADF contents than LLP. All feeds had similar GE content with the mean value being 16.5 MJ/kg DM.

Table 4.1 Feeds chemical composition (g/kg DM) and gross energy (MJ/kg DM)

Feed	Chemical composition					GE
	CP	NDF	ADF	ADFn	Ash	
Milk stage stover (MM)	48.1	696.1	448.9	1.66	63.8	16.16
Dry stage stover (MD)	28.6	733.3	452.4	1.29	60.2	15.93
Grass hay (GH)	34.3	664.7	463.7	1.46	91.2	16.28
Early flowering stage lespedeza (ELP)	187.4	408.1	282.5	11.7	57.9	17.24
Late flowering stage lespedeza (LLP)	96.8	588.7	436.4	4.79	50.0	16.90

CP- crude protein, NDF –neutral detergent fibre, ADF – Acid detergent fibre, ADFn – nitrogen in ADF, GE- gross energy.

The pH values at the end of incubation were stable near neutral (6.65 to 6.99) and (6.63 to 7.08) among ELP and LLP rations, respectively, indicating normal fermentation was maintained. The results of degradability, microbial yield, gas production and partitioning factor (PF) of the rations are shown in Table 4.2 for ELP and Table 4.3 for LLP rations. Degradability was slightly higher ($P < 0.01$) in MM as compared to MD and GH (mean 704.9 *vs.* 676.6 and 685.0 g/kg, respectively) among ELP rations. The roughages had similar but lower degradability in LLP rations with mean values of 633.4, 632.6 and 631.1 g/kg for MM, MD and GH rations, respectively. Increasing the proportion of ELP or LLP resulted to decreased ($P < 0.001$) degradability in all the roughages. Roughages had similar microbial yield in ELP rations, but differed ($P < 0.001$) in LLP rations whereby GH had the highest value and MD the lowest. Microbial yields were highly variable (cv = 24.7 and 23.9 in ELP and LLP rations, respectively). They were similar in roughages with ELP whereas in LLP rations GH had highest ($P < 0.001$) values compared to MM and MD (mean 252.6 *vs.* 211.7 and 187.3, respectively). Roughage type affected ($P < 0.001$) GP with MD having the highest and GH the lowest values. The values ranged from 167.4 to 209.8 and 160.4 to 221.0 ml among ELP and LLP rations, respectively. There was a decrease ($P < 0.001$) in GP as the proportion of ELP or LLP increased and roughage type x supplement level interaction had effect ($P < 0.05$). The stovers showed the same trend in reduction of GP with increase in lespedeza ratio, but for the GH, there was an increase in GP from 100:0 to 80:20 RGH:SUP

ratio and a decrease as lespedeza ratio increased thereafter. The ratio of degradability to GP i.e. the partitioning factor (PF) differed ($P < 0.01$) among the roughages in both ELP and LLP rations but was not affected by supplement level. They ranged from 3.43 to 4.74 and 3.13 to 4.23 in ELP and LLP rations, respectively whereby MD rations had lowest values. These results are detailed in Tables 4.2 and 4.3.

The kinetic parameters of gas production are shown in Table 4.4 for ELP and Table 4.5 for LLP rations. Gas produced from the soluble fraction (A) was only affected ($P < 0.05$) by the roughage type whereby GH had the lowest and MM the highest values (range 37.4 to 72.3 ml among ELP and 31.1 to 64.0 ml among LLP rations). The rations had similar rate of gas production from the soluble fraction (a_1).. The gas produced from the fibre fraction (B) was affected by both roughage type and supplementation level in the ELP rations whereby MM had the lowest value (mean 113.9 ml) as compared to MD and GH, which were similar (mean 129.6 and 129.8 ml, respectively). The rate of gas production from fibre fraction (b_1) was affected by roughage type in ELP rations only with GH having the lowest and MM the highest rate (range 0.023 to 0.026/h). The lt tended to be affected ($P < 0.09$) by supplement level in ELP rations (range 1.83 to 6.59 h) whereby it decreased as the proportion of ELP increased. In LLP rations, roughage type, supplement level and supplement level x roughage type interaction all had effects ($P < 0.001$) on lag (range 0.88 to 9.61 h). Among the roughages MM had the shortest and GH the highest lag. The lag shortened as the supplementation level increased across all the roughages. The interaction between roughage type and supplement level is depicted in Figure 4.1.

Table 4.2 Mean degradability, microbial yield and gas production of Sericea lespedeza harvested at early flowering stage (ELP), mixed with different roughages stages and fermented using rumen fluid *in vitro*

Roughage type (RG)	RG: ELP	Degradability g/kg DM	Microbial yield g/kg DM	Gas production ml/g DM	Partitioning factor
Milk stage maize stover	100:0	776.5	173.0	199.1	3.91
	80:20	751.7	135.0	189.5	3.98
	60:40	701.9	199.5	193.4	3.64
	40:60	657.6	264.8	167.4	3.94
	20:80	666.9	185.5	167.6	3.99
	0:100	664.5	236.1	170.8	3.89
Dry stage maize stover	100:0	729.6	150.6	209.8	3.50
	80:20	713.7	140.4	198.9	3.59
	60:40	689.0	193.3	183.6	3.75
	40:60	626.8	166.6	184.7	3.43
	20:80	651.0	226.7	173.9	3.78
Grass hay	100:0	742.6	190.3	204.0	3.65
	80:20	698.4	194.2	176.4	4.74
	60:40	667.5	254.0	176.6	3.79
	40:60	660.7	230.4	173.1	3.82
	20:80	671.3	169.6	172.5	3.92
	LSD	52.90	74.9	24.4	0.66
	P	0.001	0.05	0.001	0.01
	cv	4.9	24.7	8.5	10.9

Table 4.3 Mean degradability, microbial yield and gas production of Sericea lespedeza harvested at late flowering stage (LLP), mixed with different roughages stages and fermented using rumen fluid *in vitro*

Roughage type	Roughage: LLP	Degradability g/kg DM	Microbial yield g/kg DM	Gas production ml/g DM	Partitioning factor
Milk stage maize stover	100:0	709.5	229.5	205.4	3.50
	80:20	667.8	214.1	199.4	3.35
	60:40	645.9	184.7	186.2	3.47
	40:60	607.7	223.9	171.8	3.55
	20:80	562.0	212.0	166.7	3.37
	0:100	533.5	252.3	148.2	3.61
Dry stage maize stover	100:0	742.5	185.8	221.0	3.36
	80:20	679.0	225.7	216.6	3.13
	60:40	599.7	143.4	186.7	3.21
	40:60	611.4	216.4	181.6	3.38
	20:80	565.7	171.7	163.8	3.47
	0:100	533.5	252.3	148.2	3.61
Grass hay	100:0	719.2	295.9	172.5	4.23
	80:20	659.5	243.3	190.4	3.47
	60:40	627.9	241.7	174.8	3.62
	40:60	596.2	228.7	171.9	3.47
	20:80	578.5	261.0	160.4	3.62
	0:100	533.5	252.3	148.2	3.61
	LSD	45.37	77.17	17.12	0.45
	P	0.001	0.001	0.001	0.01
	cv	4.8	23.9	6.3	8.6

Table 4.4 Mean gas production parameters[1] of Sericea lespedeza harvested at early flowering stage (ELP), mixed with different roughages stages and fermented using rumen fluid *in vitro*

Roughage type	Roughage: ELP	A	B	a_1	b_1	lag
Milk stage maize stover	100:0	57.7	133.6	0.152	0.029	4.88
	80:20	72.3	114.9	0.123	0.027	3.48
	60:40	66.4	119.7	0.097	0.025	1.83
	40:60	53.5	106.8	0.104	0.026	3.15
	20:80	57.2	97.3	0.135	0.025	3.47
	0:100	54.1	110.6	0.105	0.026	2.97
Dry stage maize stover	100:0	57.4	145.1	0.103	0.024	4.61
	80:20	58.3	132.5	0.128	0.025	4.83
	60:40	48.0	128.6	0.129	0.025	5.02
	40:60	38.6	141.4	0.094	0.023	2.89
	20:80	53.5	107.1	0.150	0.025	4.03
	0:100	54.1	110.6	0.105	0.026	2.97
Grass hay	100:0	39.8	153.9	0.140	0.024	6.59
	80:20	46.3	108.6	0.137	0.025	5.82
	60:40	37.4	131.4	0.150	0.024	5.30
	40:60	39.8	126.9	0.101	0.024	3.34
	20:80	42.5	130.4	0.52	0.024	2.35
	0:100	54.1	110.6	0.105	0.026	2.97
	LSD	27.05	30.14	0.177	0.005	3.03
	P	0.01	0.01	Ns	0.05	ns
	Cv	29.0	13.4	69.7	10.3	41.1

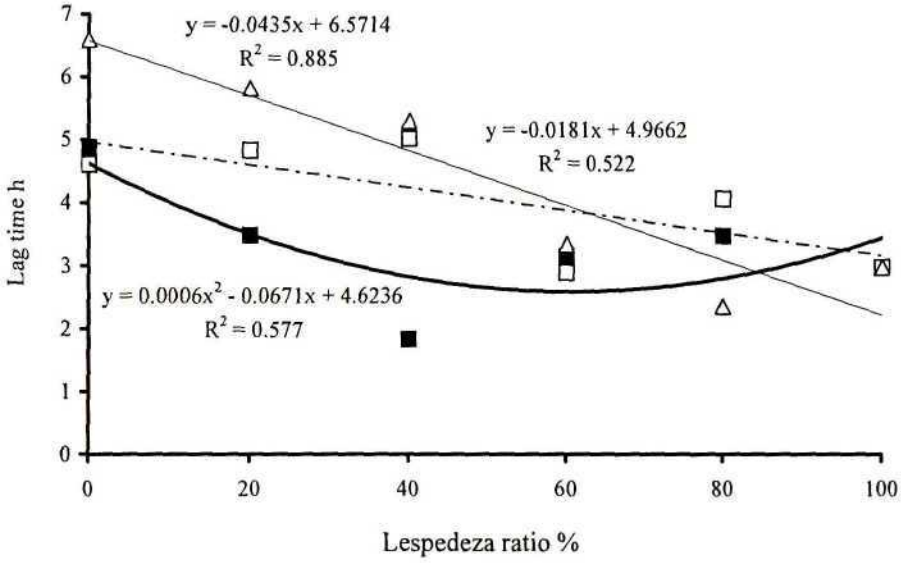
[1]based on equation by Campos *et al.* (2004) where, A and B are the gas volume (ml) from fast (soluble)and slowly (fibre) degradable fractions, respectively, a_1 and b_1 are the degradation rates (per h) of soluble and fibre fractions, respectively, lag is lag time.

Table 4.5 Mean gas production parameters[1] of Sericea lespedeza harvested at late flowering stage (LLP), mixed with different roughages stages and fermented using rumen fluid *in vitro*

Roughage type	Roughage: LLP	A	B	a_1	b_1	lag
Milk stage maize stover	100:0	64.0	135.9	0.096	0.026	2.38
	80:20	59.2	133.8	0.067	0.023	2.42
	60:40	67.4	110.9	0.099	0.025	2.36
	40:60	61.0	101.3	0.101	0.025	1.51
	20:80	58.2	102.3	0.089	0.025	1.23
	0:100	66.1	75.7	0.096	0.027	0.88
Dry stage maize stover	100:0	60.4	153.0	0.105	0.024	4.06
	80:20	61.2	147.8	0.088	0.025	3.49
	60:40	56.7	122.7	0.103	0.025	3.28
	40:60	66.4	107.6	0.098	0.025	1.85
	20:80	53.6	102.9	0.078	0.024	1.48
	0:100	66.1	75.7	0.096	0.027	0.88
Grass hay	100:0	31.1	154.3	0.087	0.024	9.61
	80:20	56.4	126.7	0.097	0.024	3.63
	60:40	60.0	106.8	0.090	0.023	2.48
	40:60	51.1	113.9	0.076	0.023	1.40
	20:80	51.1	100.3	0.083	0.023	1.33
	0:100	66.1	75.7	0.096	0.027	0.88
	LSD	21.8	29.0	0.14	0.003	1.86
	P	0.05	0.001	Ns	Ns	0.001
	Cv	19.9	15.1	85.0	7.7	37.5

[1]based on equation by Campos *et al.* (2004) where, A and B are the gas volume (ml) from fast (soluble)and slowly (fibre) degradable fractions, respectively, a_1 and b_1 are the degradation rates (per h) of soluble and fibre fractions, respectively, lag is lag time.

(a) Lespedeza harvested at early flowering stage (ELP)



(b) Lespedeza harvested at late flowering stage (LLP)

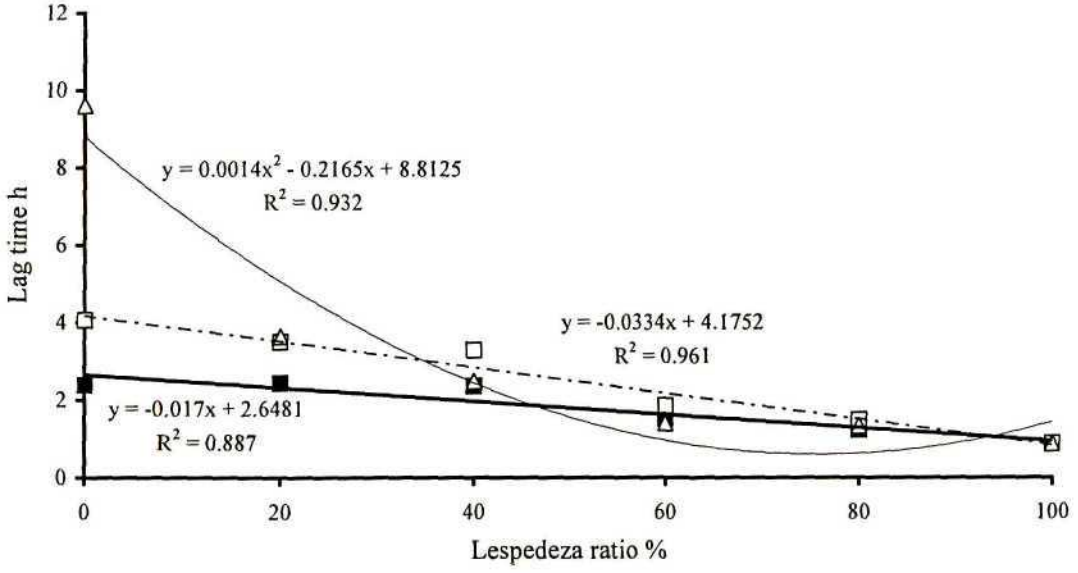


Figure 4.1 Effect of increasing ratio of lespedeza on fermentation lag time of milk stage (—■—), dry stage (– ·-□-· –) maize stover and grass hay (—△—) rations fermented *in vitro*

4.4 DISCUSSION

The maize stover exhibited high nutritive potential, especially as reflected from the highest degradability values where there was no supplementation. Although it was anticipated that there would be substantial decline in nutritive value of MD as compared to MM, the result indicated the decline was minimal. Similar observations have been made by Akbar *et al.* (2002) where 72 h degradability of early harvested stovers ranged from 70.1 – 76.8% and those of late harvested range from 69.4 to 74.6% in several maize varieties. The present findings thus support the observation of Sutton *et al.* (1999) where small and non-significant decrease in whole tract digestibility by dairy cows fed maize silage of different maturity was observed. Sheep fed maize stover supplemented with various multipurpose trees containing tannins and other antinutritive compounds had degradability ranging from 490 to 518 g/kg (Hindrichsen *et al.*, 2004). Although these values are lower than those of the current study (range 533.5 to 776.5 g/kg), they are reflective of the lower degradability often observed *in vivo* compared to *in vitro*. Flachowsky *et al.* (1991) showed that cultivars with higher straw quality were not consistently associated with lower grain yield, and this gives an opportunity to develop or select a variety with high quality crop residue without sacrificing grain yield. As for the GH used, its CP (3.4%) content was within the range of 2.33 – 5.09% reported by Ali *et al.* (2001) in several tropical grasses. These values are fairly low compared to those reported by Aganga & Tshwenyane (2004) ranging from 5.3 to 12.6% of a tropical grass, hence this emphasizes the role of proper management to maintain the quality of grass pastures and/or supplementation with CP or nitrogen sources where these poor quality roughages must be utilized. The importance of a forage such as lespedeza stems from this. Muyekho *et al.* (2003) found that farmers considered factors relating directly to the animal as more important than factors relating to the agronomic characteristics of the forage. These included palatability and potential to increase production. Agronomic factors valued by the farmers included high yields, drought tolerance and diseases/pests resistance. Their results also suggested that farmers were reluctant to adopt forage crops that require planting on an annual basis. Other criteria that needed to be considered include factors that may cause direct harm to the livestock, e.g. feeding of Lucerne was reported to have the possibility of causing bloat. These observations closely corroborate with those of a farmer in KwaZulu-Natal in preferring lespedeza forage. The farmer who had endeavoured for 30 years to find a suitable forage

described lespedeza as a 'miracle' pasture that can produce cost-effective grazing and hay on a sustainable basis (H. Botha, 2005, Personal communication, Harmonie Trust, P.O. Box 27, Matatiele 4730, South Africa). Often scientists ignore many of these factors at early stages of species/variety development, yet inclusion of farmers' criteria could minimize the costs of research and enhance the uptake of technology. This concern has also been pointed out by Muyekho *et al.* (2003).

The reduction in degradability and gas production as the lespedeza ratio increased can be attributed to its chemical composition, particularly the tannin content. Although tannin content was not determined in the current study, Turner *et al.* (2005) observed high tannin content of 23.1 mg/g in lespedeza, and a much lower value of 0.24 mg/g in lucerne. Similar to the present findings, the presence of tannins in browses depressed the *in vitro* gas and SCFA production (Getachew *et al.*, 2000). The depression in the fermentation could be a result of either direct interaction between tannins and the bacterial cell wall (Jones *et al.*, 1994) or the effect of tannin on microbial enzymes (Bae *et al.*, 1993; Ngwa *et al.*, 2003), with the net effect of hampering microbial growth as reported by Field & Lettinga (1992). Getachew *et al.* (2000) showed that the inhibitory effect of tannin can be removed by adding of polyethylene glycol (PEG), implying that a solution to this anti-nutritive factor is available. On the other hand, it is noteworthy that both advantageous and negative effects of tannin containing feeds have been reported from *in vivo* findings. Lespedeza offered to sheep had low digestibility due to high tannin concentration (Terrill *et al.*, 1989). Condensed tannin (CT) containing forages had slower rates of digestion in the rumen, but greater ruminal escape values (Albrecht and Broderick, 1990). Kaitho *et al.* (1993) speculated that sheep fed high-tannin legumes had an overall shortage of rumen-degradable-N resulting in impaired fibre digestibility and reduced weight gain. On the positive side, Caygill & Mueller-Harvey (1999) stated that some tanniniferous feeds can produce beneficial effects in ruminants, e.g. improved amino acid absorption and anthelmintic effects. Douglas *et al.* (1995) reported greater live weight gain when lambs grazed birdsfoot trefoil versus alfalfa, which was attributed to the influence of CT in the ruminant digestive tract. Medium concentrations of CT (45 to 55 g CT/kg DM) in forages can improve N-use efficiency in ruminants (Min *et al.*, 2003).

Efficiency of rumen microbial protein synthesis is a key factor in the protein evaluation systems for ruminants. However, accurate determination of microbial yield *in vitro* is still an elusive aspect of ruminant nutrition. This is because of several limitations of the batch culture applied in the *in vitro* systems (Dijkstra *et al.*, 1998, Rymer *et al.*, 2001). In the current work, the two main issues causing difficulty in making inference to microbial yield values are the duration of incubation and possibility that tannin-protein complexes were formed (Haggerman & Robbins, 1987) and were washed away by NDS, hence increasing the values estimated. Blümmel *et al.* (2003) found that PF at the time of the estimated peak microbial production *in vitro*, but not after 16 or 24 °h of incubation, was well correlated with observed microbial efficiency *in vivo*. Rymer *et al.* (2001) found the time factor to play an important role with regard to the fermentation products stoichiometry and microbial recycling during incubation. They found good relationships between predicted and observed gas volumes at early hours (8 h) which was lost by 48 h. Thus the present 72 h incubation could therefore be regarded as far too long, but was essential in assessing maximal degradability. Nevertheless, from PF values it can be deduced that GH was superior to the stovers as it had higher values with both ELP and LLP rations.

From the GP kinetics, the higher A value of MM depicts higher availability of soluble and readily fermentable compounds at the early stover stage and this is an indication of superior nutritive quality over MD in particular. In ruminant nutrition, the fermentation of fibre component depicted by B and the rapidity by which this happens are crucial. Krishnamoorthy *et al.* (1995) pointed out that information on lt and rate of fermentation as indices of rapidity with which organic matter is fermented in the rumen can be useful in formulating diet with desirable rate of fermentation. From the current results it appears that supplementing the roughages with lespedeza induces a positive impact by reducing the lag, the greatest being with GH. Two factors can be inclusively stipulated to be responsible for the shorter lag of stovers. First, the fact that unsupplemented stovers had higher A values as compared to GH indicated that the former had higher content of soluble fraction, which boosted microbial colonization. Second, grinding the much coarser stovers through a 1 mm screen resulted in finer material as compared to GH; implying the stovers had higher surface area exposed for colonization. Both of these factors would cause reduction in lt. It appeared that fibre degradation was optimal at the RGH:SUP ratio of 60:40 and 40:60 for in ELP rations with

MM and MD, respectively, when B was maximal and lag minimal. For GH rations, the pattern was not as clear although 40:60 rations could be recommended as they had modest values of B and lag. Among the LLP rations, it was interesting that the three roughages had nearly the same lag at RGH:SUP ratio of 40:60, which was near the inflection point for GH rations (Figure 4.1). Perhaps this can be regarded as a suitable supplementation level given that B remained nearly constant as the level of LLP increased beyond this point. The other parameters (a_1 and b_1) did not conspicuously reflect the impacts of supplementation within roughage types, hence were difficult to be used in making inferences.

4.5 CONCLUSIONS

Although the roughages were of poor quality reflected in low CP and high fibre contents, they showed high potential due to high degradability. The maturity stage influenced the nutritive characteristics of the forages whereby depreciation in quality with age was observed in both maize stovers and lespedeza, but the effect was small in the stovers, particularly with regard to degradability. The positive impacts arising from supplementation with lespedeza included enhancing fermentation of fibre fraction and decreasing rumen microbial colonization lag time. It was apparent that there is a limit to which lespedeza can be added to optimise the positive impacts it induces. The results indicate that when using roughages with similar quality as those studied, the lespedeza ratio of 40-60% of DM consumed can be beneficial. This needs ratification through *in vivo* trial.

Chapter 5

THE RELEVANCE AND POTENTIAL USE OF *IN VITRO* GAS PRODUCTION PARAMETERS TO EVALUATE POOR QUALITY ROUGHAGE BASED RATIONS FOR RUMINANTS[c]

ABSTRACT

This study explored the relevance and potential of measurements obtainable from the *in vitro* gas production technique (IVGPT) in predicting nutritive value of ruminant rations comprised of poor quality roughages (RG) and different protein supplements (PS). The RG used were grass (GH) and maize stover (MS). The PS were Lucerne (LH), Sericea lespedeza (LPZ) and sunflower oil cake (SFC). RG:PS rations used were 100:0, 80:20, 60:40, 40:60 20:80 and 0:100 on DM basis. Evaluation measurements included degradability (Deg), gas production (GP), time taken to produce half total gas ($T_{½}$), Deg/total gas ratio (PF) and Deg/(half total gas x $T_{½}$) ratio (DEF). The crude protein (CP) content of GH, MS, LH, LPZ and SFC were 34, 39, 199, 73 and 391 g/kg, respectively. Corresponding neutral detergent fibre (NDF) values were 665, 715, 355, 525 and 324 g/kg. Deg increased linearly as ratio of LH ($p<0.003$) or SFC ($p<0.03$) increased in GH rations. In MS rations there were quadratic trends in Deg as the ratios of LH or SFC increased. Increasing the ratio of LPZ in MS rations caused a decrease in Deg in both GH and MS rations. The PS differed ($p<0.001$) in GP in GH rations but increasing their ratios had no effect. In MS rations, the PS, PS ratio and PS x PS ratio all affected ($p<0.001$) GP. There was a linear decrease ($p<0.02$ to 0.002) in $T_{½}$. as PS increased in GH rations. In MS rations, increasing LH or SFC caused linear decrease in $T_{½}$. ($p<0.002$ or 0.009, respectively) while increasing LPZ ratio had a quadratic trend ($p=0.17$). The PF was only affected by PS ratio in GH rations. In MS rations, it was affected by PS type ($p<0.001$), PS ratio ($p<0.001$) and PS type x PS ratio interaction ($p<0.01$). in MS rations. There were quadratic trends in PF as PS ratios increased in both GH and MS rations, but the consistency of the trends were generally poor. The DEF values increased linearly ($p<0.002$ to 0.001) as the ratios of LH or SFC increased in both rations with GH or MS. Increasing LPZ ratio caused quadratic trend in DEF with significant slopes in GH and non-significant slopes in MS

[c] J. O. Ouda and I. V. Nsahlai. The relevance and potential use of *in vitro* gas production parameters to evaluate poor quality roughage based rations for ruminants. *South African Journal of Animal Science* (Submitted)

rations. It was concluded that Deg and GP measurements are useful in showing the nutritive potential of feeds subjected to extensive *in vitro* incubation, but can only provide distinctive ranking in feeds having substantial differences in these aspects. Thus they need to be supported by other measurements. The authenticity of PF is influenced by many conflicting factors making it difficult to be a determinant measurement, especially where there is extended *in vitro* incubation. $T_{½}$ and DEF are potentially useful alternative measurements and should be considered when evaluating rations using IVGPT.

5.1 INTRODUCTION

Ruminants, especially in the tropical regions, predominantly depend on roughages deficient in essential nutrients available for degradation. This leads to low rates and extent of degradation, reduced voluntary feed intake, and consequently poor animal performance (Leng, 1990, Goodchild and McMeniman, 1994, Wilson and Kennedy, 1996). Use of feeds high in readily fermentable carbohydrates and protein to supplement the poor quality forages is an option for farmers to meet the nutrient needs of the animals. Evaluation of the nutritive value arising from supplementation should be ideally done through *in vivo* methods. However, it is commonly recognized that *in vivo* evaluation of feedstuffs is time-consuming, laborious, expensive, requires large quantities of feed and is unsuitable for large-scale feed evaluation (Dijkstra *et al.*, 2005). As a result, *in vitro* methods involving incubation of feeds with rumen microorganisms as in the work of Tilley and Terry (1963) or in the gas production method (Menke *et al.*, 1979) are commonly used to predict what would happen *in vivo*.

The basic principle applied is derived from theoretical understanding of the stoichiometry of rumen fermentation: Feed degraded in the rumen *in vitro* may be partitioned either to microbial biomass production, or to fermentation (Wolin, 1960; Beever, 1993; Getachew *et al.*, 1998). The microbes degrade and ferment the degraded substrate in order to obtain energy (ATP) for their own growth. Subsequently, microbes multiply into large numbers, are passed to the lower gut and digested to become the major source of protein for the host ruminant (McDonald et al., 2002, Beever, 1993). Short chain fatty acids (SCFA) and gases are produced in the process of fermentation. The SCFA are absorbed through the gut wall and

metabolized to become the source of energy for the host ruminant (McDonald et al., 2002, Beever, 1993). The most important SCFA are acetate, propionate and butyrate and their energy concentration are 14.6, 20.8 and 24.9 MJ kg^{-1}, respectively (Moss, 1993). The important gases produced are methane (CH_4) and carbon dioxide (CO_2). The production of these gases represent energy and cell carbon losses, besides methane being a greenhouse gas (Moss, 1993). Makkar (2004) remarked that in most studies, the rate and extent of gas production has been wrongly considered to be equivalent to rate and extent of substrate (feed) degradation. Blümmel *et al.*(1997) showed that gas production and microbial yields per unit of substrate degraded are inversely related. The implication is that selecting diets on the basis that higher gas production is synonymous with higher quality might result in selecting against maximal microbial yield, which would be inappropriate (Makkar, 2004). In recognition of these underlying factors, the present ruminant nutrition concepts aim at high microbial efficiency, which cannot be measured by gas production alone. Consequently, the importance of including other concomitant measurements along side gas production has been advocated (Blümmel *et al.*, 1997). The additional measurements commonly considered include degradability (Deg) and microbial yield (MIC). Blümmel *et al.* (1997) calculated Deg:GP ratio, which they termed the 'partitioning factor' (PF). The above authors reported that feeds with a high PF stimulated higher microbial biomass production.

Although several workers have reported good correlations between *in vitro* and *in vivo* measurements (Brown *et al.*, 2002; Rymer and Givens 2002; Getachew, *et al.*, 2005), poor correlations among *in vitro* measurements and between *in vitro* and *in vivo* measurements have also been reported. For instance, Rymer *et al.* (2001) observed a poor correlation between predicted and observed GP and attributed this to variation in fermentation stoichiometry and microbial recycling over time when protein-rich feeds were fermented. Blümmel *et al.* (2005) found that PF reliably predicted feed intake when measured at early hours of incubation (4-8 h), but a poor correlation was observed beyond 24 h. On the other hand, the measurement of fermentation products at fixed incubation periods has been criticized for ignoring the dynamics of degradation and fermentation (Dijkstra *et al.*, 2005). As a result, *in vitro* (automated, semi automated or manual) gas production techniques (IVGPT) capable of providing information on the kinetics of fermentation are now popular. Given that microbial activity is close to maximum at half maximum gas volume (France *et*

al., 1993; Davies *et al.*, 2000; Makkar, 2004), it can be stipulated that incorporating the time taken to attain half gas volume ($T_{½}$) among evaluation measurements can improve the prediction of nutritive value. This study explored the relevance and potential use of measurements obtained from IVGPT in predicting nutritive effects arising from rations comprised of poor quality roughages and different protein supplements.

5.2 MATERIALS AND METHODS

Roughages (RG) including grass (GH) and maize stover (MS) and their mixtures with different types of protein supplements (PS) were used. The MS was from white maize hybrid PAN 6479 grown in Kwa-Zulu Natal Province, South Africa. The PS included lucerne hay (LH), Sericea lespedeza hay (LPZ) and sunflower seed cake (SFC). Analogous procedures as described in Chapter 3 (Section 3.2) were followed for sample preparation and conducting automated *in vitro* gas production technique. True degradability (Deg) was also determined as explained in Section 3.2. The rations partitioning factor (PF) and Degradation efficiency factor (DEF) were calculated as follows:

$$PF = \frac{Deg}{V}$$

$$DEF = \frac{Deg}{T_{½} \times V_{½}} = \frac{2PF}{T_{½}}$$

Where: Deg = True degradability (mg)
V = total gas volume (ml)
$T_{½}$ = time taken to produce $V_{½}$ (h)
$V_{½}$ = half of V (ml)

Data from rations of GH or MS were separately subjected to an analysis of variance (ANOVA) to determine treatment effects on nutritive measurements. Supplement type, supplement ratio and the interactions between these two were used as sources of variation. The means were compared by least significant difference (LSD). Regression analysis was performed using supplement ratio as independent variable and Deg, $T_{½}$, PF or DEF as

dependent variables. The significance of the slopes was used to assess the effect of the supplement ratio on the dependent variables. Genstat (ver. 8) statistical software was used in the analyses.

5.3 RESULTS

Chemical composition of the feeds is shown in Table 5.1. NDF content was higher in MS than GH but their ADF contents were comparable. The PS types had varied CP content with LPZ having the lowest and SFC the highest. The results of IVGPT measurements are presented in Tables 5.2 and 5.3. Terminal pH ranged from 6.75 to 6.95 and 6.68 to 7.06 in GH and MS rations, respectively. Deg was affected by PS type ($p<0.001$), PS ratio ($p<0.01$ to 0.001) and PS type x PS ratio interaction ($p < 0.01$ to 0.001) in GH and MS rations. Deg increased linearly as ratio of LH or SFC increased ($p < 0.003$ or 0.03, $R^2 = 0.91$ or 0.74, repectively) in GH rations. There was a quadratic trend in Deg as LH or SFC ratios increased in MS rations. The Deg decreased ($p<0.001$) in MS and tended to decrease ($p=0.12$) in GH rations with increased ratio of LPZ (Figure 5.1).

There was relatively high variability in MIC values (cv = 20.2 and 33.9 in GH and MS rations, respectively, Table 5.2). Only PS type had effect ($p<0.001$) in GH rations whereby highest values were obtained in rations with LH. The values ranged from 188-368 mg/g DM incubated. The MIC in MS rations, were similar across PS types and ratios. The values ranged from 195-389 mg/g DM incubated. The volume of gas produced was affected by only PS type ($p<0.001$) in GH rations whereby rations of LPZ had lower values than those of LH or SFC. The values ranged from 132 to 155, 103 to 117 and 132 to 149 ml/g DM incubated for LH, LPZ and SFC rations, respectively. In MS rations, gas volume was affected by PS type ($p<0.001$), PS ratio ($p<0.001$) and PS type x PS ratio interaction ($p<0.001$). The corresponding values for the PS types ranged from 159 to 168, 104 to 156 and 131 to 164 ml/g DM incubated. The $T_{½}$ was affected by PS type ($p<0.001$) and PS ratio ($p<0.001$) in both GH and MS rations. The values ranged from 14.7 to 22.2, 11.9 to 26.2 and 9.3 to 21.8 h in GH rations and 10.6 to 14.7, 14.8 to 18.3 and 10.6 to 14.2 h in MS rations with LH, LPZ and SFC, respectively. Except for MS:LPZ rations which had quadratic trend ($R^2 = 0.64$) in $T_{½}$, the rest of the rations showed linear decrease ($p<0.02$ to 0.002, $R^2 = 0.79$ to 0.93) as the

ratio of PS increased (Figure 5.3). The PF was only affected by PS ratio in GH rations, but was affected by PS type ($p<0.001$), PS ratio ($p<0.001$) and PS type x PS ratio interaction ($p<0.01$) in MS rations. The values ranged from 4.9 to 6.3, 4.6 to 5.4 and 4.9 to 5.9 in GH rations with LH, LPZ and SFC, respectively. The corresponding values in MS rations were 4.7 to 5.2, 4.4 to 5.2 and 4.7 to 6.0. There were quadratic trends (R^2 range = 0.19 to 0.97) in PF as PS ratios increased in both GH and MS rations. The significance of the slopes were generally poor with only the quadratic slopes of GH:SFC, MS:SFC and MS:LH rations being significant ($p<0.02$, $p<0.05$ and $p<0.05$, respectively, Figure 5.2).

Table 5.1 Chemical composition and energy content of roughages and protein supplements

Feed	Chemical composition g/kg DM					Energy content MJ/kg DM
	CP	NDF	ADF	ADFn	Ash	GE
Maize stover	38	715	451	1.5	62	16
Grass hay	34	665	464	1.5	91	16
Lucerne	199	355	270	0.4	86	16
Lespedeza	73	522	395	4.8	50	17
Sunflower cake	390	324	215	0.9	54	17

CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, GE = gross energy

The DEF were affected by PS type ($p<0.001$) and PS ratio ($p<0.001$) in both GH and MS rarions. The effect of PS type x PS ratio interaction was only significant ($P<0.001$) in MS rations. The values ranged from 0.46 to 0.91, 0.33 to 0.84 and 0.46 to 1.34 in GH rations with LH, LPZ and SFC, respectively. The corresponding values in MS rations ranged from 0.65 to 1.02, 0.54 to 0.68 and 0.68 to 1.21.There was a linear increase ($p<0.002$ to 0.001, $R^2 \geq 0.93$) in DEF as the ratio of LH or SFC increased in both GH and MS rations, while increasing LPZ ratio resulted in a quadratic trend ($R^2 = 0.96$ and 0.67 in GH and MS rations, respectively, Figure 5.4).

Table 5.2 Degradability and gas production of grass hay mixed with different protein supplements and fermented *in vitro* using rumen fluid

Supplement (PS)	RG:PS	pH	Degradability (Deg) mg/ g DM	Microbial yield mg/ g DM	Gas production (V) ml/g DM	Time to half gas volume ($T_{½}$) h	PF	DEF
	100:0	6.76	608	259	119	26.0	4.8	0.37
Lucerne	80:20	6.82	712	319	145	22.2	4.9	0.46
	60:40	6.72	740	342	139	18.3	5.4	0.60
	40:60	6.85	761	373	155	15.2	4.9	0.66
	20:80	6.85	789	346	139	14.3	5.7	0.83
	0:100	6.86	836	368	132	14.7	6.3	0.91
Sericea lespedeza	80:20	6.71	557	216	110	26.2	5.1	0.33
	60:40	6.72	572	221	117	26.1	4.9	0.33
	40:60	6.80	495	168	109	21.3	4.6	0.39
	20:80	6.83	543	277	103	20.3	5.4	0.49
	0:100	6.95	532	188	115	11.9	4.8	0.84
Sunflower cake	80:20	6.75	732	282	149	21.8	4.9	0.46
	60:40	6.94	749	260	149	19.6	5.0	0.50
	40:60	6.90	769	317	145	14.5	5.3	0.78
	20:80	6.95	778	238	140	11.5	5.8	1.12
	0:100	6.95	781	265	132	9.3	5.9	1.34
	LSD	0.225	56	99	25	6.7	1.0	0.27
	P	ns	0.001	0.001	0.001	0.001	0.05	0.001
	cv	2.2	4.6	20.2	10.7	20.4	10.4	23.0
Sources of variation effects								
	PS	ns	***	***	***	***	ns	***
	PS ratio	ns	**	ns	ns	***	*	***
	PS*PS ratio	ns	**	ns	ns	ns	ns	ns (p=0.07)

PF = Deg/V, DEF = Deg/ $T_{½}$ x $V_{½}$, $V_{½}$ = half of V, ns = not significant,

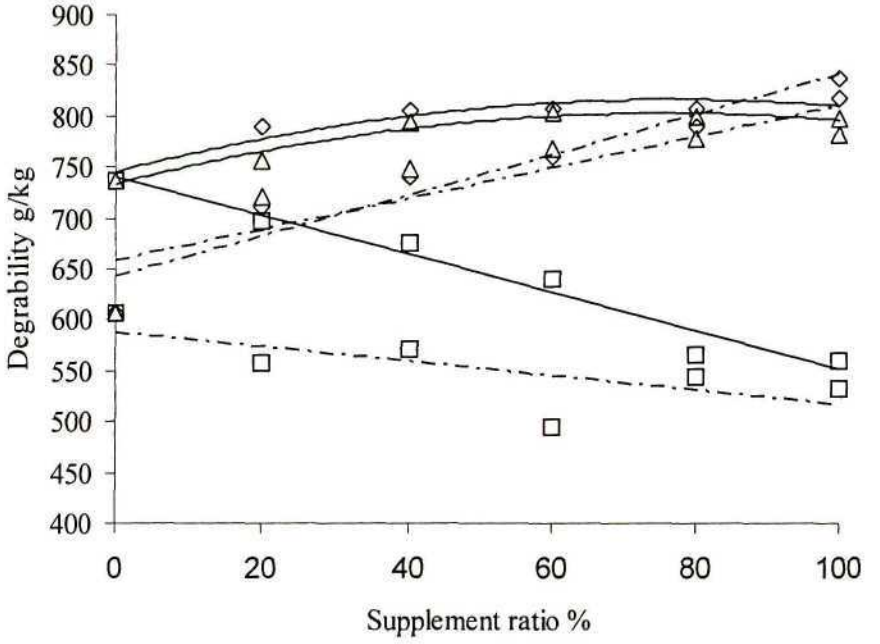
* p<0.05, **p<0.01, *** p<0.001

Table 5.3 Degradability and gas production of maize stovers (MS) mixed with different protein supplements and fermented *in vitro* using rumen fluid

Supplement (PS)	MS:PS	PH	Degradability (Deg) mg/ g DM	Microbial yield g/ kg	Gas production (V) ml/g DM	Time to half gas volume ($T_{½}$) h	PF	DEF
	100:0	6.79	730	288	148	18.8	5.0	0.53
Lucerne	80:20	6.83	786	248	167	14.7	4.7	0.65
	60:40	6.79	805	235	168	13.0	4.8	0.77
	40:60	6.68	807	265	164	12.7	5.0	0.82
	20:80	6.83	807	344	163	11.8	5.0	0.87
	0:100	6.85	792	389	159	10.6	5.2	1.02
Sercea lespedeza	80:20	6.84	698	297	145	18.3	5.1	0.57
	60:40	6.78	678	330	156	17.2	4.4	0.57
	40:60	6.88	642	288	141	17.3	4.6	0.54
	20:80	7.06	565	289	118	18.0	5.2	0.58
	0:100	7.00	509	218	104	14.8	5.0	0.68
Sunflower cake	80:20	6.80	771	238	164	14.2	4.7	0.68
	60:40	6.89	787	243	159	13.9	5.0	0.74
	40:60	6.94	798	246	148	12.6	5.4	0.89
	20:80	6.94	799	195	136	11.6	5.9	1.07
	0:100	6.87	793	263	131	10.6	6.0	1.21
	LSD	0.27	41	122	20	2.7	0.6	0.13
	P	Ns	0.001	ns	0.001	0.001	0.001	0.001
	cv	2.2	4.3	33.9	8.5	14.8	9.1	13.2
Sources of variation effects								
PS		Ns	***	ns (p=0.06)	***	***	***	***
PS ratio		Ns	***	ns	***	***	***	***
PS*PS ratio		Ns	***	ns	***	ns	**	***

PF = Deg/V, DEF = Deg/ $T_{½}$ x $V_{½}$, $V_{½}$ = half of V, ns = not significant,

* p<0.05, **p<0.01, *** p<0.001



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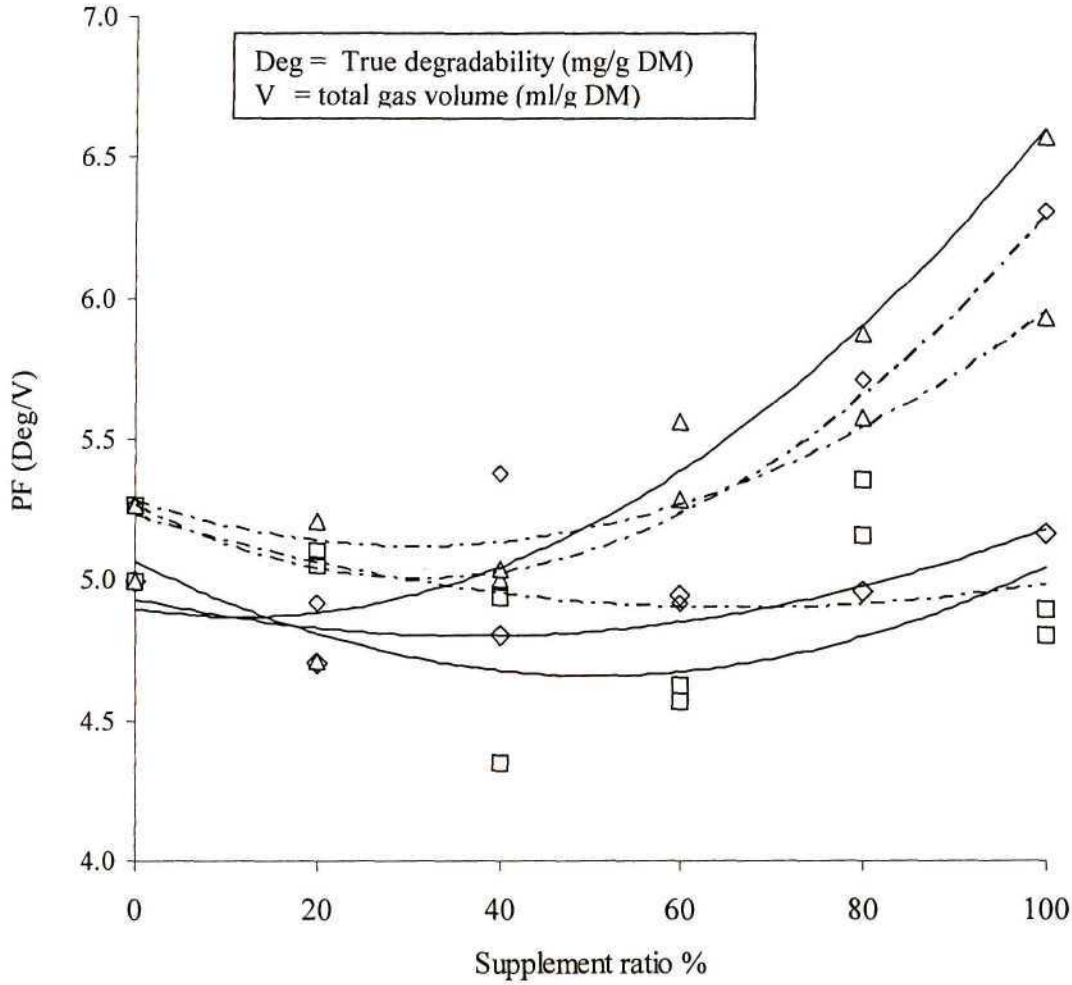
(a) $y_{\Diamond} = 1.99x + 641.2$ $R^2 = 0.91$, $p_1<0.003$
$y_{\square} = -0.71x + 586.4$, $R^2 = 0.49$, $p_1=0.12$
$y_{\Delta} = 1.51x + 658.6$ $R^2 = 0.74$, $p_1 < 0.03$

(b) $y_{\Diamond} = -0.01x^2 + 1.87x + 745.1$, $R^2 = 0.90$,
$p_1<0.03$, $p_2 = 0.08$
$y_{\square} = -1.8967x + 740.7$, $R^2= 0.96$, $p_1 < 0.001$
$y_{\Delta} = -0.0121x^2 + 1.8406x + 733.6$, $R^2 = 0.96$,
$p_1 < 0.01$, $p_2 < 0.03$

p_1 = significance of linear slope, p_2 = significance of quadratic slope

Figure 5.1 Degradability of (a) grass hay (–·–·–) and (b) maize stover (——) rations supplemented with lucerne (◊),*Sericea lespedeza,* (□) or sunflower cake (Δ) and fermented *in vitro* using rumen fluid inoculum

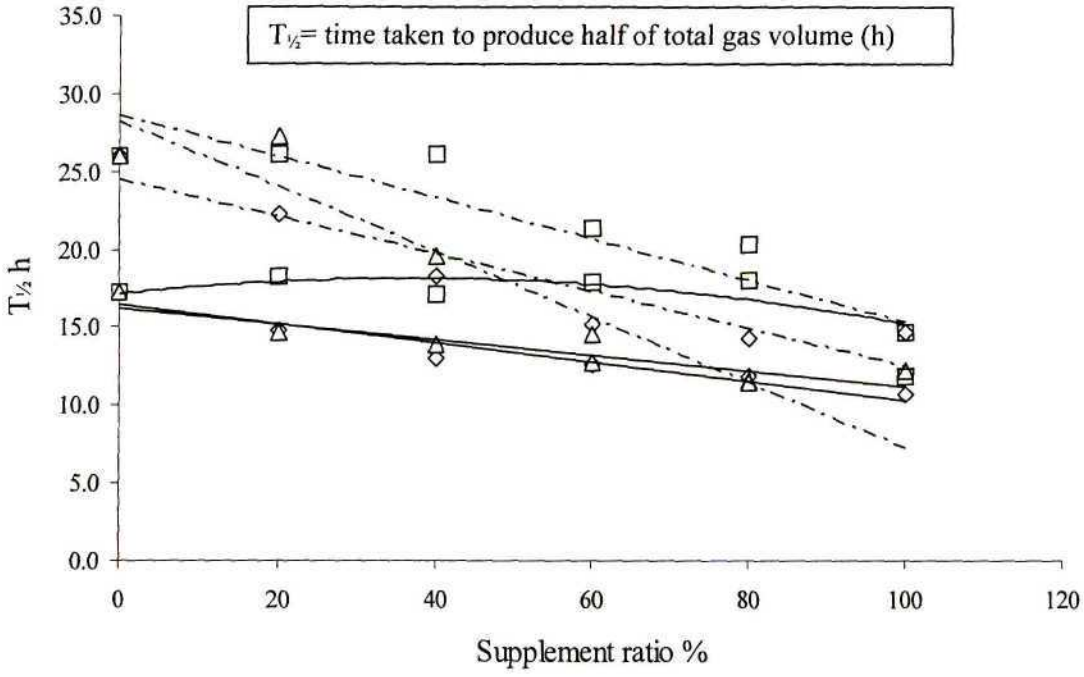


(a) $y_{\Diamond} = 0.0003x^2 - 0.017x + 5.3$, $R^2 = 0.83$,
$p_1 = 0.26$, $p_2 = 0.11$
$y_{\square} = 7E\text{-}05x^2 - 0.01x + 5.2$, $R^2 = 0.19$,
$p_1 = 0.49$, $p_1 = 0.58$
$y_{\Delta} = 0.0002x^2 - 0.01x + 5.3$, $R^2 = 0.97$,
$p1 = 0.07$, $p_2 < 0.02$

(b) $y_{\Diamond} = 1E\text{-}04x^2 - 0.0072x + 4.9$, $R^2 = 0.77$
$p_1 = 0.10$, $p_2 < 0.05$
$y_{\square} = 0.0002x^2 - 0.016x + 5.1$, $R^2 = 0.29$,
$p_1 = 0.39$, $p_2 = 0.37$
$y_{\Delta} = 0.0002x^2 - 0.005x + 4.9$, $R^2 = 0.97$,
$p_1 = 0.55$, $p_2 < 0.05$

p_1 = significance of linear slope, p_2 = significance of quadratic slope

Figure 5.2 Partitioning factor (PF) of (a) grass hay (_._._) and (b) maize stover (____) rations supplemented with lucerne (◊),*Sericea lespedeza,* (□) or sunflower cake (Δ) and fermented *in vitro* using rumen fluid inoculum

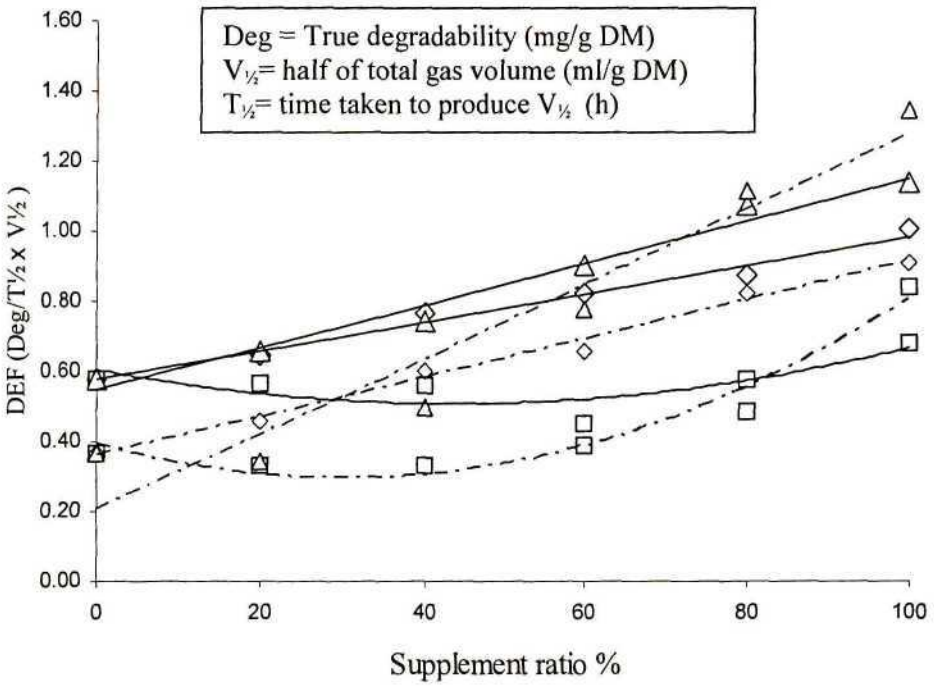


a) $y_{\Diamond} = -0.12x + 24.4$, $R^2 = 0.88$, $p_1 < 0.006$
$y_{\square} = -0.13x + 28.6$, $R^2 = 0.79$, $p_1 < 0.02$
$y_{\Delta} = -0.2091x + 28.1$, $R^2 = 0.91$, $p_1 < 0.002$

(b) $y_{\Diamond} = -0.06x + 16.4$, $R^2 = 0.93$, $p1 < 0.002$
$y_{\square} = -0.0007x^2 + 0.05x + 17.2$, $R^2 = 0.64$,
$p_1 = 0.30$, $p_2 = 0.17$
$y_{\Delta} = -0.05x + 16.3$, $R^2 = 0.85$, $p_1 < 0.009$

p_1 = significance of linear slope, p_2 = significance of quadratic slope

Figure 5.3 Time taken to produce half gas volume ($T_{½}$) of (a) grass hay (_ . _ . _) and (b) maize stover (——) rations supplemented with lucerne (◊),*Sericea lespedeza,* (□) or sunflower cake (Δ) and fermented *in vitro* using rumen fluid inoculum



(a) $y_{\Diamond} = 0.005x + 0.36$, $R^2 = 0.99$, $p_1<0.001$
$y_{\square} = 0.0001x^2 - 0.006x + 0.3919$, $R^2 = 0.96$,
$p_1 < 0.05$, $p_2 <0.01$
$y_{\Delta} = 0.0107x + 0.21$, $R^2 = 0.93$, $p_1<0.002$

(b) $y_{\Diamond} = 0.004x + 0.58$, $R^2 = 0.98$, $p_1 < 0.001$
$y_{\square} = 5E\text{-}05x^2 - 0.004x + 0.61$, $R^2 = 0.67$,
$p_1 = 0.17$, $p_2 = 0.12$
$y_{\Delta} = 0.006x + 0.55$, $R^2 = 0.98$, $p_1<0.001$

p_1 = significance of linear slope, p_2 = significance of quadratic slope

Figure 5.4 Rumen degradability efficiency factor (DEF) of (a) grass hay (_ _ _) or (b) maize stover (———) rations supplemented with lucerne (◊),*Sericea lespedeza,* (□) or sunflower cake (Δ) fermented *in vitro* using rumen fluid inoculum

5.4 DISCUSSION

Extensive *in vitro* incubations (beyond 24 h) are often employed to establish the maximal degradation potential of substrates. Besides observing anaerobic and suitable temperature conditions (39°C), maintenance of appropriate pH for microbial activities is crucial when simulating rumen fermentation *in vitro*. For these reasons, culture media containing buffering agents (Williams *et al.*, 1998) are normally added to the *in vitro* fermentation culture, to help in stopping the drop in pH due to production of fermentation metabolites, particularly acids and hydrogen ions. This study used buffer solution described by McDougall (1948) and it contained bicarbonate as buffer agent. Rymer *et al.* (2005) stated that bicarbonate is an important component of the rumen buffering system, and is usually included in all media to simulate the rumen conditions more closely. Of fundamental importance in this study, and ruminant nutrition in general, was fibre breakdown or cellulolysis. Cellulolysis became partially inhibited as the rumen pH of sheep receiving roughage was reduced from 6.6 to 6.2 and ultimately totally inhibited as the pH fell below 6.0 (Mould and Ørskov, 1983). Since the terminal pH of this study ranged from 6.68 to 7.05, it can be deduced that cellulolysis was not impaired.

Present results indicate that LH and SFC induced maximal Deg at their low levels (20% inclusion) in MS rations as opposed to GH rations where there was a substantial linear increase in Deg as LH or SFC ratios increased (Figure 5.1). On the other hand, the decrease in Deg and GP as LPZ ratio increased may be attributed to its chemical composition, particularly the tannin content. Although the tannin content was not analysed in the present study, Turner *et al.* (2005) observed high tannin content of 23.1 mg/g in *Sericea lespedeza,* and a much lower value of 0.24 mg/g in lucerne. Condensed tannins have been reported to inhibit ruminal microbial activity and/or form undegradable complexes with substrates (Ngwa *et al.*, 2003), which could reduce degradability.

The importance of predicting rumen microbial production has been recognized for a long time (e.g. Bergen, 1977). However, accurate determination of MIC *in vitro* is still an elusive subject. Methods commonly applied to estimate MIC include gravimetric procedures (Van Soest, *et al.*, 1991; Blümmel *et al.*, 1997), nitrogen balance determination (Getachew *et al.*, 2000), use of

markers (Krishnamoorthy *et al.*, 1991; Blümmel *et al.*, 1997) and purine derivatives analysis (Makkar and Becker, 1999). With the use of the gravimetric method, Van Soest (1994) reported that extraction of residue with NDS entirely removes microbial matter. This has been validated from *in vitro* fermentations by Blümmel *et al* (1997) who observed close agreement (r = 0.93) between MIC estimated by marker method (^{15}N incorporation into microbial cells) and that estimated gravimetrically. Ranilla *et al.* (2001) also obtained closely comparable values of MIC estimated using ^{15}N marker or gravimetrically. The MIC values obtained in this study which ranged from 195-389 mg/g DM incubated were within MIC range of 168-462 mg/g DM incubated reported by Ranilla *et al.* (2001) in an *in vitro* study of effect of different fibre sources on efficiency of microbial synthesis. They were also closely comparable to MIC obtained by Jaurena *et al.* (2003) which ranged from 285 -376 g/kg of organic matter degraded using *in vitro* rumen simulation technique. *In vivo* microbial DM ranging from 266-285 g/kg of rumen particulate DM were reported by Yang *et al.* (2001) in dairy cattle fed diets differing in chemical and physical composition. Craig *et al.* (1987) obtained microbial DM ranging from 168-301g/kg of rumen particulate in a study where rumen samples were taken at different times within 10 h after feed withdrawal. Under *in vivo* situation, the washing effect and passage of digesta to the lower gut is expected to prevent high accumulation of microbial matter. Under *in vitro* situation, there is continuous accumulation of microbial matter before the substrate is exhausted and microbial lysis sets in (Rymer, 1999). This can be responsible for some higher values obtained *in vitro* than the cited *in vivo* values. On the other hand, several factors have been reported to confound the validity of *in vitro* MIC values. For instance, in a study where protein-rich feeds were incubated, Rymer *et al.* (2001) reported that with a more limited supply of fermentable (although not necessarily degradable) material, microbial recycling begins early, and this would lower MIC estimated at the end of incubation. This can partly explain lower MIC values among SFC rations as compared to those of LH. The former had higher CP content. The results therefore supported the suggestion by other workers (Blümmel *et al.*, 1997a; Rymer *et al.*, 2001; Makkar, 2004) that when using the GP technique to estimate the dynamics of protein metabolism, correct timing is an important consideration. With LPZ rations, it was also possible that tannin-protein complexes were formed (Haggerman and Robbins, 1987) and were washed away by NDS, hence confounding the values of MIC estimates as explained by Blümmel and

Lebzien (2001). It is therefore difficult to draw concrete conclusions from the present MIC values.

Although PF values have been positively correlated with microbial yield (Blümmel *et al.*1997) and feed intake (Blümmel *et al.*1997; Blümmel *et al.*, 2005), there can be confounding factors affecting the reliability of PF values from rations with varied protein contents such as those in the present study. Cone and Van Gelder (1999) observed lower gas production from proteins than from carbohydrates, and this may have been responsible for the increase in PF as the ratio of LH or SFC increased. Rymer (1999) obtained PF values ranging from 2.77 to 30.3 in incubations lasting between 9 and 48 h and cautioned that if PF is recorded too early, the microbial growth, and consequently degradation, will not be as great as the feed's potential, and if measured too late, then microbial recycling of unknown magnitude will have occurred. Blümmel *et al.* (2005) obtained high and biologically doubtful values from fermentation of a tropical tree legume (*Leucaena)*. Makkar *et al.* (1998) demonstrated that PF derived from many browse species deviated from conventional values. These anomalies have been partly attributed to the fact that the degradability of browses was overestimated as a result of the solubilization of unfermented substances, such as tannins and their complexes, from the residue. Given that poor quality roughages are commonly supplemented with browses to boost their utilization, it can be said that PF cannot be a sufficient measurement to evaluate the nutritive value of such rations. This was demonstrated in the present study where PF was similar among MS rations supplemented with LH or LPZ despite remarkable differences in the extent and rate of degradation induced by the two supplements. Furthermore, the quadratic trend shown in PF, with most rations having similar values up to 60% supplementation, indicated the inability of PF to distinguish nutritive impact arising from moderate levels of supplementation. It is therefore evident that caution needs to be exercised when interpreting and ranking diets on the basis of PF since there can be many confounding factors.

The fact that rations showed high consistency in $T_{½}$ was an important revelation, given that it is a measurement obtainable directly from IVGPT alongside Deg and GP. It can be argued that short $T_{½}$ coupled with high degradability depicts high microbial efficiency. This is in agreement with the findings by Cone and Van Gelder (2000) who observed increased microbial efficiency

in substrates with higher fermentation rates. It may be possible that confounding results obtained in other measurements (Deg, GP, MIC and PF), can be moderated by including $T_{½}$ among the evaluation measurements. The DEF depicted the rapidity by which the feed is degraded to make nutrients available and the extent to which the degraded material is fermented. The fact that it showed high consistency across rations showed it is an important measurement which should be included in IVGPT evaluations. This can further help in resolving confounding results which may be obtained in other measurements.

5.5 CONCLUSIONS

Roughages used in the present study were characterized by high fibre and low protein content, which is typical of roughages commonly available in tropical regions. The results generally showed that the nutritive value can be improved by mixing the roughages and protein supplements. Specifically, supplementation improved the overall degradability, $T_{½}$ and DEF. However, the supplementation needs to be done carefully because these nutritive attributes were affected differently by the supplement type, supplement ratio and the interactions between the two.

Although IGVPT is dynamic and can give several measurements to help in understanding the fate of the substrates under fermentation, caution should be taken in ranking the rations based on Deg, GP, MIC or PF values. This is because several conflicting factors can affect the validity of these measurements. The present results indicated that other derived variables such as $T_{½}$ and DEF were important measurements that should be included in IVGPT evaluation to help in moderating the confounding results obtainable when only Deg, GP, MIC and PF are used. Further work should investigate the relationship between *in vivo* responses and the *in vitro* measurements, particularly $T_{½}$ and DEF.

Chapter 6

TYPOLOGY OF RUMINANT DIETS FROM NUTRITIVE MEASUREMENTS DETERMINED USING *IN VITRO* GAS PRODUCTION TECHNIQUE

ABSTRACT

In vitro gas production technique (IVGPT) is one of the biological methods presently receiving much attention in ruminant feed evaluation. The objective of this study was to determine whether measurements derived from IVGPT can be used to consistently and meaningfully classify rations into homogeneous groups of nutritive characteristics. A multivariate cluster analysis was used to create clusters of diets with similar nutritive characteristics. Forty two diets used in the analysis comprised of different roughages (RG) and protein sources (PS). The RG were grass (GH) and maize stover harvested at grain milk (MM) or dry (MD) stages. The PS were Lucerne (LH), Sericea lespedeza (LPZ) and sunflower oil cake (SFC). The classifying variables included rate of gas production (GP) from soluble (a_1) and insoluble (b_1) feed components, degradability (Deg), microbial yield (MIC) and Deg/GasVol ratio (PF). Using a variable reduction technique, two canonical variables accounting for 85% of the variation were generated and used in clustering. The cubic clustering criterion (CCC), pseudo-F and pseudo-t analyses were unanimous for a three cluster solution, which accounted for approximately 60% of the variation. Total gas volume (GasVol), gas volumes from soluble (A) and insoluble (B) feed components, lag time (lag), contents of protein(CP) and fibre (NDF and ADF), time taken to produce half GasVol ($T_{½}$) and DEF (Deg/($T_{½}$ x half GasVol) were analyzed to check the validity of the clusters. Cluster 1 had the highest Deg, GasVol, B and MIC, but lowest PF and was predominated by rations containing either MM or LH. Cluster 2 had similar b_1, Deg, A, $T_{½}$, and lag as Cluster 1, but had the lowest MIC and highest DEF. It was predominated by SFC rations with either GH or MD. Cluster 3 was predominated by LPZ rations with GH or MD. It had the lowest Deg, b_1, A and GasVol, and the longest $T_{½}$ and lag. The study showed that rations can be consistently classified into distinct homogeneous groups of IVGPT derived measurements.

6.1 INTRODUCTION

The nutritive value of ruminant feeds is principally governed by their chemical composition, intake, digestibility and efficiency by which the nutrients are released. There are several methods which have been developed to study the nutritive value of feeds. Biological based methods have been recommended as being more meaningful because the microorganisms and enzymes involved are more sensitive to factors influencing the rate and extent of *in vivo* digestion than are chemical methods (Van Soest., 1994). *In vitro* gas production technique (IVGPT) is one of the biological methods presently receiving much attention in ruminant feed evaluation (Getachew *et al.*, 1998; Dijkistra *et al.*, 2005; Rymer *et al.*, 2005). The IVGPT became a routine method of feed evaluation after the work of Menke *et al.* (1979), where high correlation between gas production and *in vivo* apparent digestibility was reported. In the present use of IVGPT, it has been recognized that gas measurement alone is insufficient and should be complemented with residue determination (Blümmel *et al.*, 1997). Also, several models have been developed to generate information about fermentation kinetics from gas production profiles (Beuvink and Kogut, 1993; Groote *et al.*, 1996; Campos *et al.*, 2004). In general the advantage of IVGPT is the ability to generate diverse information regarding the fate of feed undergoing rumen metabolism. The objective of this study was to determine whether measurements derived from IVGPT can be used to consistently and meaningfully classify rations into homogeneous groups of nutritive characteristics.

6.2 MATERIALS AND METHODS

In vitro experiments reported in Chapters 3 and 5 were performed concurrently in a series of nine incubations. The incubator had a capacity of 24 samples. Groups of roughages (RG): protein supplements (PS) rations were formed and randomly allocated to three different periods of incubation. The incubations were replicated three times. There were 21 samples and 3 blanks per incubation. The rations and schedule of incubations is shown in Table 6.1.

Table 6.1 The incubation schedule of Roughage:Protein supplement rations

Roughage / Supplements	Milk stage maize stovers (MM)	Dry maize stovers (MD)	Grass hay (GH)
Lucerne (LH)	100:0 80:20 60:40 P1 40:60 20:80 0:100	100:0 80:20 60:40 P3 40:60 20:80 0:100	100:0 80:20 60:40 P2 40:60 20:80 0:100
Lespedeza(LPZ)	100:0 80:20 60:40 P3 40:60 20:80 0:100	100:0 80:20 60:40 P2 40:60 20:80 0:100	100:0 80:20 60:40 P1 40:60 20:80 0:100
Sunflower oil cake (SFC)	100:0 80:20 60:40 P2 40:60 20:80 0:100	100:0 80:20 60:40 P1 40:60 20:80 0:100	100:0 80:20 60:40 P3 40:60 20:80 0:100

P1, P2, P3 = Periods when the rations were incubated.

Data collected included degradability (Deg), gas production (GP), time taken to produce half gas volume ($T_{½}$) and fermentation kinetics derived from fitting the Model of Campos *et al.* (2004) detailed in Chapters 3. Cluster multivariate analysis technique (SAS, 2002), was performed with the aim of classifying the rations into homogeneous groups (clusters) based on IVGPT derived parameters. The analysis involved four steps. Firstly, a variable reduction technique (ACECLUS) was performed. ACECLUS procedure summarizes variation in a multivariate set of data into separate functions called canonical variables, which are orthogonal to each other. The classifying variables used included Deg, degradation rate of soluble (a_1) and fibre (b_1) fractions of the rations, microbial yield (MIC) and degradability:gas production ratio i.e. the partitioning factor (PF). Two canonical variables accounting for 85% of the variation were generated. In the second step, the canonical variables were used in hierarchical cluster analysis using Ward's method (William, 1994) to ensure that both within cluster differences and between

clusters linkages were minimized. Because it was important to establish a suitable number of clusters to keep, in the third step cubic clustering criterion (CCC), pseudo-F and pseudo-t analyses were unanimous for a three cluster solution, which accounted for approximately 60% of the variation. The diets were then assigned into the clusters through use of the TREE procedure. Finally the clusters were finely adjusted using FASTCLUS, which is a non hierarchical procedure. To check the validity of the clusters, general linear model (GLM) analysis was performed. The classifying variables used in the creation of the clusters and other variables not used in cluster creation were included in the GLM procedure. The validation variables not used in cluster creation included total gas volume (GasVol), gas volumes from soluble (A) and insoluble (B) feed components, lag time (lag), contents of protein(CP) and fibre (NDF and ADF), time taken to produce half GasVol ($T_{½}$) and degradation efficiency factor-DEF (see Chapter 5). The hypothetical trends of these variables were used in assessing the validity of the clusters.

6.3 RESULTS

There were 25, 9 and 8 diets grouped into clusters 1, 2 and 3, respectively (Table 6. 2). The GLM analysis of cluster classifying variables showed that Cluster 1 had the highest MIC ($p<0.001$), lowest PF ($p<0.05$), and had similar Deg and b_1 as Cluster 2. It was predominated by rations containing MM or LH. Cluster 2 had the highest PF and lowest MIC. It was predominated by SFC rations with GH or MD. Cluster 3 was predominated by LPZ rations with GH or MD. It had the lowest Deg ($p<0.001$) and b_1 ($p<0.02$) while the MIC was intermediate. All the clusters had similar a_1 value. The GLM analysis of the variables not used in clusters creation further showed that Cluster 2 had the highest CP ($p<0.01$) and DEF ($p<0.003$), but was similar with Cluster 1 in GasVol, A, B, lag, NDF, ADF and $T_{½}$ values. Cluster 3 had the longest lag ($p<0.05$) and $T_{½}$ ($p<0.01$), lowest GasVol ($p<0.001$), A ($p<0.003$) and B ($p<0.05$), highest NDF ($p<0.001$) and ADF ($p<0.001$), lowest CP and lowest DEF. These results are summarized in Table 6.3.

Table 6.2 Diet clusters formed from measurements derived from *in vitro* rumen fermentation

RG	SUP	SUPL	A	a_1	B	b_1	lag	GasVol ml/g	Deg mg/g DM	MIC mg/g DM	PF	DEF	CLUSTER
MM	NONE	100:0	22	0.17	141	0.04	4.2	159	765	267	4.6	0.59	1
MM	LH	80:20	25	0.16	139	0.04	3.6	165	803	260	5.0	0.67	1
MM	LH	60:40	43	0.11	121	0.04	3.5	172	812	223	4.8	0.82	1
MM	LH	40:60	43	0.12	114	0.04	3.8	162	800	222	5.0	0.88	1
MM	LH	20:80	40	0.14	119	0.04	2.9	162	798	329	4.9	0.87	1
MM	LPZ	80:20	15	0.18	152	0.04	4.4	172	782	297	4.6	0.53	1
MM	LPZ	60:40	21	0.24	132	0.03	3.8	152	721	255	4.8	0.58	1
MM	LPZ	40:60	15	0.47	136	0.03	3.3	144	672	226	4.7	0.49	1
MM	LPZ	20:80	17	0.23	109	0.03	3.3	134	657	254	4.9	0.56	1
MM	SFC	80:20	28	0.12	140	0.04	3.6	167	811	309	5.0	0.66	1
MM	SFC	60:40	33	0.16	129	0.04	2.9	162	817	295	5.1	0.76	1
MM	SFC	40:60	34	0.16	109	0.04	3.9	151	813	338	5.4	0.91	1
MM	SFC	20:80	33	0.16	96	0.04	4.0	140	783	298	5.7	1.00	1
NONE	LH	0:100	37	0.13	107	0.04	2.4	140	776	310	5.7	1.00	1
MD	NONE	100:0	22	0.17	117	0.04	8.6	146	727	245	5.0	0.57	1
MD	LH	80:20	32	0.17	132	0.04	4.8	148	759	304	5.3	0.62	1
MD	LH	60:40	33	0.15	125	0.04	4.0	166	779	311	4.7	0.72	1
MD	LH	40:60	36	0.14	121	0.04	3.8	162	789	260	4.9	0.76	1
MD	LH	20:80	47	0.14	113	0.04	2.1	163	787	301	4.9	0.88	1
MD	LPF	80:20	24	0.18	111	0.04	7.4	136	676	289	5.1	0.60	1
GH	NONE	100:0	11	0.14	128	0.03	8.8	139	667	272	4.8	0.37	1
GH	LH	60:40	20	0.14	118	0.03	6.3	133	688	315	5.5	0.60	1
GH	LH	40:60	25	0.14	120	0.04	4.7	162	717	343	5.4	0.66	1
GH	LH	20:80	30	0.12	104	0.04	4.7	142	730	315	5.5	0.83	1
GH	LPZ	80:20	5	0.13	128	0.03	6.0	116	708	314	5.9	0.33	1
MD	SFC	80:20	34	0.15	118	0.04	4.1	154	719	176	4.7	0.66	2
MD	SFC	60:40	30	0.09	117	0.03	3.5	147	744	156	5.1	0.71	2
MD	SFC	40:60	34	0.14	110	0.04	2.8	151	776	138	5.2	0.89	2
MD	SFC	20:80	38	0.17	94	0.04	2.9	139	798	138	5.8	1.14	2
GH	SFC	80:20	34	0.16	111	0.03	3.5	143	732	171	5.1	0.78	2
GH	SFC	60:40	14	0.47	140	0.03	4.8	145	712	193	5.1	0.50	2
GH	SFC	40:60	32	0.20	111	0.03	3.6	133	749	174	5.8	0.78	2
GH	SFC	20:80	37	0.18	98	0.04	2.7	135	777	196	7.7	1.12	2
NONE	SFC	0:100	32	0.26	93	0.04	3.7	132	792	215	6.1	1.19	2
MD	LPZ	60:40	12	0.22	92	0.03	10.2	114	630	227	5.7	0.53	3
MD	LPZ	40:60	24	0.16	87	0.04	7.3	126	592	265	4.7	0.41	3
MD	LPZ	20:80	15	0.27	84	0.03	7.3	99	551	261	5.7	0.59	3
GH	LH	80:20	16	0.18	131	0.03	10.7	113	640	280	6.0	0.46	3
GH	LPZ	60:40	3	0.12	127	0.03	7.6	108	605	266	5.3	0.33	3
GH	LPZ	40:60	11	0.34	112	0.03	3.6	105	553	258	5.1	0.39	3
GH	LPZ	20:80	11	0.29	101	0.03	3.8	107	566	278	5.3	0.49	3
NONE	LPZ	0:100	28	0.16	82	0.035	2.6	100	556	255	5.6	0.68	3

Table 6.3 Mean values of clusters classifying and validation variables

Nutritive parameter	Cluster			SED	P
	1	2	3		
Cluster classifying variables					
a	0.17	0.17	0.17	0.26	ns
B	0.036	0.039	0.032	0.002	0.001
Deg	754	736	577	26	0.001
MIC	305	163	265	21	0.001
PF	5.1	5.6	5.5	0.1	0.05
Cluster validation variables					
GasVol	150	144	113	6	0.001
A	27	30	14	4	0.003
B	118	112	104	6	0.05
Lag	4.4	3.4	7.1	0.8	0.05
CP	121	242	68	27	0.01
NDF	515	480	592	36	0.001
ADF	340	313	411	21	0.001
DEF	0.69	0.82	0.46	0.07	0.003
$T_{½}$	15.6	14.7	20.0	1.5	0.01

A and B are the gas volume (ml) from fast (soluble)and slowly (fibre) degradable fractions, respectively, a_1 and b_1 are the degradation rates (per h) of soluble and fibre fractions, respectively, lag is microbial colonization lag time. RG = roughage, PS = protein supplement, TruDeg = true degradability, MIC = microbial yield, GasVol = total volume of gas produced, $V_{½}$ = Half of GasVol, $T_{½}$ = Time (h) taken to produce $V_{½}$, PF = partitioning factor (TruDeg/GasVol), DEF = degradability efficiency factor (TruDeg/ ($T_{½}$ x $V_{½}$)

6.4 DISCUSSION

Validity of the clusters

Given that this study sought to establish whether parameters derived from evaluation of ruminant feeds using IVGPT can meaningfully separate diets into distinct groups of nutritive attributes, it was important to establish the authenticity of the clusters through assessing the consistency of the hypothetical trends. For instance, it is expected that the soluble component of the feeds should ferment faster than the fibre component (Beuvink and Kogut, 1993; Groot *et*

al., 1996; Campos *et al.*, 2004). This was confirmed in the present results where a_1 values were the same among the clusters and were higher than b_1. From the classifying variables used in clusters creation, Cluster 2 showed superiority over Cluster 1 by having the higher b_1 ($p<0.02$) and PF ($p<0.05$). Cluster 3 showed poorest nutritive attributes by having the lowest b_1 as well as Deg. The results of the validation variables not used in clusters creation were generally consistent with hypothetical expectations. As shown in Table 6.3, it was consistently shown that Cluster 2 had the most superior qualities by having the shortest lag ($p<0.05$), highest CP ($p<0.01$) lowest fibre (NDF and ADF, $p<0.001$) and highest DEF ($p<0.007$). The opposite was true for Cluster 3, which was similarly characterized by the poorest nutritive attributes from the classifying variables. Cluster 1 maintained being of intermediate quality in all validation variables except for GP measurements (GasVol and B) where it had the highest ($p<0.001$) values.

It can be said that diets comprising Cluster 1 as compared to those of Cluster 2 were of high degradability coupled with high gas production, and this was responsible for the lowest PF exhibited by the former. The fact that MIC was highest in Cluster 1 and lowest in Cluster 2 was unexpected. Cluster 2 had the highest CP and lowest fibre content and these are expected to promote a higher microbial growth. The explanation to the unexpected low MIC in Cluster 2 could partly relate to the stipulation by Rymer *et al.* (2001) that under limited supply of fermentable material (although not necessarily degradable) microbial recycling begins early, hence lowering MIC values estimated at the end of a prolonged incubation. Thus in reality, the diets of this cluster most likely had the highest MIC, as depicted by their shortest lag (Table 6.3).

In vivo testing of the diets from the clusters

It is widely recognized that utilization of fibrous roughages, which form substantial component of ruminant diets in the tropical livestock systems, can potentially be improved through supplementation with protein sources. To this end, several workers (Nsahlai *et al.*, 1998; Ngwa *et al.* 2003; Hindrichsen *et al.*, 2004) have reported suitability of different protein supplements to boost utilization of the roughages. The clusters formed in present study showed that the PS

were the principal determinants of the diets nutritive value. This is demonstrated by the fact that there was segregation of the PS in the three clusters. As shown in Table 6.2, nearly all LH containing diets were found in Cluster 1 (except for GH: LH 80:20 diet in Cluster 3). Cluster 2 was entirely comprised of SFC containing diets while Cluster 3 was predominated by LPZ diets. However, Cluster 1, which was of intermediate characteristics also had rations of SFC and LPZ. In general, the results indicated that the PS had different and distinct nutritive properties. This was an important revelation as it suggested that the PS would have different impacts on the utilization and productivity when mixed with poor quality roughages similar to those of the present study. Among the PS, lucerne is perhaps the most popular worldwide for ruminants, but requires good soils, high moisture availability and high agronomic input. Thus the superiority shown SFC rations (Cluster 2) over those of LH (Cluster 1) was important given that substantial amount of SFC is produced in Sub-Saharan Africa (Table 2.2). Although SFC has been used extensively in ruminant feeding in temperate countries, Bedingar and Degefa (1990) reported that there is scarce information on SFC utilization in ruminant feeding in SSA. Thus exploring the potential of SFC mixtures with typical roughages for ruminant production in SSA as those of the present study is essential. The necessity to explore the role of SFC in ruminant production is further emphasised by the fact that being an agro-industrial by-product, SFC may be cheaply available, hence can be a good alternative for smallholder farmers practicing zero- or semi zero-grazing in small plots where cultivation of high demanding forages such as lucerne may be prohibitive. On the other hand, the poor nutritive attributes exhibited by rations of LPZ (Cluster 3) could be expected. High tannin content in LPZ is stipulated to be responsible for the inferior performance of these rations. Turner *et al.* (2005) observed high tannin content of 23.1 mg/g in lespedeza, and a much lower value of 0.24 mg/g in lucerne. However, Lespedeza has been recommended as a pasture species in the south-eastern U.S.A. where its deep rooting habit improves vigor and survival during summer drought (Guernsey, 1970; Schimidt, 1982). Lespedeza root growth in acid soils is less inhibited than that of lucerne (Joost and Hoveland,1986). Hay from lespedeza often cures more rapidly than many other common hay species (Guernsey, 1970, 2005; H. Botha, pers. Comm., Harmonie Trust, P.O. Box 27, Matatiele 4730, South Africa). In addition to utilization by cattle (Guernsey, 1970; Griffith, 1996; Ohlenbusch, *et al.*, 2001), goats also make good gains grazing lespedeza (Silanikove, *et al.*, 1996; Escobar, 1998). Thus the poor quality shown by Cluster 3 may not necessarily imply that the rations of this cluster are of low benefit in ruminant production.

6.5 CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that the measurements derived from gas production technique can be used in multivariate cluster analysis to logically separate feeds into distinct homogeneous groups of nutritive characteristics. Generally, rations of Cluster 2 could be described as being of the fastest and most efficient fermentation, hence of highest nutritive value. Rations of Cluster 3 could be described as being of inferior nutritive characteristics depicted by slow fermentation and low degradability. Cluster 1 was generally of intermediate properties. *In vivo* testing of rations selected from all the three clusters is essential, given that besides the nutritive attributes, rations from all the three clusters may have other advantages. In the testing of the diets, it would be important to determine the relationship between animal performance and *in vitro* parameters. This would help to establish the authenticity of IVGPT and cluster analysis as tools for evaluating and ranking ruminant diets.

Chapter 7

PERFORMANCE OF SHEEP FED ON DIFFERENT DIETS AND RELATIONSHIPS WITH *IN VITRO* GAS PRODUCTION PARAMETERS

ABSTRACT

This study used ten diets comprised of varied roughages (RG) and protein supplements (PS) to investigate the influence the diets on performance of sheep in a feeding trial. There were six yearling rams per diet and feeding the trial lasted for four months. The relationships among measurements obtained from *in vivo*, chemical analyses and *in vitro* gas production technique (IVGPT) evaluations were also determined. The roughages included maize stover harvested at grain milk (MM) or dry (MD) stages and natural pasture grass hay (GH), whereas the PS included Lucerne hay (LH), Sericea lespedeza hay (LPZ) and sunflower oil cake (SFC). They had crude protein (CP) and fibre (NDF) contents ranging from 55-230 and 486-660 k/kg DM, respectively. Diets affected ($p < 0.02$ to 0.001) DM intake (DMI/LW), digestibility (DMD), energy loss in faeces (GELF) or urine (GELU) and weight gain (WtGain). The diets also influenced ($p < 0.04$ to 0.001) IVGPT measures of Apparent (ApDeg) and True (TruDeg) degradability, gas volume (GasVol) and time taken to produce half of V ($T_{½}$). In prediction of feed intake, models incorporating degradability (ApDeg or TruDeg), NDF, $T_{½}$ and roughage physical form (Rform) had the highest R^2 (Range = 0.75-0.85). DMD was predicted with 89% of the variation accounted for by models incorporating NDF, GasVol and $T_{½}$. WtGain was predicted by models having Rform, NDF, CP and ApDeg or TruDeg (R^2 range 0.77 to 0.82). TruDeg and $T_{½}$ accounted for 88% of the variation in GELF. GELU was accounted for by diet CP and $T_{½}$ with moderate R^2 of 0.60. The results demonstrated that diets IVGPT and chemical composition measurements plus Pform can be used in different combinations to reliably predict *in vivo* responses. The diets comprised of GH supplemented with 40% SFC and those of MM or MD supplemented with 40% LH showed high potential to optimise productivity.

7.1 INTRODUCTION

Many parts of the world, especially the tropical regions, experience seasonal dry periods during which the available feeds for livestock depreciate in quality. Consequently, feed intake declines and animal productivity is curtailed. Strategic supplementation, to provide limiting nutrients, has received much attention in the recent years as a way to improve ruminant feeding systems. Identification of suitable dietary combinations for specific agro-ecological systems remains a major challenge to stakeholders in the farming industry. The value of diets in ruminant production should be ideally determined through feeding trials. However, testing all possible feeds for all animals in all possible situations is not only expensive but also impracticable. For these reasons laboratory based (*in vitro*) methods that can evaluate nutritive value of ingredients and be able to predict the fate of ingested feeds, are commonly used as alternative to *in vivo* trials. *In vitro* gas production technique (IVGPT) has gained wide popularity in evaluation of ruminant feedstuffs. The advantages of IVGPT include versatility and ability to provide many parameters that can be used to predict animal response (Dijkstra *et al.* 2005). Concerns have been raised (Rymer *et al.*, 2005) about scarcity of information in the literature presenting simultaneous IVGPT and *in vivo* data, yet this is highly essential towards development and perfection IVGPT as a routine method.

Good relationships between some *in vitro* and *in vivo* measures have been reported (Brown *et al.*, 2002; Rymer and Givens, 2002; Blummel, *et al.*, 2005; Getachew, *et al.*, 2005). Conversely, poor relationships among or within *in vitro* and *in vivo* measures have also been recorded. For instance Blummel *et al.* (2005) reported that the ratio of degraded material to gas produced i.e. the partitioning factor (PF) could only predict feed intake reliably when measured at early hours of incubation (4-8 h), and that poor relationships were observed beyond 24 h. Rymer *et al.* (2005) cautioned that maximum fermentability of a substrate *in vitro* may not emulate what occurs in animals. The aim of this study was to establish *in vivo* benefits obtainable from diets which had been selected on the basis of IVGPT evaluation and develop models for predicting *in vivo* responses from IVGPT derived measurements.

7.2 MATERIALS AND METHODS

7.2.1 Feeds

This study used ten diets comprised of different roughages (RG) and protein supplements (PS). The diets were randomly selected from each of the three clusters presented in Chapter 6. The RG were natural pasture grass hay (GH) and maize stover (MS). MS were from white maize hybrid PAN 6479. The PS were lucerne hay (LH), Sericea lespedeza hay (LPZ) and sunflower seed cake (SFC). The maize was grown at Ukulinga Research Farm, University of Kwa-Zulu Natal. The MS were harvested at grain milk (MM) or grain dry (MD) stages. The GH was harvested from Ukulinga farm. The rest of the feed resources were outsourced. The treatment diets were as shown below:

Diet	RG:PS	Ration (DM basis)	Cluster
1	MM:SFC	60:40	1
2	MM:LH	60:40	1
3	MM:LPZ	60:40	1
4	GH:SFC	40:60	2
5	MD:LH	60:40	1
6	MD:SFC	80:20	2
7	MD:SFC	40:60	2
8	GH:SFC	60:40	2
9	GH:LH	80:20	3
10	GH:LPZ	60:40	3

7.2.2 Experiment 1. Sheep feeding trial

Sixty yearling Damara rams were used. They were kept in pens at the Ukulinga Research Farm, University of KwaZulu-Natal (Appendix 9). The rams were tagged, dewormed with broad spectrum antihelmintic (levamisole) and weighed at the beginning of the trial. They were then grouped by weight and within weight group randomly allocated to the diet treatments so as to balance for weight. The initial mean weight per treatment was 23.1 (sd = 4.0) kg. There were six

rams per diet and the pens had a permanent supply of fresh water. The feeding trial lasted for twelve weeks of which the first three were for adaptation. The feeds were milled through 10 mm screen using a hammer mill. MM was air dried after milling. The DM content of the feeds was determined before separately weighing diet proportions of RG and PS on DM basis. Sub samples were taken at each weighing sessions and kept for laboratory analysis. The rations were mixed and fed in roughly two equal portions twice a day at 9.00 am and at 4.00 pm. Feed allowance was maintained to have roughly 10% as left-over. Left-overs for each ram were weighed daily, bulked for each week, sub-sampled, oven dried at 50°C overnight, ground through 1-mm screen and stored until required for analysis. The rams were weighed weekly (every Tuesday before morning feeding).

From the fourth week of the experiment period, a ram from each of the ten treatments was harnessed with faeces collection bags (Appendix 10) and moved to a metabolic crate (Appendix 11). The rams lasted ten days in the crates of which the first four days were for adaptation. Movement of rams to crates were done in turn until a total of four rams per treatment were covered. The crates had permanent supply of fresh water and feeding continued as usual. Faeces and urine were collected daily before morning feeding. The faeces was weighed and sub-samples of roughly 50-100 g taken for the determination of DM content. Another set of sub-samples of roughly 10% of the total amount were taken, sealed and kept in the freezer at -15°C. After the last set of rams had gone through the metabolism study, sub-samples were removed from the freezer, bulked for the individual rams and returned to the freezer to await further analyses. Urine was collected into 6% sulphuric acid solution, diluted to 3 L with water and thoroughly mixed. Sub-samples 50 ml were taken daily, accumulated into labelled bottles per sheep and refrigerated at 4°C. At the end of the collection week, the bottles were moved into the freezer (-15°C) to await further analyses.

7.2.3 Experiment 2. 72-h *in vitro* gas production

Rations of diets fed to sheep in Experiment 1 were weighed and subjected to automated *in vitro* gas production fermentation. Similar procedures as described in Section 3.2 were followed, except that the incubation lasted for 72 h, and $T_{½}$ and DEF were calculated as described in Section 5.2.

7.2.3 Experiment 3. Incubation stoppage at half gas volume.

An analogous procedure to Experiment 2 was followed with the only difference being stoppage of incubations at $T_{½}$ of respective rations. The calibration relationship was used to calculate the pressure (kPa) at which $V_{½}$ was to be attained for each bottle and incubation stopped when this pressure was attained, and time recorded.

7.2.4 Data collection and analyses

The individual feeds, diets, left overs and faeces were analysed for chemical composition and GE content. Urine was analysed for GE and N content. Nitrogen was determined by micro-Kjeldahl method (AOAC, 1990) and CP was calculated as N x 6.25. Methods described by Van Soest *et al.* (1991) were used to determine the neutral detergent fibre (NDF) and acid detergent fibre (ADF). *In vivo* measures determined included DM intake (DMI), DM digestibility (DMD), GE intake (GEI), GE losses in urine (GELU) and faeces (GELF) and daily weight gain (WtGain). The WtGain was calculated as the total weight gained during the experiment period divided by the total number of days. Gross energy (GE) of feeds and faeces was determined using DDS isothermal CP500™ bomb calorimeter (Digital Data Systems Ltd., 188 Arbeid Avenue, Johannesburg, SA). To determine urine GE, known volume (10 ml) was added to a crucible containing filter of known weight. Control crucible had filter paper but no urine added. The crucibles were placed in a dessicator containing Phosphorus pentoxide to dry up the urine as explained by Nijkamp (1969). The dried samples were then bombed and the GE of urine calculated as the difference between the control and the respective samples. The detailed procedure is described in Appendix 15.

Parameters determined from IVGPT included degradability (TruDeg and ApDeg), microbial yield (MIC), total gas volume (V), time taken to produce half of V ($T_{½}$), PF and DEF. Statistical analysis was performed using Genstat (Ver. 8) software. Analysis of variance (ANOVA) was performed to determine diet effects and means compared using least significant difference (LSD). The relationships among diets parameters derived from chemical composition, IVGPT and *in vivo* evaluations were determined using correlation analysis. SPSS software was used to perform ridge regression analysis to derive models for prediction of *in vivo* performance from IVGPT and chemical composition parameters.

7.3 RESULTS

The chemical composition of individual feeds and treatment diets comprised of their mixtures is shown in Table 7.1. The roughages had low CP content (range 29 to 48 g/kg DM) whereby MM had higher CP and lower fibre contents compared to MD. GH had the lowest fibre content among the roughages. The PS had highly varied CP contents with SFC having the highest and LPZ the lowest (range 97 to 360 g/kg DM). All diets had similar GE and OM contents, which ranged from 16.0 to 16.9 MJ/kg DM and 911 to 942 g per kg DM, respectively. Diets had varied chemical composition with CP ranging from 55 to 230, NDF from 486 to 660 and ADF from 311 to 452 g/kg DM.

Performance of sheep on different diets is shown on Table 7.2 whereby the diet effect was significant ($p < 0.02$ to 0.001) for all variables. The values of DMI/LW, GEI, DMD and DMDIG/LW ranged from 25.1 to 46.0 %, 11.3 to 22.5 MJ/d, 374 to 608 g/kg and 9.5 to 25.2 g/kgLW, respectively. Values of GELU and GELF ranged from 1.1 to 9.4 % and 29.4 to 62.4% of GEI. The highest weight gain was 172 g/d where 60:40 GH:SFC was fed (diet 8). The poorest performance of 11g/d weight loss was recorded from 60:40 MS:LPZ diet (Diet 3).

Results from IVGPT experiments are shown on Table 7.3. The 72-h incubation (Exp 2) had higher Deg, PF and DEF values than the corresponding values obtained where incubation was stopped at $T_{½}$ (Exp 3). TruDeg ranged from 679 to 820 and from 468 to 659 g/kg in Exp 2 and Exp 3, respectively. There was relatively high variability in MIC (cv = 33%) and the values of

Exp 2 were markedly lower than those of Exp 3. Diets differed ($p<0.04$) in PF in Exp 2 (range 3.9 to 4.9) but had similar values in Exp 3 (range 2.8 to 3.6). Diets also differed ($p<0.001$) in DEF in both experiments with values ranging from 0.44 to 0.70 in Exp 2 and 0.30 to 0.58 in Exp 3.

The correlation matrices of *in vivo*, IVGPT and chemical composition measurements are shown in Appendices 13 and 14. Generally, as compared to Exp. 2 (72 h incubation), Exp. 3 ($T_{½}$ stoppage) had poorer correlations between IVGPT and *in vivo* or chemical composition measurements. As compared to PF, both DEF and $T_{½}$ had higher correlation with CP, NDF and ADF. Also $T_{½}$ and DEF showed high correlation with *in vivo* DMD accounting for 75 and 67% of variation in DMD, respectively. They, however, had poorer correlation with intake and WtGain as compared to PF (Table 7.2).

In predicting intake (DMI/LW), the physical form of the roughage (RForm) was an outstanding determinant, for which inclusion in the models improved the precision (R^2). Equations incorporating RForm, degradability (ApDeg or TruDeg), NDF and $T_{½}$ had the highest R^2 of 0.85 followed by those incorporating, NDF and DEF or PF and lastly those incorporating CP and DEF or PF (Table 7.4).

Digestibility (DMD) was highly predictable with up to 89% the variation being accounted for (Table 7.4) by a combination of variables. The NDF, degradability measures (ApDeg or TruDeg) and $T_{½}$ were the most important variables for predicting DMD. Models incorporating only these variables had R^2 of at least 0.83. DMD was also reasonably predictable ($R^2 = 0.70$) by model incorporating only NDF and DEF. Weight gain/loss was predicted (R^2 range 0.76 to 0.83) by models having Rform, NDF, ApDeg or TruDeg and $T_{½}$. TruDeg and $T_{½}$ accounted for 88% of the variation in GELF, and incorporating CP and GP into the model resulted to marginal improvement in the R-square. Loss of energy in urine (GELF) was only correlated with diet CP content which accounted for 60% of variation (Table 7.5).

Table 7.1 Feeds and Diets chemical composition (g/kg DM) and gross energy (MJ/kg DM)

Feed	OM	CP	NDF	ADF	ADFn	Ash	GE
Milk stage stover (MM)	936	48	696	449	1.7	64	16.2
Dry stage stover (MD)	940	29	733	452	1.3	60	15.9
Grass hay (GH)	910	34	665	464	1.5	91	16.3
Lucerne hay (LH)	920	140	445	376	4.2	81	16.2
Lespedeza hay (LPZ)	950	97	589	436	4.8	50	16.9
Sunflower oil cake (SFC)	920	360	367	217	9.3	80	17.2
Diet							
1	930	173	565	356	6.6	70	16.6
2	929	85	596	420	2.7	70	16.2
3	942	68	653	444	2.9	58	16.9
4	915	230	486	316	6.2	85	16.9
5	932	73	617	422	6.4	70	16.0
6	936	102	660	405	2.9	65	16.2
7	928	227	514	311	6.1	72	16.7
8	913	165	546	365	4.6	87	16.7
9	911	55	621	446	2.0	90	16.3
10	925	59	634	453	2.8	75	16.9

OM = organic matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADFn = nitrogen in ADF, GE = gross energy.

Table 7.2 Mean performance of sheep fed diets composed of different roughages and protein supplements and correlation with gas production parameters

Diet	DMI g/kg LW	GEI MJ/d	DMD g/kg DM	DMDIG/LW g/kg	GELU %	GELF %	WtGain g/d
1	31.4	13.7	528	16.5	6.8	41.7	62
2	29.1	11.3	539	15.8	1.8	42.4	54.7
3	25.1	10.4	374	9.5	1.8	61.5	-11
4	38.8	19.5	588	22.9	9.4	32.8	153.7
5	31.0	12.7	465	14.3	4.9	46.3	59.4
6	29.2	12.1	466	13.9	2.5	43.7	27.6
7	33.1	14.7	608	20.0	4.7	29.4	87
8	42.6	22.5	584	25.2	4.8	35.6	171.5
9	34.3	16.1	455	15.7	3.2	54.2	96.4
10	30.8	14.0	381	11.7	1.1	62.4	33.1
ANOVA results							
LSD	0.2	3.8	48.8	2.3	4.4	8.7	29.3
P	0.001	0.001	0.001	0.001	0.02	0.001	0.001
Cv	10.6	17.8	17.1	24.1	72.8	13.2	27.3
Correlations with IVGPT[1] parameters							
ApDeg	0.49*	0.41*	0.79***	0.70***	0.67***	-0.83***	0.52*
TruDeg	0.60**	-0.03	-0.87***	0.81***	0.67***	-0.83***	0.65**
GasVol	-0.05	-0.15	0.36	0.15	0.17	-0.50*	-0.02
MIC	-0.11	-0.15	-0.38	-0.23	0.17	-0.50*	-0.08
$T_{½}$	-0.27	-0.22	-0.86***	-0.63**	-0.69***	0.81***	-0.40*
PF	0.66**	0.70***	0.42*	0.63**	0.54*	-0.38	0.66**
DEF	0.44*	0.41*	0.83***	0.70***	0.75***	-0.77***	0.54*

DMI = dry matter intake, GEI = gross energy intake, DMDIG/LW = dry matter digested per kg liveweight, GELU = portion of GEI lost in urine, GELF = portion of GEI lost in faeces, WtGain = sheep weight gain, [1]Data from 72-h incubation used, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 7.3 Mean *in vitro* gas production parameters determined after a 72-h incubation or when incubation was stopped when half of expected maximum gas volume was produced

Diet	TruDeg g/kg DM		MIC g/kg DM		V ml/g DM		$T_{½}$		PF		DEF		X/Y %
Incubation length (h)	72	$T_{½}$	72	$T_{½}$	72	$T_{½}$	72	[1]$T_{½}$	72	$T_{½}$	72	$T_{½}$	
1	787	603	79	241	174	88	14.1	12.0	4.5	3.4	0.64	1.16	76.6
2	753	548	82	261	176	89	15.2	15.4	4.3	3.1	0.56	0.78	72.3
3	679	515	138	177	159	79	17.9	17.9	4.3	3.3	0.48	0.74	75.6
4	820	644	110	276	169	89	13.9	13.8	4.9	3.6	0.70	1.04	78.5
5	724	627	122	196	185	90	18.5	15.2	3.9	3.5	0.44	0.92	86.6
6	794	567	62	163	196	102	18.4	19.7	4.1	2.8	0.44	0.56	71.4
7	820	659	95	175	179	92	13.9	13.9	4.6	3.6	0.66	1.06	80.3
8	790	617	94	259	172	89	16.6	16.4	4.6	3.5	0.56	0.84	78.2
9	741	590	105	211	165	84	19.9	19.8	4.5	3.5	0.45	0.72	79.5
10	704	468	97	277	158	77	20.4	20.7	4.5	3.1	0.44	0.60	66.4
LSD	65	109	52	108	10	9	2.5	2.6	0.5	0.7	0.06	0.16	0.06
P	0.001	0.04	Ns	Ns	0.001	0.002	0.001	0.001	0.04	ns	0.001	0.001	0.001
Cv	5.0	10.9	30.8	28.2	3.2	6.0	8.8	9.1	6.6	11.6	6.3	11.3	6.3

TruDeg = true degradability, MIC = microbial yield, V = total volume of gas produced, $V_{½}$ = Half of V, $T_{½}$ = Time (h) taken to produce $V_{½}$, PF = partitioning factor (TruDeg/V), DEF = degradability efficiency factor (TruDeg/ $T_{½}$ x $V_{½}$), X= TruDeg in incubation stopped at $T_{½}$, Y= TruDeg in incubation stopped at 72-h, [1]Time taken to produce half of expected maximum gas volume.

Table 7.4 Regression models for predicting dry matter intake and digestibility by sheep using *in vitro* gas production and chemical composition measurements

	Variable	Coefficient	R^2	p	Overall p
Dry matter intake (g/kg LW) models					
(1)	NDF	- 0.03	0.81	0.03	0.01
	ApDeg	0.02		0.07	
	Rform	-4.87		0.006	
	Constant	47.83		0.002	
(2)	NDF	- 0.03	0.82	0.04	0.01
	TruDeg	0.31		0.04	
	Rform	-4.90		0.006	
	Constant	31.77		0.04	
(3)	NDF	- 0.04	0.76	0.01	0.007
	Rform	- 4.67		0. 01	
	Constant	63.40		0.001	
(4)	CP	0.03	0.75	0.01	0.008
	Rform	-5.40		0.005	
	Constant	37.24		0.001	
Dry matter digestibility (g/kg)					
(1)	NDF	- 0.74	0.89	0.03	0.003
	V	2.27		0.03	
	$T_{½}$	-10.96		0.10	
	Constant	725.88		0.005	
(2)	NDF	- 0.73	0.83	0.01	0.002
	ApDeg	0.56		0.02	
	Constant	561.4		0.03	
(3)	NDF	- 0.55	0.83	0.06	0.002
	TruDeg	0.87		0.03	
	Constant	155.23		0.37	
(4)	NDF	- 0.51	0.70	0.06	0.015
	DEF	267.14		0.02	
	Constant	657.71		0.001	
(5)	ApDeg	0.49	0.85	0.008	0.001
	$T_{½}$	- 16.59		0.002	
	Constant	456.53		0.008	
(6)	TruDeg	0.72	0.87	0.004	0.001
	$T_{½}$	- 14.39		0.004	
	Constant	190.15		0.17	

ApDeg = Apparent degradability (g/kg DM), P = Crude protein (g/kg DM),DEF = degradability efficiency factor (TruDeg/ $T_{½}$ x $V_{½}$), NDF = Neutral detergent fibre (g/kg DM), Rform = Physical form of roughage (1 = fine, 2 = coarse), TruDeg = True degradability (g/kg DM), $T_{½}$ = Time (h) taken to produce $V_{½}$, V = Total gas production (ml/g DM),$V_{½}$ = Half of V

Table 7.5 Regression models for predicting gross energy losses (%) in faeces (GELF), urine (GELU) and weight gain (WtGain) by sheep using *in vitro* gas production and chemical composition

	Variable	Coefficient	R^2	P	Overall p
GELF models					
(1)	CP	- 0.04	0.91	0.02	0.009
	TruDeg	- 0.08		0.01	
	V	- 0.19		0.07	
	$T_{½}$	1.19		0.03	
	Constant	122.67		0003	
(2)	TruDeg	- 0.11	0.90	0.002	0.002
	V	0.17		0.08	
	$T_{½}$	1.66		0.009	
	Constant	126.24		0.002	0.001
(3)	TruDeg	-0.12	0.88	0.007	
	$T_{½}$	1.59		0.007	
	Constant	111.10		0.001	
(4)	V	- 0.31	0.76	0.04	0.007
	$T_{½}$	2.72		0.002	
	Constant	52.45		0.06	
GELU model					
	CP	0.01	0.60	0.02	0.007
	$T_{½}$	-0.24		0.12	
	Constant	46.09		0. 07	
WtGain models					
(1)	NDF	- 0.41	0.81	0.01	0.01
	ApDeg	0.19		0.10	
	RForm	- 42.30		0.02	
	Constant	287.77		0.02	
(2)	NDF	- 0.35	0.82	0.02	0.01
	TruDeg	0.31		0.05	
	RForm	42.83		0.01	
	Constant	109.68		0.26	
(3)	NDF	- 0.50	0.77	0.003	0.005
	Rform	-40.40		0.02	
	Constant	433.24		0.001	

ApDeg = Apparent degradability (g/kg DM), CP= Crude protein content (g/kg DM), NDF = Neutral detergent fibre content (g/kg DM), Rform = Physical form of roughage (1 = fine, 2 = coarse), TruDeg = True degradability (g/kg DM), $T_{½}$ = Time (h) taken to produce $V_{½}$, V = Total gas production (ml/g DM), $V_{½}$ = Half of V

7.4 DISCUSSION

7.4.1 Feed chemical composition and sheep performance

Feed evaluation has been defined (Madsen *et al.*, 1997) as basically a description of feeds in terms that allow for a prediction of the performance of animals offered the feeds with the aim of giving guidance about the best feeding methods and, if possible, how feeds should be best processed, stored, fed and combined or supplemented. The nutritive value of feeds generally depends on chemical composition, and benefits to the animal are derived from metabolic processes that release the nutrients. From the chemical composition attributes of the present study, it can be deduced that the feeds were comparable in terms of GE, but they differed in fibre and protein contents. Given that ruminants require at least 60-70 g/kg of CP (Minson, 1990) in the diet, it was apparent that the present roughages needed substantial supplementation with CP sources. The production (WtGain) attained by sheep fed MS or GH based rations provided evidence of complementary roles these roughages can play in enhancing productivity of a maize-ruminant system. Ahmed and Abdalla (2005) fed sheep iso-nitrogenous diets containing roughly 14% CP from different oil seed cakes and obtained weight gains ranging from 72.9 to 97.7 g/d whereas Akinlade *et al.* (2002) obtained weight gains of 13.2 to 33.3 g/d in sheep fed diets comprised of a tropical grass supplemented with different forage legumes. These findings are inclusive of the values obtained in this study, confirming that the present diets have potential to support normal or even better production. However, current results demonstrated that care needs to observed in choosing the supplements and supplement levels because some rations can be unproductive e.g. Diet 3. Nevertheless, under a practical farming situation, even such marginally unproductive rations can be beneficial in the short run e.g. during dry seasons to enable the animals to survive until the rainy season when feeds are expected to be abundant.

7.4.2 Relationships among *in vivo* and *in vitro* parameters

Intake is a principal component governing the nutritive value of feeds, since if the feed is not adequately consumed all other aspects are of little or no value as far as the animal is concerned. Generally, it would be expected that feeds with high digestibility also have high intake and vice versa. Lack of strong correlations between DMI/LW and DMD or *in vitro* degradability measures (ApDeg or TruDeg) were therefore unexpected. This observation

agrees with previous findings (Khazaal *et al.*, 1993; Carro *et al.*, 2002) where poor relationship between digestibility and intake were obtained. These findings thus indicate that there could be other factors influencing intake, which are not related to digestibility. This is supported by the remark by Madsen *et al.* (1997) that many factors, apart from degradation and passage rate, influence the intake. These authors further expressed the necessity to find out the causes of intake limitation of specific feeds. The present high correlations between RForm and intake showed that physical or textural nature of a diet can also influence intake, whereby diets having a relatively finer GH had higher intake than those having MS which is a coarser roughage. Physical/textural factors would primarily influence the bite size and chewing time length, which consequently impact on consumable amount. Furthermore, intake could also be affected by chemical composition, particularly in diets containing LPZ, since lespedeza is known (Min and Hart, 2003) to have low intake due to its high tannin content. Also, Hindrichsen *et al.* (2004) reported palatability problems with tree fodders due to presence of alkoloids and sapponins, which further supports this possibility.

The fact that chemical composition (CP, NDF, and ADF) and *in vitro* (ApDeg, TruDeg and $T_{½}$) measures showed good relationships with *in vivo* parameters (DMI/LW, DMD, GELF and GELU) indicated that they can be useful in the prediction of animal response, as opposed to GP and MIC which had poor relationships. It can be argued that short $T_{½}$ coupled with high degradability depicted high microbial efficiency, and this is supported by findings of Cone and Van Gelder (2000) who observed increased microbial efficiency in substrates with higher fermentation rates. The poor correlations shown by MIC or GP with *in vivo* measurements (Table 6.2) can be partly attributed to some confounding factors which can affect the reliability of MIC estimates *in vitro*. For example, Rymer (1999) cautioned about reliability of *in vitro* MIC due to microbial recycling of unknown magnitude which occurs under long incubations. This was evident in the present results whereby MIC values determined after a 72-h incubation (Exp. 2) were markedly lower than those at $T_{½}$ stoppage (Exp. 3). Also, determination of MIC through extraction of apparent residue with NDS, as was done in this study, has been criticized (Blummel and Lesbien, 2001) to result in unreliable values for some feeds due to a possibility of dissolving some undegraded complexes together with microbial cells, resulting to inflated MIC values. In the case of GP, it has been shown (Cone and Van Gelder, 1999; Rymer et al., 2001) that fermentation stoichiometry is influenced by feeds chemical composition whereby proteins produce less gas compared to carbohydrates. Carro *et al.* (2002) found lack of relationship between GP

parameters and *in vivo* DMD, and also attributed this to the problem of altered stoichiometry of GP in the fermentation of proteins.

7.4.3 Prediction of feed intake

Present results revealed that NDF, $T_{½}$, RForm and degradability (ApDeg and TruDeg) are the most important determinants of intake accounting for up to 85 % of the variation. Although no information was found in the reviewed literature where these paramters are considered together, individually these parameters have been found by several workers to be important in predicting intake. For instance, Blummel and Becker (1997) reported improvement in intake prediction of straws and legume-hay supplemented straws by including NDF in the equation. Using barley straw, a poor quality roughage similar to the ones used in the present study, Ørskov *et al.* (1987) reported that better predictions of intake ($r = 0.88$) and animal performance ($r = 0.95$) could be made by including the degradable fraction and its rate constant in the regression. Carro *et al.* (2002) stated that the time necessary for forage breakdown (depicted by $T_{½}$) is one of the factors determining voluntary intake. They further explained that since cell contents are rapidly degraded, the duration of digestion depends on both the proportion and rate of cell walls (fibre) degradation in the diet whereby the higher the resistance the greater the rumen fill effect on intake. Leonardi *et al.* (2005) found that DM intake by dairy cattle was linearly decreased by increasing the feed particle length, which supports the impact of RForm in the present results.

Given that PF and DEF are derived parameters, it is possible that their usefulness in prediction models may be of less importance if the inclusion of the parameters from which they are derived produce comparable or better predictions. Furthermore, there can be confounding factors affecting the reliability of PF values. Rymer (1999) obtained PF ranging from 2.77 to 30.3 in incubations lasting between 9 and 48 h and cautioned that if PF is recorded too early, the microbial growth, hence degradation, will not be as great as the feed's potential, and if measured too late, then microbial recycling of unknown magnitude will have occurred. Unreliable PF values have also been found with some feeds (Makkar *et al.*, 1998, Blummel *et al.*, 2005), emphasizing the need for taking precaution when ranking feeds using PF as indicator nutritive value, particularly MIC efficiency.

7.4.4 Prediction of digestibility

The present results showed that digestibility (DMD) is highly predictable by IVGPT and chemical composition measures whereby a combination of only three parameters accounted for over 80% of the variation in DMD. The important variables included NDF, GasVol, degradability (ApDeg or TruDeg) and $T_{½}$. Nsahlai and Ummuna (1996) reported that gas production could predict *in vivo* DMD of roughages (R^2 = 0.64, p <.001), and legumes (R^2 = 0.82, p<0.05). They also found that DM degradation, using different methods, was always strongly correlated (r = 0.87 to 0.98) with DMD, which generally agreed with the current findings. Earlier studies reported by Menke *et al.* (1979) similarly achieved high precision (R^2 = 0.98) in predicting *in vivo* organic matter digestibility using a multiple regression model based on *in vitro* gas and chemical composition measurements as independent variables. Inclusion of PF or DEF in the models resulted to inferior predicting DMD compared to models which had the measurements from which PF and DEF are derived. It can therefore be said that the use of PF and DEF in prediction of digestibility may only be necessary if the parameters from which they are derived produce inferior predictions.

7.4.5 Role of ridge regression

Data obtained from IVGPT studies have been subjected to different types of analyses to derive parameters that depict the fate of substrates in the ruminant gastro-intestinal track and their implication on production. Both good and poor relationships have been found among *in vitro* and *in vivo* measures of feed quality. In the current study, stepwise regression analysis was attempted but failed to provide biologically meaningful results. For example, despite giving regression models with high R^2 values, in a number of cases variables from stepwise regression had coefficients with opposite signs of what should be expected. This is known to occur as a result of multicollinearity among variables (Neter *et al.*, 1990). Blummel and Becker (1997) experienced similar multicollinearity problems when using stepwise regression to derive models for predicting *in vivo* response from IVGPT parameters. Given that several parameters measured from feeds are inherently correlated (see Appendices 13 and 14) yet they can cause different impacts *in vivo*, it was important to use an analytical tool that can resolve multicollinearity conflicts. Ridge regression is designed to overcome serious multicollinearity problems by modifying the method of least squares (Neter *et al.*, 1990); and its use in the present study enabled derivation of several alternative models to predict the

animal response from IVGPT and chemical composition parameters. An important feature of ridge regression is stability of estimates in the sense that they are usually little affected by small changes in the data on which the fitted regression is based, and this is in contrast to ordinary least squares estimates which may be highly unstable when the independent variables are highly multicollinear (Neter *et al.*, 1990).

7.5 CONCLUSIONS

The study clearly demonstrated that IVGPT can provide parameters having high correlation with *in vivo* responses, thus confirming its relevance and reliability as a tool for evaluating ruminant diets. The results showed that IVGPT and chemical composition measurements plus physical form attributes of diets can be used in different combinations to predict *in vivo* responses with high precision. However, some IVGPT measurements including volume of gas produced, MIC and PF estimates generally showed poor correlation with *in vivo* responses. Because $T_{½}$ and DEF showed high consistency, good correlations with *in vivo* measurements and significant contribution to prediction of *in vivo* responses, it can be deduced that inclusion of these parameters in routine use of IVGPT would greatly contribute to more meaningful determination of feeds nutritive value. The present results showed that the roughages used can support high production but care needs to be observed in choosing the type and level of supplements to obtain good animal (sheep) performance. The diets comprised of GH supplemented with 40% SFC and those of MM or MD supplemented with 40% LH showed high potential in optimising productivity.

Chapter 8

NITROGEN FLOW, MANURE QUALITY AND MINERALIZATION FROM DIFFERENT DIETS FED TO SHEEP[d]

Abstract

Nitrogen (N) is an important nutrient for both plants and animals and is available for plant uptake in ammonium (NH_4^+) and nitrate (NO_3^-) forms. NH_4^+ is an important end product of ruminant feed digestion excreted in faeces (manure). The objectives of this study were to investigate the influence of diets comprised of different roughages (RG) and protein supplements (PS) on partitioning dietary N ingested by sheep, manure quality and mineralization of N from the manure. The RG were maize stovers and grass hay (GH). The stovers were harvested at grain milk (MM) or dry (MD) stages. The PS were lucerne hay (LH), lespedeza hay (LPZ) and sunflower oil cake (SFC). Ten diets composed of different RG:PS ratios with CP contents ranging from 55 to 230 g/ kg DM were fed to sheep. Faeces and urine were separately collected and analysed for N content and faeces analysed for mineral composition. Fresh and dried faeces were further analysed for NH_4^+. Samples of fresh faeces containing150 μg N /g soil were incubated and had net N mineralised determined over a 20 weeks period. The diets differed ($p < 0.006$ to 0.001) on DM intake (DMI), DM digested (DMDIG), manure output (ManuYld), Faeces-N and weight gain (WtGain). They also differed ($p<0.001$) in NH_4^+ concentration with values ranging from 653 to 4240 and 114 to 2153 mg/kg DM in fresh and dried faeces, respectively. Substantial amount (24 to 83 %) of NH_4^+ was lost by drying the manure. The highest amounts of mineralised N were obtained in the first leaching performed after incubation lasting one week, with substantially lower amounts obtained in subsequent leaching. Net N-mineralization was maintained by fertilizer (LAN) and manure diets which had CP content above 170 g/kg. These diets were among those found to support WtGain of 62.0 to 172 g/d. Faeces from diets containing SFC showed highest N, P and K content while those containing LH showed high Na content. The results showed that appropriate supplementation of MS or GH, coupled with proper use of manure can potentially improve productivity of maize-ruminant system through N recycling at farm level.

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[d] J. O. Ouda, A. T. Modi and I. V. Nsahlai. Nitrogen flow, manure quality and mineralization from different diets fed to sheep. *Agricultural Ecosystems and Environment Journal* (Submitted)

8.1 INTRODUCTION

The world rising human population coupled with decreasing farm sizes has caused the necessity to intensify and optimise agricultural production to meet the increased demand for food. FAO (2001) reported that crop and livestock production must expand by more than 3% annually to keep pace with increased food demand. Despite the general tendency towards specialised farming over the past few decades, it appears that there is again an increasing interest in the advantages of mixed farming with crop-livestock systems covering about 2.5 billion hectares of land in the world (FAO 2001). Mixed farming systems are predominant in the developing countries where households may cultivate less than one hectare of land, leading to a decline in soil fertility and crop yields.

Nitrogen has been is widely recognized to be a limiting nutrient for the utilisation of poor quality roughages by ruminants (e.g. Kaitho *et al.*, 1993; Hindrichsen *et al.*, 2004). In vast parts of tropical regions, particularly Sub-Saharan Africa (SSA), the ruminants mostly survive on low-quality roughage basal diets. The predominant roughages include standing hay and crop residues. Maize stover is perhaps the most important crop residue, since maize grain is staple in many communities. The high content of cell wall component (fibre) in the roughages often leads to digestibility below 50% due to lack of sufficient nutrients for the fibre-degrading microbes in the ruminant gut (Leng, 1990). The adverse effects of protein under-nutrition can be alleviated through judicious feeding of ruminants with protein-rich supplements. Commercial concentrate protein sources are not only scarce in SSA smallholder systems, but also generally unaffordable. For these reasons, opportunities for improving ruminant productivity in SSA will mostly depend on production of protein sources at farm level or utilization of cheap industrial by-products such as oil seed cakes. Previous studies (Umunna *et al.*, 1995; Mpairwe *et al.*, 2003; Ngwa *et al.* 2003) have shown that forage legumes and foliage of multipurpose trees and shrubs (MPTs) can be suitable protein sources to improve ruminant production in tropical crop-livestock systems.

Biomass turnover is a major factor in crop-livestock systems, because crops and crop residues are meant to feed animals and the manures are used to maintain soil fertility. Although manure can supply other nutrients, it is commonly known (Delve *et al.*, 2001; Nhano *et al.*, 2004; Powell *et al.*, 2004) that nitrogen (N) is the most important. The major components of N flow in a crop-livestock system are depicted in Figure 8.1. The efficiency of N utilization

and system productivity are influenced by the ratios of availability and losses occurring in the N transformation pathway.

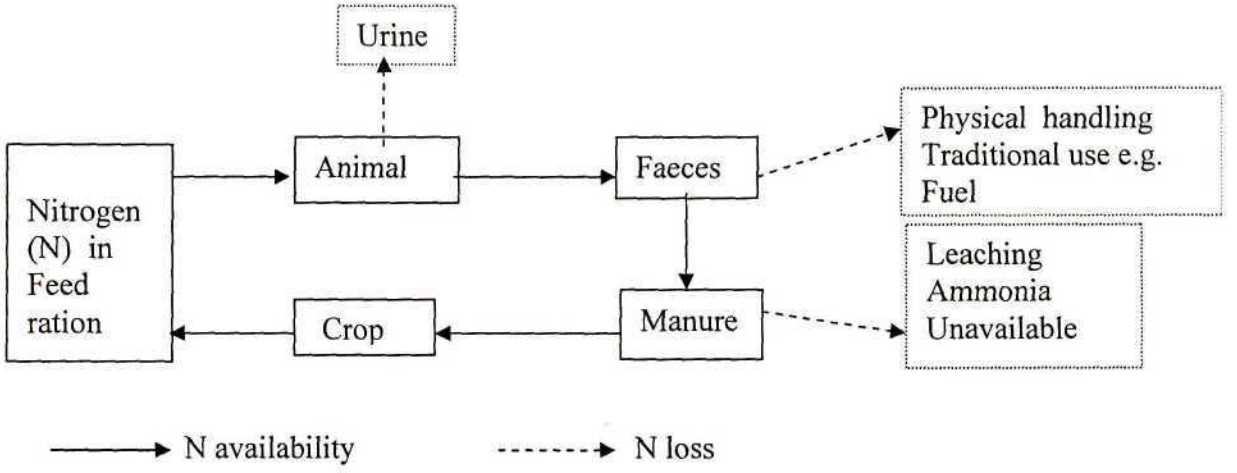

Figure 8.1. A schematic presentation of possible nitrogen cycle in a livestock-crop system.

Powell *et. al.* (1995) pointed out that studies linking nutritive characteristics of the ruminant diets to the quality of by-products such as manures and crop residues, the maintenance of soil fertility and optimisation of crops/plants production through nutrient recycling have been lacking. The broad aim of this study was to determine dietary and husbandry factors important in optimising crop-livestock productivity through N recycling when poor quality roughage based diets are used. The specific objectives were to determine the influence of diets on N partitioning, manure (faeces) quality and release of N from the manures.

8.2 MATERIALS AND METHODS

Faeces produced from the feeding trial reported in Chapter 7 were the source of manures used in this study. The collection and preservation of the faeces, analyses of individual feeds and diets for chemical composition and gross energy (GE) and data collection were as described in Section 7.2.

8.2.1 Manure chemical composition and mineralization evaluation

Sub-samples of faeces from individual rams were removed from the freezer, allowed to thaw, bulked per treatment, crushed and air dried. They were then milled through 1 mm screen using a Cyclotec mill (Perstorp Analytical Ltd, Bristol, UK) and analysed for mineral composition (C, N, P, K, Ca ,S, Mg, Zn, Cu, Fe, Mn, Al). Feed samples were also similarly milled and analysed for mineral composition.

Another group of sub-samples from individual rams were removed from the freezer and had DM content determined. Thereafter, two sets of sub-samples each weighing 10 g (DM basis) were separately removed from the freezer. One set was directly dissolved in 100 ml of 2 M KCl solution (Maynard and Kalra, 1993) to extract NH_4^+ and NO_3^-. The concentrations of NH_4^+ and NO_3^- were determined calorimetrically using a TRAACS 2000 continuous flow auto analyser. The second set of sub-samples was crushed and air dried until constant weight was attained, which took at least five days. They were then similarly processed and analysed for NH_4^+ and NO_3^- .

The N mineralization from the manures was measured using leaching tubes (Appendix 12) according to methods of Stanford and Smith (1972). To do this, a further set of faeces sub-samples were removed from the freezer, thawed and bulked per treatment. They were divided into two sub-sets. One set was analysed for DM and N content. The second set was sealed and returned to the freezer. Afterwards, 50 g of soil was mixed with acid washed sand (1: 2 w/w) and faecal sub-samples added to provide equal amounts of N (150 μg N/g soil). For this reason, the second set of sub-samples was removed from the freezer and thawed. Amounts to supply the equivalent of 150 μg N/g soil were weighed in three replicates. The corresponding DM and N content were used to calculate the amounts to be weighed. The soil used had been collected from Brookdales Farm, Howick, (South Africa) and was from a fallow field that had previously been used for maize and soybean production. The soil chemical composition was: pH (water) = 5.34, organic C = 3.5%, clay = 67.1%, Ca = 13.2 cmolc/kg, K = 0.18 cmolc/kg and Mg = 0.34 cmolc/kg. Two control treatments were included. One control had conventional fertilizer (Limestone ammonium nitrate-LAN) added to give an equivalent amount of N (150 μg N/g soil). The second had no N source added. The samples were thoroughly mixed with the soil. A layer of glasswool was placed at the bottom of the tubes followed by addition of samples and lastly a layer of sand was added at the top

to prevent evaporation and disturbance during leaching. Distilled water was added gradually to bring the mixtures to approximately maximum water holding capacity (WHC). The tops of the tubes were covered with aluminium foil. The tubes were placed in a rack, enclosed in a carton to conceal sunlight and moved to an air controlled greenhouse. The greenhouse temperature ranged from 15-27°C. A leaching solution containing 1mM $CaCl_2$, 0.9 mM KCl, 0.1 mM $MgSO_4$ was prepared (Delve *et al.*, 2001). Leaching was done by adding 150 ml of leaching solution to each tube to remove all mineral N. Leachates were collected at 1, 2, 3, 4, 6, 8, 12, 16 and 20 weeks after the initiation of incubation. The tubes were allowed to drain completely and then covered after leaching. The leachates (supernatants) were analysed for free N. The net N mineralization was calculated by subtracting N mineralization of the control which had no N source added from the rest of the treatments.

8.2.2 Statistical analyses

Analysis of variance (ANOVA) was performed using Genstat (Version 8) software to determine treatment effects, and means were compared using least significant difference (LSD). The relationships among diets chemical composition, *in vivo* performance parameters, manure quality and N partitioning measures were determined by correlation analysis.

8.3 RESULTS

The chemical composition of individual feeds and diets from their mixtures is shown in Table 7.1. The roughages had low CP content (range 29 to 48 g/kg DM), with MM having a higher CP and lower fibre contents compared to MD. The GH had the lowest fibre content among the RG. The supplements were highly varied in CP content with SFC having the highest and LPZ the lowest values (range 97 to 360 g/kg DM). The feeds had nearly equal GE concentrations (16.0 MJ/kg DM). The diets had similar GE and OM contents which ranged from 16.0 to 16.9 MJ/kg and 911 to 942 g/kg DM, respectively. The chemical composition of the diets were varied with CP (55 to 230 g/kg DM), NDF (486 to 660 g/kg DM)and ADF (311 to 452 g/kg DM).

Mineral composition of individual feeds is shown in Table 8.1. The feeds had similar carbon (C) content with mean values of approximately 43 %. SFC was the richest in N, K, Zn and P

contents while LH had markedly high Na, Fe and Al contents. Grass hay had the highest Mn and substantially high Na contents. Maize stovers and LPZ had low to moderate contents of most of the minerals except for K, which the stovers had among the highest values. The mineral composition of faeces from different treatments is shown in Table 8.2. Apart from the C content which remained stable at approximately 43%, generally the faeces mineral composition varied among the diets. Faeces from diets containing SFC had highest N, P and K values while those containing LH tended to have high Na values.

Faeces ammonium (NH_4^+) was highly varied ranging from 653 to 4240 and 108 to 2303 mg/kg in fresh and dried faeces, respectively. All faeces reduced in NH_4^+ content as a result of drying with reductions ranging from 24 to 83 % of the initial amount. Highest reductions tended to be in faeces obtained from diets of low CP content. Faeces nitrate (NO_3^-) content were stable (mean = 27 mg/kg DM). The pH values of the faeces were slightly alkaline.

Mineralization of N from different diets and fertilizer (LAN) is shown in Figure 8.2. The total net N mineralised over the experimental period is shown in Table 8.3. All treatments had positive and highest net N released at the first leaching (after one week of incubation) with drastic reduction, subsequently. Net N mineralization in consecutive leachings was maintained by Diets 1, 4 and 7 up to the eighth week (sixth leaching) while Diets 2, 5 and 9 had net N immobilization in the second week but maintained net mineralization thereafter up to the eighth week. Diets 3, 6, 8 and 10 had N immobilization in the second and third weeks, net N mineralization in the fourth week and N was undetected thereafter. The fertilizer (control) maintained net N mineralization up to the sixth week after which N was undetected.

The performance of sheep under different treatment diets is shown on Table 8.3. Diets differed ($p < 0.006$ to 0.001) in the DMI, N intake, DMDIG, Manure output, Faeces-N and WtGain. Urine-N ratio was inconsistent (cv = 116.1%) and did not differ among diets. The correlation among various measurements is shown in Table 8.4. The diets CP and NDF contents were highly correlated ($r = -0.91$). There was also a high correlation between DMI and DMDIG ($r = 0.93$) as well as between DMDIG and diet NDF or CP contents ($r = -0.83$ or 0.76, respectively). Diets NDF and CP contents showed high correlation with DMDIG, WtGain, N-intake, N-retained and Faeces-N ratio. Also, faeces NH_4^+ content was highly correlated ($r \geq 0.79$) with DMI, DMDIG, WtGain, N-intake and N-retained. ManuYld, Urine-N ratio and faeces NO_3^- content had poor correlation with other parameters.

Table 8.1. The mineral composition of feed resources used in composing diets fed to sheep during the feeding trial

Feed type	C	S	N	Ca	Mg	K	Na	Zn	Cu	Mn	Fe	P	Al	pH
	%	%	%	%	%	%	mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg	%	mg/Kg	
Milk stage stover (MM)	44.15	0.12	0.95	0.19	0.28	1.71	274.0	22	3.7	69	371	0.14	153	6.39
Dry stage stover (MD)	43.85	0.11	0.68	0.15	0.25	1.63	344.6	26	2.1	56	204	0.19	106	6.57
Grass hay (GH)	42.72	0.19	0.74	0.47	0.13	0.60	635.5	19	1.4	185	262	0.07	175	5.62
Lucerne hay (LH)	43.98	0.24	2.55	1.20	0.33	1.56	2172.7	19	8.6	33	709	0.22	500	5.38
Lespedeza hay (LPZ)	45.58	0.17	1.91	1.04	0.29	0.65	91.9	26	9.0	61	213	0.25	131	5.49
Sunflower oil cake	44.04	0.48	6.53	0.42	0.80	1.87	275.0	133	42.2	66	204	1.51	5	6.00

Table 8.2. The mineral composition of faeces manure produced by sheep fed on diets comprised of different roughages and protein supplements

Diet	NO_3	C	N	C:N	S	Ca	Mg	K	Na	Zn	Cu	Mn	Fe	P	Al	pH
		%	%		%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	%	mg/kg	
1	27.3	42.30	2.04	20.7	0.32	0.64	0.89	2.25	380.0	219	32.9	177	1632	1.47	314	7.56
2	27.8	43.22	1.57	27.5	0.25	1.43	0.52	0.90	1281.7	93	12.4	162	1907	0.50	839	7.78
3	28.7	44.99	1.54	29.2	0.18	1.14	0.35	0.92	319.4	64	8.3	125	1979	0.37	494	7.28
4	27.4	41.70	2.29	18.2	0.33	0.83	1.07	1.99	1153.5	253	51.5	285	1224	1.97	236	7.40
5	27.4	42.04	1.68	25	0.25	1.29	0.53	1.28	1306.0	138	10.3	123	2951	0.45	1167	7.68
6	17.9	42.75	1.71	25	0.26	0.56	0.65	1.80	517.3	141	17.0	152	3403	1.02	664	7.52
7	31.8	41.63	2.20	18.9	0.30	0.67	1.12	2.34	334.0	282	52.1	154	919	2.08	206	7.29
8	29.4	41.34	1.93	21.4	0.32	0.81	0.71	1.62	1481.0	211	38.5	330	901	1.42	422	7.23
9	28.4	41.64	1.30	32.0	0.25	1.05	0.27	0.79	1456.9	167	23.9	337	1486	0.22	1109	7.49
10	25.9	43.48	1.28	34.0	0.19	1.11	0.20	0.54	763.3	43	2.8	302	914	0.18	522	7.17

Table 8.3.Performance of sheep fed diets comprised of different roughages and protein supplements and partition of intake nitrogen

Diet	DMI g/d	N intake g/d	DMDIG g/d	*WtGain g/d	Manure DM output g/d	Retained N g/d	Fresh Faeces N %	Urine N %	Fresh Faeces NH_4^+ mg/kg	Dried Faeces NH_4^+ mg/kg	[1] NH_4^+ Loss %	[2]Total net N mineralized µg
1	850	27.4	459	62	390	16.4	24.2	16.8	2086	1585	24	2.5
2	788	12.4	439	54.7	349	6.9	37.8	7.4	1367	920	33	1.1
3	696	9.7	291	-11	404	4.1	61.0	4.2	653	114	83	0.5
4	1056	40.1	608	153.7	448	28.8	22.4	8.7	2728	1588	42	2.2
5	829	12.6	405	59.4	425	6.0	52.3	5.5	1892	1324	30	1.0
6	809	16.4	394	27.6	415	9.5	36.6	6.2	1805	475	74	0.7
7	882	37.7	536	87	346	27.0	18.4	10.2	2968	1865	37	2.3
8	1212	38.6	714	171.5	498	28.4	23.2	3.9	4240	2153	49	1.6
9	837	7.4	408	96.4	529	1.6	64.5	3.7	2028	715	67	0.9
10	834	9.0	330	33.1	504	2.0	67.5	14.0	1032	400	61	0.3
												$2.4^{control}$
LSD	206	5.7	191	29.3	75	5.1	5.5	13.9	403	297		1.2
p	0.004	0.001	0.006	0.001	0.001	0.001	0.001	ns	0.001	0.001		0.004
cv	15.6	18.1	28.1	27.3	11.8	26.4	9.2	116.1	23.2	32.6		49.8

DMI = dry matter intake (g/d), DMDIG= total dry matter digested (g/d), WtGain = weight gain (g/d),

[1]Decrease in NH_4^+ as a result of drying the faeces

[2] Total N mineralized from incubation of fresh faeces added to supply 150 µg N /g soil

[control]Total N mineralized from fertilizer (LAN) added to supply 150 µg N /g soil

*The same is shown in Table 7.2

Table 8.4. Relationship among animal performance, manure quality and nitrogen partitioning parameters

	DMI	DMD	DMDIG	WtGain	ManuYld	CP	NDF	N-intake	N-retained	*Faeces-N %	*Urine-N %	NO_3^-	NH_4^+
DMI	1												
DMD	0.66	1.00											
DMDIG	0.93	0.88	1.00										
WtGain	0.93	0.76	0.93	1.00									
ManuYld	0.39	-0.32	0.08	0.35	1.00								
CP	0.60	0.85	0.76	0.62	-0.31	1.00							
NDF	-0.69	-0.88	-0.83	-0.78	0.16	-0.91	1.00						
N-intake	0.78	0.86	0.88	0.74	-0.16	0.96	-0.90	1.00					
N-retained	0.78	0.87	0.90	0.75	-0.17	0.95	-0.89	1.00	1.00				
*Faeces-N %	-0.58	-0.91	-0.79	-0.57	0.46	-0.90	0.78	-0.90	-0.90	1.00			
*Urine-N %	-0.10	0.09	-0.08	-0.14	-0.23	0.32	-0.25	0.18	0.12	-0.23	1.00		
NO_3^-	0.23	0.35	0.34	0.36	-0.08	0.32	-0.55	0.33	0.33	-0.14	0.00	1.00	
NH_4^+	0.88	0.79	0.93	0.88	0.15	0.66	-0.69	0.80	0.81	-0.69	-0.18	0.30	1

DMI = dry matter intake (g/d), DMD = dry matter digestibility *in vivo* (g/kg DM), DMDIG= total dry matter digested (g/d), WtGain = weight gain (g/d), ManuYld = daily manure output, CP = diet crude protein content, NDF = neutral detergent fibre content, NO_3^-= manure nitrate content, NH_4^+ =manure ammonium content, * As percent of N-intake

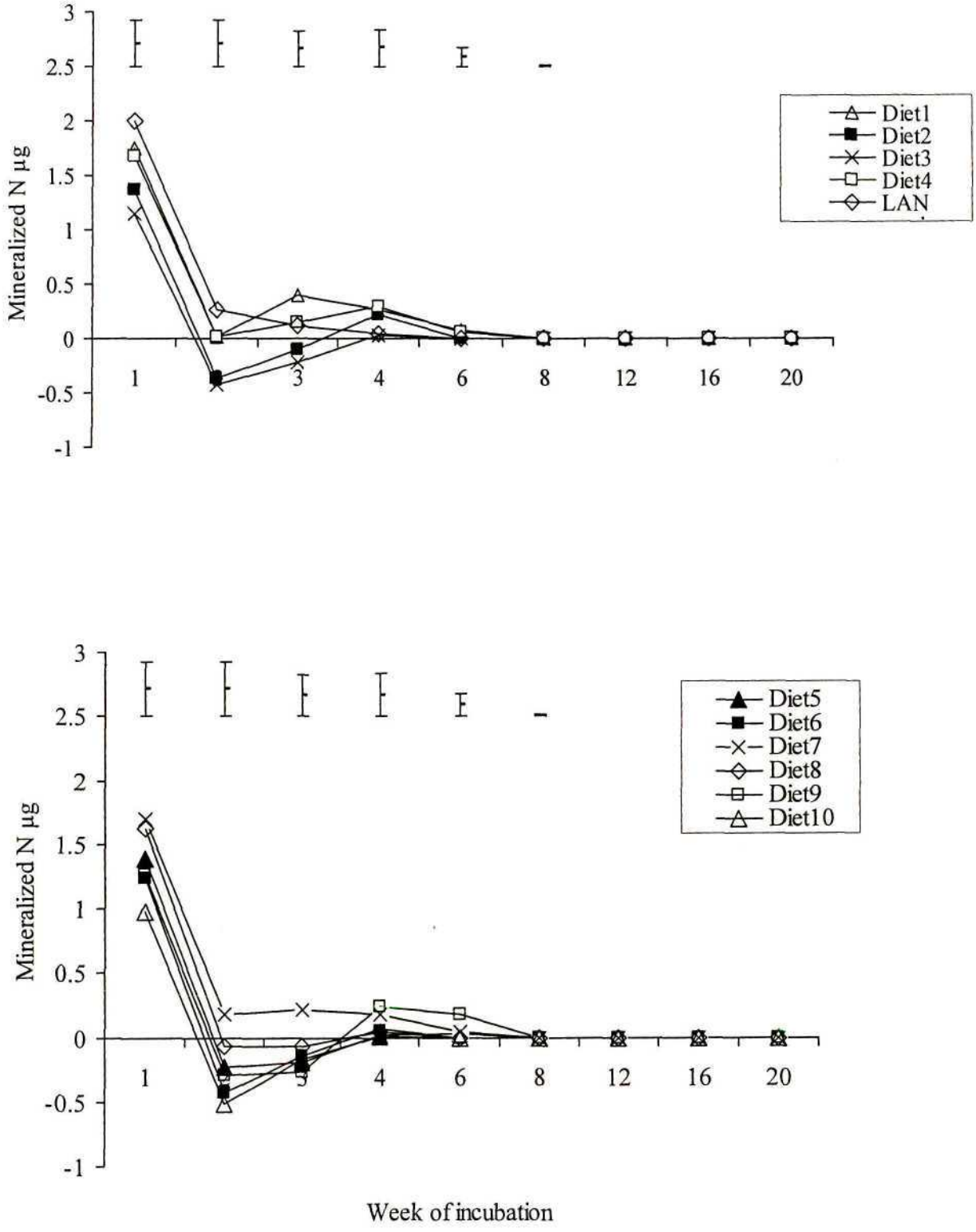

Figure 8.2. Net mineralization patterns of manure obtained from feeding ten diets and incubated with initial N content of 150 µg/g of soil

8.4 DISCUSSION

8.4.1 The effects of diets on animal performance and manure production

Interest and efforts towards utilization of manures from livestock to improve soil fertility and crop production have continued for a long time. Epstein (2003) reported that as early as 1863, even the most ignorant farmers were aware of the value of dung on their fields. However, there is scarcity of information directly linking livestock production performance and manure fertiliser quality. Feed intake is perhaps the most important factor in livestock production. Other factors only become relevant after the animal can adequately consume and survive on a feed. This study demonstrated that appropriate supplementation of poor quality feeds can lead to improvement in animal production with a simultaneous yield of high quality manure. The fact that feeding some diets resulted in marginal weight gain (Diets 6 and 10) or weight loss (Diet 3) were important findings as they demonstrated that it would be risky to depend on these diets even if they produced excellent manures. Thus, although Delve *et al.* (2001) suggested that low quality plant materials should be first fed to livestock (instead of composting) followed by faeces collection and use as manure, it can be deduced from the present results that such strategies can only be of practical value if the animal performance is not compromised.

8.4.2 The effects of diets in N partitioning and manure quality

Ammonium (NH_4^+) is the most important soil fertility nutrient arising from manure. It is a product of microbial protein degradation in the ruminant gastrointestinal track. The microbes multiply into large numbers using ammonia (NH_3) in reconstituting their own proteins, with excreted NH_4^+ being surplus to microbial requirements or spilled from imbalanced anabolic processes. From the animal production point of view, production of NH_3 and NH_4^+ is nutritionally wasteful since they represent waste of dietary protein. The high positive correlation among N-intake, retained-N and faeces NH_4^+ implied that feeds with high N-intake also had high microbial growth coupled with high degradation of protein into NH_4^+. Microbial growth is expected to be positively correlated with DMDIG and WtGain, which explains the high correlation shown by NH_4^+ with these measurements. The negative correlation between Faeces-N ratio and digestibility measures (DMD and DMDIG) and

WtGain indicated that having high amounts of N in faeces is not necessarily beneficial with regard to animal production.

While higher intake should result in higher manure production, higher digestibility causes a reverse effect. The opposite is true for diets with lower digestibility i.e. although they should have higher manure yield, they also have lower intake, which physically limits the quantity that can be produced. It can be deduced that these conflicting scenarios are responsible for the poor correlations shown by ManuYld, hence the difficulty to design diets for increased quantity of manure.

The present Urine-N ratio was highly variable (cv =116%) and had poor correlation with all the measured parameters. Low Urine-N ratio (range 0.2-11.4% of N-intake) was recorded by Delve *et al.* (2001), which is inclusive of the present ratios except for Diet 1 and Diet 10 which had slightly higher values. To the contrary, Stevens *et al.* (2004) reported high Urine-N ratio, reaching up to 97% of intake-N. Thus despite the poor correlation with other measurements shown by the present Urine-N ratio, urine can be a major source of excreted N, and should not be ignored when diets are to be designed to optimize N recycling. This is because the N in urine is mostly contained in urea and is largely lost through volatilization as opposed to N in faeces which is contained in more stable forms. The fact that the present Faeces-N accounted for most (59-90%) of the excreted N demonstrated that the diets were of high potential to promote N recycling for improved crop-livestock productivity and soil fertility.

8.4.3 Supply of nutrients by manure

The huge loss (24 – 83 %, Table 8.3) of NH_4^+ as a result of drying can be attributed to NH_3 volatilisation. White and Sharpley (1996) reported that losses of N as a result of NH_3 volatilisation can be drastic and reach 20% within four days if fresh manures are exposed, which corroborates the present results. The above authors pointed out that such losses can be reduced substantially (e.g. to 5%) by ploughing fresh manure into soil whereby NH_4^+ can be used directly by plants or converted to NO_3^-, which is another available form. The fact that highest release of mineralised N, was obtained in first leaching indicated that fresh faeces are of great potential as source of N. The findings emphasized the need to observe proper care in conservation and handling in order to maintain high manure quality. However, it is ironically

believed by many farmers, e.g. among communities in Eastern and Southern Africa, that fresh faeces (manure) is harmful to crops by causing 'burning'. As a result, there is low use of fresh faeces on the farms. Such beliefs may relate to the high NH_4^+ concentration in fresh faeces as evident in the present study. The burning may result from localised high concentrations caused by poor distribution. This is a challenge requiring further investigation and technology development because, as demonstrated by the present results, a great value is liable to be lost if fresh manure is not appropriately conserved and applied.

The immobilization of N in the interim period (between first and fifth week of incubation) and lack of detection of the N beyond the eighth week (Figure 8.2) indicated that as decomposition continued, the soil microbes multiplied and exhausted the available mineral N from faeces and, in addition, utilized N from the soil. Delve *et al.* (2001) and Nhamo *et al.* (2004) reported similar findings. Thus use of manure can be counterproductive, particularly if there is prolonged immobilization phase followed by slow N mineralization. This fear was also expressed by Delve *et al.* (2001). The fact that the present diets differed in N immobilization patterns implies that identification of underlying factors responsible for such scenarios is key to a more informed feeding strategy with a dual aim of improving manure quality. In this regard, it appeared that Diets 1, 4 and 7 were the least affected by N immobilization, hence could be regarded as the most advantageous to use. Rees *et al.* (1996) reported that the main mechanism for loss of N in a conventional fertilizer appeared to be by NH_3 volatilization, rather than leaching. The lack of distinct superiority of LAN in N mineralization could therefore partly be attributed to the possibility of NH_3 volatilization. Otherwise the similarity shown by the manures and LAN emphasize the potential of the manures and the need to undertake appropriate conservation and application in manure utilization. Similar sentiments have been expressed by Lekasi *et al.* (2002) who reported that many farmers are collecting and composting manure by methods that result in unnecessary losses of quantity and quality.

Besides N, this study showed that manures were also rich in other important elements that can improve soil fertility, including C, P and K (Table 8.2). Maintenance of soil humus is a crucial fertility factor and is dependent on the presence of organic C. Lekasi *et al.* (2003) reported C content and C:N ratio ranging from 6.5 to 49.2% and 5.3 to 81.0%, respectively among 281 manures/composts, which are inclusive of the values obtained in the present study. Exhibition of similarity in C content by the present faeces implied that the ability to

supply C per se was not a quality distinguishing characteristic. On the other hand, C:N ratio has been used as an index to indicate immediate net N immobilization or mineralization (Senesi, 1989). Delve *et al.* (2001) argued that by C:N ratio of soil bacteria being around 6-8, and given that 45-50% of carbon is lost during decomposition, a C:N ratio greater than 12-16 should result in net N immobilization. However, conflicting results on the relationships of N mineralization or immobilization with different C and N concentrations were reported by Nhano *et al.* (2004). The authors concluded that neither %N nor C:N can individually explain mineralization pattern in manures. In the present study, no attempt was made to relate N immobilization or mineralization to C:N ratio, although there could be linkages. Faeces from diets containing SFC showed high amounts of P and K compared with those from diets containing LH and LPZ . Lekasi *et al.* (2003) and Kimani and Lekasi (2004) reported K concentrations in manures ranging from 0.43-7.0 and 0.84-1.29%, respectively, which are inclusive of the present values. Lekasi *et al.* (2003) reported P values ranging from 0.06 to 0.75%. Those of manures in the present study ranged from 0.18 to 2.08%, indicating the superiority of some of the diets in supplying of P. Na was also an important mineral excreted in manures with lucerne (LH) containing-diets having the highest concentrations of Na. Hence, caution needs to be observed when manures from such diets with high Na are to be used, particularly where there is risk of soil salinity.

8.5 CONCLUSIONS

This study showed that appropriate supplementation of poor quality roughages, which are predominant in tropical smallholder crop-livestock systems, can lead to improved livestock production with high potential of nitrogen recycling for enhanced productivity. Specifically, the results demonstrated that maize stover or grass hay supplemented with sunflower cake or lucerne may support sustainable sheep production while at the same time producing good quality manure. For practical purposes, diets containing 20-40% of DM contributed by these supplements can be recommended. However, caution needs to be observed when lespedeza is to be used as a supplement since sheep growth was hampered in lespedeza:maize stover diet. The fact that 24 to 83 % of NH_4^+ was lost as a result of drying fresh faeces showed the need to take precaution in manure conservation in order to maximise quality preservation. The results of this study also showed that manure from diets with crude protein content above 170 g/kg could be comparable to LAN in supplying N. Furthermore, the manures would have additional advantage of improving soil humus and supply more nutrients as it decomposes

Chapter 9

GENERAL DISCUSSION

The constraint to utilization of poor quality roughages by ruminants is widely recognized to be due to high fibre and low protein content of the roughages. The literature reviewed (Chapter 2) provided details about the potential role of the studied roughages and protein sources, particularly in Sub-Saharan Africa crop-ruminant production system. The Chapters reporting the results of the experiments (Chapters 3 to 8) were prepared with intention of publication in scientific journals. For this reason, the results are discussed extensively in each of the Chapter, and the conclusions were drawn according to the Chapter's stated objectives. Consequently, there were some inevitable repetitions, particularly in chemical composition of the feeds and *in vitro* procedures. The main aim of this General Discussion is to show linkages between and among the experiments, and to illustrate how the experiments contributed towards accomplishment of the overall study goal. Some aspects encountered during the study implementation considered to be of practical importance, particularly at farm level, are also discussed.

The overall goal of this study was to identify nutritive attributes and opportunities for improvement of ruminant production using diets comprised of maize stovers or grass hay mixed with different protein supplements, and to determine the influence of the diets on manure quality with regard to the chemical composition and nitrogen mineralization.

9.1 The effect of the protein supplements on nutritive value of maize stovers and grass hay diets

The principal challenge in improving utilization of low quality roughages, such as maize stovers, is to increase their intake. Efforts to improve intake of maize stovers have been mainly through chemical treatment or use of supplementary feeds. However, as has been pointed out by Ndlovu (1992), chemical treatments present several practical problems for smallholder agriculture. On the other hand, use of supplements has been shown by several workers (Chakeredza, *et al.*, 2001; Mpairwe *et al.,* 2003; Hindrichsen *et al.*, 2004; Wambui

et al., 2006) to be more practicable. Ndlovu (1992) explained that the effect of adding higher quality feeds to improve poor quality feeds utilization need to be properly understood for practical application. If the addition improves overall intake without reducing the intake of the basal poor quality feed, then supplement effect exists. If the addition results in reduced intake of the basal diet but an increase in total intake, then a substitution effect exists. In this regard, since high quality feeds are expected to be more expensive and may be available in small quantities, it would be more advantageous if supplementation effect is realized rather than substitution effect (Ndlovu, 1992). Nevertheless, under practical situations, the merits and demerits of supplementation or substitution effect will depend on the overall economic value including nutrients recycling.

This study showed that in choosing alternative forage legumes, and in particular between LH and LPZ, the former showed nutritive superiority (Chapters 3 and 7). However, this does not out rightly imply that LH would always be a better choice. LPZ can be a suitable alternative due to economic and/or ecological adaptation factors. The economic factors in LPZ utilization can emanate from high yields, possibility to moderate anti-nutritive factors due to high tannin content (Getachew *et al.*, 2000) and the possibility of anti-helmintic effects (Caygill and Mueller-Harvey, 1999). Furthermore, reports in the literature indicate that the tannin content of LPZ is lower at early growth stages. This was demonstrated in Chapter 4 where early harvested lespedeza showed superior nutritive attributes as compared to the one harvested at late maturity stage. This indicates that husbandry practices can be used as another strategy to improve nutritive value of LPZ. With respect to ecological adaptation, LPZ can be valuable in soil restoration and conservation (Powell *et al.*, 2003), hence may be suitable for rehabilitation of denuded rangelands, which are common in SSA. When used this way, livestock utilization can be regarded as an added advantage.

As for SFC, availability and costs are the most important factors for its utilization in maize-ruminant systems. Bedingar and Degefa (1990) pointed out that much of SFC produced in SSA has probably been fed to monogastric animals rather than ruminants. This implies that its utilization can be highly competitive among the livestock enterprises. Thus , there is need to take precaution with regard to sustained availability of SFC before basing ruminant production on such a competitive product, especially considering that ruminants generally have lower feed conversion efficiency as compared to monogastricts (McDonald *et al.*, 2002).

As shown on Table 3.4, MS on its own is potentially highly degradable. However, despite the supplementation of MS based diets with protein sources, the digestibility was markedly lower *in vivo* as compared to what was attained *in vitro* (see Tables 3.4 and 6.4). Although a lower digestibility *in vivo* as compared to *in vitro* incubations is generally expected (Rymer *et al.*, 2005), it cannot be simply concluded that the high degradabilities are merely artefacts of *in vitro* systems which are unattainable *in vivo*. The strong relationships between DMD and CP, NDF, ADF, $T_{½}$, and DEF (Appendices 13 and 14) indicate that there are opportunities to beneficially manipulate these parameters for improvement of DMD. For instance, as can be depicted from Table 3.2, the fibre content (NDF and ADF) can be moderated by early harvesting, which corroborates with other findings (Tolera *et al.*, 1999; Akbar *et al.*, 2002). The $T_{½}$ and DEF can be manipulated by choosing appropriate supplement and supplement level as demonstrated in Chapter 5 where both PS and PS x PS level interactions caused significant effects on these measurements. Manipulating the diet by beneficially altering these parameters can improve DMD to approach the *in vitro* degradability, hence are worth pursuing.

9.2 Predicting *in vivo* performance from *in vitro* gas production measurements

Rymer *et al.* (2005) posed a question that it may be necessary to re-examine the objectives of gas production techniques: Should the objective be to simulate the animal, or should substrate fermentability *in vitro* be considered as a characteristic per se? The present results indicate that it may be difficult to draw distinction between these two objectives since the information generated can be used in answering both. Generally, this study showed that the strength of IVGPT was in the assessment of fermentability of feeds. In particular, the results presented in Cahpter 5 corroborate with what other authors (Blümmel and Bullerdick, 1997, Getecahew *et al.* 1998; Makkar, 2004) have advocated that gas measurement alone is not satisfactory and needs to be complemented with other measurements. This study revealed that the important directly obtainable complementary measurements included chemical composition, degradability (ApDeg or TruDeg), gas profile and $T_{½}$.From these measurements, PF and DEF can be derived. The gas profile may also be fitted in models, such as that of Campos *et al.* (2004) used in this study (Chapters 3 and 4), in order to obtain more fermenation kinetics parameters. In Chapter 5, it was demonstrated that $T_{½}$ and DEF had high consistency among rations (Figure 5.3 and 5.4). These measurements were shown

(Chapter 7) to be correlated (Table 7.2) with various *in vivo* measurements and were useful in improving precisions of models predicting *in vivo* performance (Tables 7.4 and 7.5).

Although Blümmel *et al.* (1997b) and Blümmel *et al.* (2005) advocated the usefulness of PF as a predictor of partitioning efficiency (particularly rumen microbial yield) and feed intake, there are several factors discussed in Chapter 5, that confound the validity of PF. These factors include fermentation stoichiometry differences due to feeds chemical composition (Cone and Van Gelder, 1999; Rymer *et al.*, 2001), the length of incubation (Rymer 2001) and presence of anti-nutritive compounds e.g. tannins in legume forages (Makkar *et al.*, 1998, Blümmel eta al., 2005). The measurements which showed consistency and an/or distinctiveness were degradability measurements (TruDeg and or ApDeg), gas production profile and $T_{½}$. Therefore, it can be said that when using IVGPT to evaluate varied mixtures of roughages and supplements, these measurements should be included. The DEF may then be calculated and used in providing more distinctions where necessary. To determine maximum or potential degradability, the samples should be allowed to ferment until asymptote gas volume is attained. For fibrous roughages, such as those studied, at least 48 h incubation is recommendable, assuming similar procedures are applied. To increase accuracy of predicting *in vivo* responses, the IVGPT derived measurements should be complemented with those of chemical composition. The important chemical composition measurements include fibre (NDF and ADF) and protein (CP) contents.

In Chapters 3 and 4, it was demonstrated that fitting the gas production profiles to a model described by Campos *et al.* (2004) yielded fermentation kinetic information which were useful in screening individual diets. Important measurements revealed by the kinetics included impacts of forage type, maturity stage, supplementation levels and interactions among these aspects (Chapters 3 and 4). The kinetic information combined with other measurements were useful in creating clusters of diets with nutritive similarity (Chapter 6), and this was instrumental in selecting diets for *in vivo* trial presented in Chapters 7 and 8. Although it can postulated that kinetics parameters can be valuable for inclusion in models to predict *in vivo* responses, the fact that directly measured variables (e.g. chemical composition, ApDeg or TruDeg and $T_{½}$) gave high accuracies in predictions (Chapter 7) deplore the necessity for kinetic parameters for use in predicting *in vivo* response.

There can be inherently high correlations between nutritive measurements (Appendices 13 and 14). Problems of multicollinerity have been experienced in attempts to include some *in*

vitro measurements in prediction models (Blümmel *et al.*, 1997b). For this reason use of ridge regression (Chapter 7) demonstrated that selection of an appropriate method designed to handle multicollinearity can enable accurate prediction of *in vivo* responses from correlated *in vitro* and or chemical composition measurements.

9.3 Effect of diets on manure quality and nutrient recycling

Results presented in Chapter 8 and evidence from literature indicate that fresh (non-composted) manure will generally have a higher mineralised N content than composted manure. In this regard, use of fresh manure would be advantageous with respect to N supply to the crops. This poses a management challenge. Continuous direct application of freshly produced manure (faeces) may only be of practical value to perennial crops. In the case of seasonal crops, it would be recommendable to bulk the manures in heaps to minimise ammonia volatilization, while at the same time allowing decomposition to continue. The manure can then later be collected from the heaps and strategically applied. Application can be done by incorporating manure into the soil during seed bed preparation (ploughing), at planting or during weeding. In manure use, precaution need to be undertaken to avoid burning of crops due to high concentration of ammonium (Rankin, 2006). Uniformity of spreading and correct rate of nutrients supply to the crops are crucial considerations in order to prevent the burning. For these reasons, it is recommendable that in routine applications, the manure being used should be analysed for nutrient content, particularly total nitrogen (N), ammonium-N, phosphate (P_2O_5) and potash (K_2O). The crops requirements should be matched with the nutrient supply from the manures and soil. Availability of affordable analytical services is therefore necessary to support correct use of manures. Proper sampling is crucial for accurate analysis, and this will require that several sub-samples are collected and mixed to make up the samples submitted for analyses.

An example of a management system where fresh manure is utilized is in Sahel regions of West Africa. In this system, manure is directly deposited onto land by grazing animals (Powell *et al.*, 2003). Besides maximising N supply, this management system also has the advantages of storage and labour savings. However, as revealed in this study (Table 8.3), caution needs to be observed because of the risk of losing a high proportion of N due to ammonia volatilization (see Figure 2.3). These losses can be minimized by immediate mechanical incorporation of manure into the soil after herding animals on a plot. Therefore,

it is recommendable that land tilling should be done soon after the manure is deposited. Powell *et al.* (2003) suggested that another strategy to minimise losses is to maintain a vigorous biological community (e.g., beetles, earthworms, chickens, wild turkeys) that can chop, bury, and decompose the manure so that it is quickly fixed into forms that are not easily leached or volatilized. Another important factor to consider when planning direct manure deposit is irregular distribution. Inevitably, more manure is deposited near watering, feeding, and bedding areas.

9.4 Opportunities and challenges to conservation of maize stovers

Maximal utilization of maize stovers is constrained by many factors, of which the most commonly recognised is chemical composition, particularly the high fibre and low protein contents. As a result several studies have focused on improving the utilization of stovers through supplementation with protein sources (Mpairwe *et al.*, 2003; Hindrichsen *et al.*, 2004; Wambui *et al.*, 2006). This study demonstrated that supplementation is a viable option for improving utilization, but needs to be carefully planned with regard to the type and level of supplements. In addition, as explained in Chapters 3 and 7, physical or textural limitations, due to the bulkiness of stovers, can also pose serious constraints not only to the utilization by livestock, but also to the mode of conservation. Some options for conservation, especially under small scale operation, are briefly discussed.

Careful harvesting and storage should be the first step in maximising stovers utilization. If the stovers are to be conserved in heaps of dry material, thorough drying after harvesting is vital, in order to avoid decay and/or fungal attack, which can lead toxicity and total feed loss. This can be done by cutting the stovers and stooping in pyramidal heaps to minimise moisture accumulation during rains. This is a common practice in East Africa, and was applied in the current study (Appendix 4). However, this method of conservation is vulnerable to pests infestation as was experienced in this study where heavy attack by moths occurred, particularly in stovers harvested at milk stage (Appendix 5). The stovers conserved using this method are also more liable to decay and fungal attack, particularly in cold and/or humid weather. An alternative conservation method can be by milling the stovers followed by drying and stacking in bags (Appendix 6). The MM stovers used in the present feeding trial were mostly conserved using this method. Also, when maize is

harvested at young stage, the stovers can be cut and spread in the field to dry (Appendix 7), as is done in hay making. However, this method is liable to substantial wastage as a result of weeds and/or pest infestation; hence precautions need to be observed in case of such unprecedented occurrances. Silage making is also another conservation option. Under small scale operation, stovers can be chopped using motorised or manual chaff cutter and conserved as silage by adding molasses as reported by Otieno *et al.* (1990).

9.5 Conclusions and recommendations

Although the roughages of this study were of poor nutritive quality due to high fibre and low protein contents, the *in vitro* results showed that they were highly valuable by having degradabilities beyond 700 g/kg DM where there was no supplementation (see Tables 3.4 and 4.3). In the contrary, much lower *in vivo* digestibilities ranging from 381-608 g/kg DM were obtained from diets comprised of roughage mixtures with different protein supplements. This poses a serious challenge to the traditional approach of improving digestibility of poor quality roughages through strategic supplementation with protein or N sources. Given that higher degradabilities are attainable from the roughages, the future pursuits should explore means of improving their digestibility through a multi-factorial approach rather than depending on supplementation alone. Identification of factors influencing degradability (ApDeg and TruDeg), $T_{½}$, DEF, CP and fibre (NDF and ADF) content can be useful in improving digestibility, since these parameters showed high correlation with DMD. This study showed that processing (physical form of the roughage), the type and level of supplement and maturity stage are some of the factors that can be manipulated towards this goal.

As would be expected, the maize stovers harvested at milk stage had superior nutritive quality as compared to those harvested at dry stage. However, both *in vitro* and *in vivo* results indicated that the difference between these two stover types was not remarkable. At farm level, it may be therefore unnecessary to pay much attention to the loss of quality due to maturity stage. Much attention should instead be in conservation. This study experienced serious pests attack, particularly by moths, on milk stage stovers heaped in the field to dry. It can be therefore suggested that in livestock-maize system where large quantities of milk stage stovers are produced, such as in commercial baby corn production, making silage may be a viable undertaking to optimise stovers quantity. Besides conservation challenges,

another serious limiting factor to optimal utilization of the stovers is their physical form. From the present *in vitro* results, un-supplemented stovers showed superior degradability compared to un-supplemented grass (see Table 5.2 and 5.3). From *in vivo* results, comparable digestibilities were obtained in stover based and grass based diets supplemented with similar protein source. This is clearly demonstrated by the results of Diets 1 and 8 (Table 7.2). However, there was a remarkable difference between these diets in terms of DM intake. Diet 1 which was stover based had a much lower intake than Diet 8 which was grass based (31.4 vs 42.6 g/kg liveweight). A high correlation (Appendices 13 and 14) was obtained between intake and physical form of the roughage (RForm). The coarse stovers had lower intake. These findings emphasize the need to consider processing stovers to improve intake.

It was demonstrated that the measurements derived from gas production technique can be used in multivariate cluster analysis to logically separate feeds into distinct homogeneous groups of nutritive characteristics. The results also showed that IVGPT and chemical composition measurements plus physical form attributes of diets can be used in different combinations to predict *in vivo* responses with high precision. Among the IVGPT parameters, $T_{½}$ and DEF showed high consistency, good correlations with *in vivo* measurements and significant contribution to prediction of *in vivo* responses. Determination of these parameters should therefore be included in routine use of IVGPT to improve the authenticity of the evaluation.

The diets comprised of GH supplemented with 40% SFC and those of MM or MD supplemented with 40% LH showed high potential in optimising productivity. Supplementation with LPZ showed poor performance by sheep, and this was attributed to high tannin content in LPZ. Evidence from literature indicates that the tannin content in lespedeza varies among cultivars and maturity stage. Further work to improve utilization of lespedeza is therefore necessary. The investigations can focus on moderating the negative effects of tannins through chemical additives e.g. polyethylene glycol as reported in the literature. However, such intervention may be difficult under small scale operation. Hence there is need to explore other options such as identifying or developing adapted lespedeza cultivars with low tannin contents and exploring the impact of agronomic practices such as stage of harvesting.

Appropriate supplementation of poor quality roughages was shown to have good potential to improve livestock production while at the same have high nitrogen recycling for enhanced productivity. However, 24 to 83 % of NH_4^+ was lost as a result of drying fresh faeces. This emphasized the need to take precaution in manure conservation in order to maximise quality preservation. The results of this study also showed that manure from diets with crude protein content above 170 g/kg could be comparable to LAN (a commercial fertilizer) in supplying mineralised N. Agronomic trials to practically ratify this possibility are essential future pursuits.

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APPENDICES

Appendix 1 Different stages of maize maturation as used in the study: (a) whole plant at milk stage (b)dry stovers after harvest.

Appendix 2 Lucerne: (a) good growth under suitable environmental conditions (b) morphological features

Appendix 3 Sericea lespedeza: (a) good growth in poor climatic condition and (b) Morphological features.

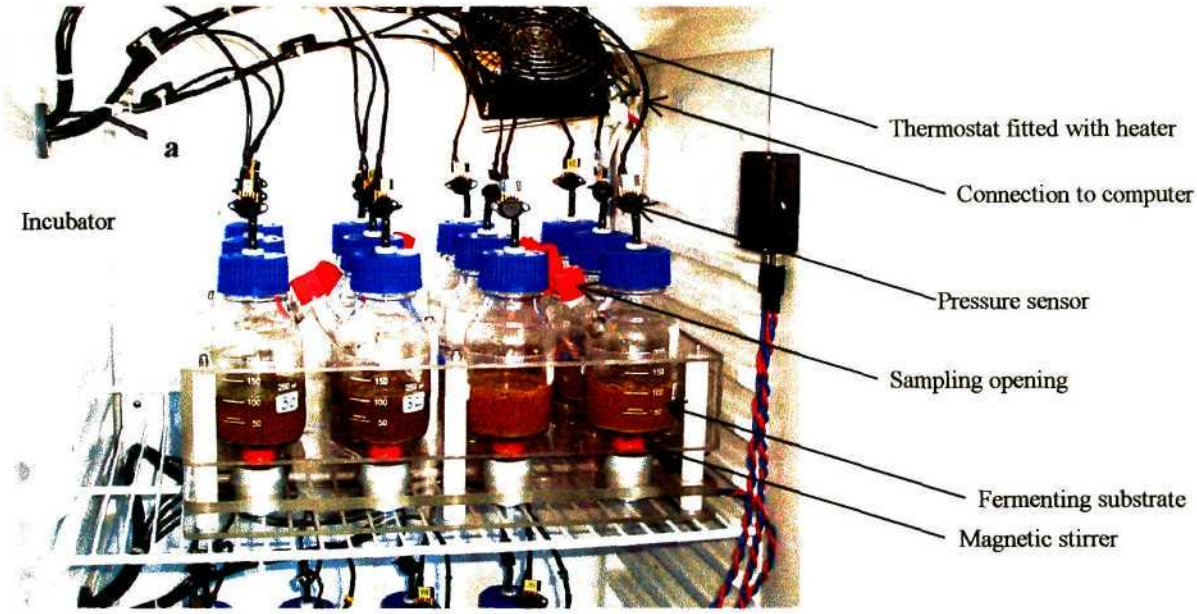
Appendix 4 Conservation of maize stovers by stooping in pyramidal heaps in the field.

Appendix 5 Pest attack on maize stovers conserved in heaps.

Appendix 6 Conserving milk stage maize stovers by grinding, drying and keeping in bags

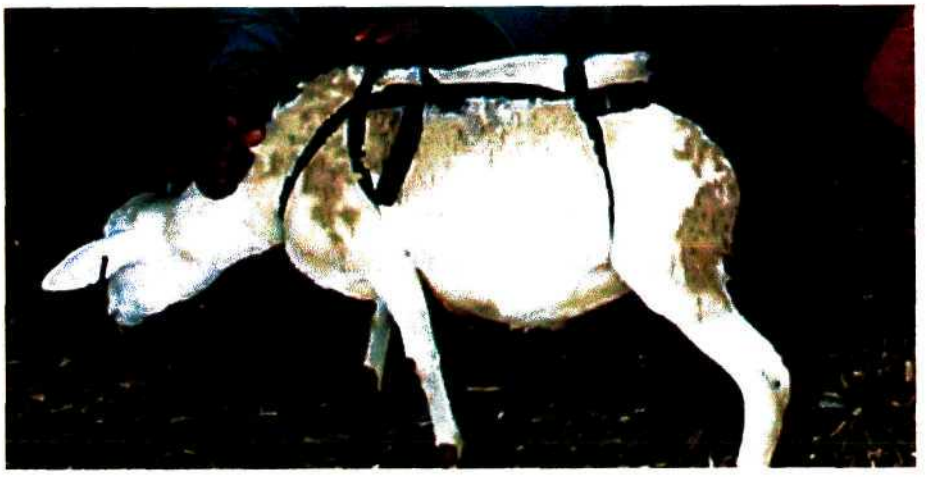
Appendix 7 Conservation of maize stovers by field spreading and drying.

Appendix 8 Automated *In vitro* gas production technique apparatus components (a) set up in incubator (b) closed system in operation.



Appendix 9. Sheep in individual pens during the trial

Appendix 10. Sheep harnessing for faeces collection during the trial



Appendix 11. Sheep in metabolic crates during the trial

Appendix 12 Manure mineralization apparatus components

Appendix 13 Correlation between *in vivo* and *in vitro* gas production technique parameters (72 h incubation)

	DMILW	DMD	DMDLW	WtGain	GEI	GELU	GELF	CP	NDF	ADF	ApDeg	TruDeg	MIC	GasVol	$T_{½}$	PF	DEF	RForm
DMILW	1																	
DMD	0.53	1.00																
DMDL W	0.90	0.84	1.00															
WtGain	0.98	0.64	0.94	1.00														
GEI	0.99	0.43	0.85	0.94	1.00													
GELU	0.59	0.62	0.69	0.67	0.56	1.00												
GELF	-0.58	-0.97	-0.86	-0.67	-0.49	-0.68	1.00											
CP	0.57	0.82	0.79	0.62	0.54	0.79	-0.86	1.00										
NDF	-0.70	-0.83	-0.87	-0.78	-0.66	-0.80	0.82	-0.91	1.00									
ADF	-0.55	-0.86	-0.80	-0.62	-0.51	-0.80	0.91	-0.99	0.91	1.00								
ApDeg	0.49	0.79	0.70	0.52	0.41	0.50	-0.83	0.73	-0.59	-0.75	1.00							
TruDeg	0.60	0.87	0.81	0.65	0.52	0.67	-0.92	0.86	-0.75	-0.89	0.96	1.00						
MIC	-0.11	-0.38	-0.23	-0.08	-0.03	0.04	0.37	-0.20	0.04	0.23	-0.78	-0.57	1.00					
GasVol	-0.05	0.36	0.15	-0.02	-0.15	0.17	-0.50	0.20	0.04	-0.29	0.55	0.49	-0.50	1.00				
$T_{½}$	-0.27	-0.86	-0.63	-0.40	-0.22	-0.69	0.81	-0.86	0.81	0.87	-0.60	-0.71	0.17	-0.18	1.00			
PF	0.66	0.42	0.63	0.66	0.70	0.54	-0.38	0.67	-0.77	-0.60	0.37	0.48	0.01	-0.51	-0.47	1.00		
DEF	0.44	0.82	0.70	0.54	0.41	0.75	-0.77	0.92	-0.92	-0.91	0.60	0.73	-0.13	-0.03	-0.94	0.73	1.00	
RForm	0.71	-0.12	0.37	0.62	0.76	0.17	0.10	0.04	-0.25	0.02	-0.01	0.04	0.12	-0.54	0.29	0.62	0.00	1

DMLIW = dry matter intake (g DM/kg liveweight), DMD = dry matter digestibility in vivo (g/kg DM), DMDLW = dry matter digested (g DM/kg liveweight), WtGain = weight gain (g/d), GEI = gross energy in take (MJ/d), GELU = portion of GEI lost in urine, GELF = portion of GEI lost in faeces, CP = diet crude protein content, NDF = neutral detergenet content, ADF = acid detergent content, ApDeg = apparent degradability, TruDeg = true degradability, MIC = microbial yield, GasVol = total volume of gas produced (V), $V_{½}$ = half of V, $T_{½}$ = Time (h) taken to produce $V_{½}$, PF = partitioning factor (TruDeg/V), DEF = degradability efficiency factor (TruDeg/ $T_{½}$ x $V_{½}$), Rform = roughage physical form (fine = 1, coarse = 2).

Appendix 14 Correlation between *in vivo* and *in vitro* gas production technique parameters (incubation stopped at the time when half of the potential maximum gas volume was produced).

	DMILW	DMD	DMDLW	WtGain	GEI	GELU	GELF	CP	NDF	ADF	ApDeg	TruDeg	MIC	GasVol	$T_{½}$	PF	DEF	RForm
DMILW	1																	
DMD	0.53	1.00																
DMDLW	0.90	0.84	1.00															
WtGain	0.98	0.64	0.94	1.00														
GEI	0.99	0.43	0.85	0.94	1.00													
GELU	0.59	0.62	0.69	0.67	0.56	1.00												
GELF	-0.58	-0.97	-0.86	-0.67	-0.49	-0.68	1.00											
CP	0.57	0.82	0.79	0.62	0.54	0.79	-0.86	1.00										
NDF	-0.70	-0.83	-0.87	-0.78	-0.66	-0.80	0.82	-0.91	1.00									
ADF	-0.55	-0.86	-0.80	-0.62	-0.51	-0.80	0.91	-0.99	0.91	1.00								
ApDeg	0.16	0.50	0.36	0.24	0.10	0.45	-0.61	0.46	-0.33	-0.53	1.00							
TruDeg	0.57	0.78	0.75	0.67	0.49	0.78	-0.85	0.72	-0.73	-0.78	0.84	1.00						
MIC	0.48	0.15	0.37	0.48	0.49	0.25	-0.06	0.15	-0.40	-0.10	-0.67	-0.16	1.00					
GasVol	0.17	0.58	0.40	0.22	0.07	0.29	-0.69	0.39	-0.19	-0.47	0.64	0.57	-0.37	1.00				
$T_{½}$	-0.21	-0.75	-0.53	-0.36	-0.16	-0.74	0.70	-0.72	0.73	0.75	-0.42	-0.67	-0.16	-0.24	1.00			
PF	0.52	0.40	0.53	0.60	0.52	0.67	-0.38	0.50	-0.68	-0.50	0.45	0.69	0.12	-0.20	-0.57	1.00		
DEF	0.31	0.70	0.56	0.44	0.28	0.82	-0.66	0.76	-0.79	-0.78	0.47	0.73	0.15	0.13	-0.96	0.75	1.00	
RForm	0.71	-0.12	0.37	0.62	0.76	0.17	0.10	0.04	-0.25	0.02	-0.39	-0.06	0.62	-0.37	0.35	0.26	-0.18	1.00

DMLIW = dry matter intake (g DM/kg liveweight), DMD = dry matter digestibility in vivo (g/kg DM), DMDLW = dry matter digested (g DM/kg liveweight), WtGain = weight gain (g/d), GEI = gross energy in take (MJ/d), GELU = portion of GEI lost in urine, GELF = portion of GEI lost in faeces, CP = diet crude protein content, NDF = neutral detergenet content, ADF = acid detergent content, ApDeg = apparent degradability, TruDeg = true degradability, MIC = microbial yield, GasVol = total volume of gas produced (V), $V_{½}$ = half of V, $T_{½}$ = Time (h) taken to produce $V_{½}$, PF = partitioning factor (TruDeg/V), DEF = degradability efficiency factor (TruDeg/ $T_{½}$ x $V_{½}$), Rform = roughage physical form (fine = 1, coarse = 2).

Appendix 15

PROCEDURE FOR DETERMINITATION OF GROSS ENERGY IN URINE

Materials

Dessicator
Phosphorus pentoxide
Large deep glass evaporating basin or similar open glass container
Whatman ® filter paper No. 1
Nickel bomb crucibles
Koki

Method

1. Cut up filter paper into 20mm x 20mm squares and use 3 per sample

CAUTION

2. **Read safety hazard data sheet for Phosphorus pentoxide. It is toxic and may be fatal if inhaled, swallowed or absorbed through the skin. Use a fume extraction cupboard and wear gloves, eye-goggles and a respirator.**

Prepare the dessicator by placing Phosphorus pentoxide into a suitable container under the supporting mat. Do not place Phosphorus pentoxide straight into the the dessicator bottom because it forms a dangerous sludge which is difficult to remove.

3. Label crucible, weigh and record mass {crucible [1]}
4. Place 3 squares of filter paper into crucible, weigh and record mass {crucible + filter [2]}.
5. Pipette 3ml urine onto the filter paper and dry in a dessicator over Phosphorus pentoxide until just dry
6. Remove the crucibles from the dessicator and immediately record mass {crucible + filter paper + dried urine[3] }.
7. Record mass of filter paper plus dried urine (3 – 1) for Gross energy (GE) analyser to in put
8. Place the crucible in the bomb canister with ignition wire just touching the filter paper. Carry out r bomb calorimetric determination of GE
9. Carry out a few GE determinations on 3 squares of filter paper
10. Calculate urine GE by subtracting the GE value of the filter paper from the GE of the urine + filter paper
11. At the of drying the Phosphorus pentoxide will be partly liquid partly solid. Using a fume extraction cupboard and personal protective equipment, carefully remove the dish and with pastic spoon, CAREFULLY spoon the mixture into a bucket containing atleast 5 litres of water. When the dish is empty immerse in the water and wipe clean. Pour the liquid waste into a suitably labelled waste container for collection and safe disposal.