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EFFICIENCY OF PROTEIN UTILISATION OF FORAGE LEGUMES FOR MILK PRODUCTION IN GOATS

BY

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Thesis submitted to the School of Agriculture University of Venda in fulfilment of the requirement for the degree of

Doctor of Philosophy in Agriculture

Department of Animal Science,

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University of Venda

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SOUTH AFRICA

2014

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DECLARATION

I, Katsande Simbarashe hereby declare that this thesis was submitted in fulfilment of the requirements for the Doctor of Philosophy Degree in Agriculture at the University of Venda has not previously been submitted for a degree at this or any other university, and in execution and that all reference material contained therein has been duly acknowledged.

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DEDICATIONS

I dedicate this thesis to my father Aaron and my late mother Felistus Katsande.

"I thank you".

Glory is to Elohim Jehovah the father of our Saviour Jesus Christ.

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ACKNOWLEDGEMENTS

I would like to thank my major promoter Dr J. J. Baloyi for the professional guidance and inspiration through out my studies. I greatly appreciate Dr F. V. Nherera-Chokuda for all the ultramodern vision and meticulous effort. My sincere thanks are also given to the Professors N. T. Ngongoni and G. Matope for the direction and all the support. I am gratiful to all the laboratories (Lacto-lab ARC Irene, Agrilabs Zimbabwe, Animal Science University of Zimbabwe, Biochemistry University of Venda, Animal Science University of Venda) and laboratory technicians (M. Pitsi, N. Mnonopi, K. Chigayo, S. Chikambi, Sena, Irene and C. Mashegede) who participated directly and indirectly in the analysis of the samples. My brother Mlaudzi I thank him for the assistance and unconditional love.

I extend my gratitude to the supporters of this study University of Venda, Agricultural Research Council (Irene) Pretoria, Henderson Research Institute Zimbabwe, University of Zimbabwe farm and the Department of Animal Science University of Zimbabwe.

All my colleagues Mr J. Gusha, Ms P. I. Zvinorova, Dr T. E. Halimani, Mr Makiwa, Mr L. Muronda, Mr M. Lubisi and Mr K. Chigayo, I greatly appreciate your input. I express thankfulness to my family, and all my fellow brethren and sisters in Christ for all the moral support. Promise Kondo thank you for always being there for me. All those who participated directly and indirectly to this research I am very indebted to you.

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ETHICAL APPROVAL

All the experiments carried out in this thesis were approved by the University of Venda, Research Ethics Committee and Project number issued SARDF/13/ANS/01/0522.

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ABSTRACT

Protein is the one of the limiting nutrients in ruminants relying on natural rangelands due to seasonal fluctuation in quality in response to seasonality of rainfall. Understanding rumen degradability, digestion and utilisation for milk production of protein from forage legumes will aid in improving their use by smallholder farmers and decrease dependency on expensive, protein-rich supplements.

Six experiments were conducted to evaluate *Desmodium uncinatum* (Silverleaf desmodium), *Mucuna pruriens* (Velvet bean) and *Vigna unguiculata* (L.) Walp (Cowpea) forage legumes as protein supplements for goats. Rumen disappearance of amino acids, dry matter (DM), crude protein (CP), phosphorus (P) and calcium (Ca) were determined using the *in sacco* (Nylon-Bag technique) and *in vitro* (Daisy^{II} technique). Rumen degradability patterns were assessed using the Ankom^{RF} gas technique whilst feeding trials were carried out to determine apparent digestibility, milk production and utilisation of protein for milk production in goat.

Dry matter, CP and P effective disappearance of Cowpea were the highest and significantly ($P < 0.05$) different from Velvet bean and Silverleaf desmodium. Dry matter effective degradability of Velvet bean was higher than that of Silverleaf desmodium. However, the CP effective degradability of Silverleaf desmodium was significantly higher than Velvet bean ($P < 0.05$). Disapperance of Ca was significantly higher ($P < 0.05$) in Velvet bean forage followed by Cowpea. The volumes of gases produced ranged from 61.4 to 70.4 ml, with an average of 64.2 ml, whilst the fractional rate of gas production varied between 4.6 and 5.6 % h^{-1} with a mean of 5 % h^{-1}. Effective gas production, (a), (b) and (c) were significantly ($P < 0.05$) different for the three legumes. Cowpea produced significantly ($P < 0.05$) the highest amount of gas followed by Velvet bean.

When both *in sacco* and *in vitro* techniques were correlated on dry matter degradability of the three forage legumes, a significant linear relationship was obtained at $P < 0.001$. When the values for the legume dry matter degradability were considered, there were significant ($P < 0.001$) correlations 0.93, 0.72 and 0.82 for Velvet bean, Silverleaf desmodium and Cowpeas respectively. The effective degradability values of both techniques were within the same ranges.

Total nitrogen intake was significantly ($P < 0.05$) highest in the commercial goat feed followed by hay supplemented with Cowpea, Silverleaf desmodium and Velvet bean respectively.

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Microbial protein supply, calculated microbial true protein and digestible microbial true protein were affected ($P < 0.05$) by legume supplementation.

Total dry matter intake (DMI) and metabolisable energy (ME) intake were not significantly different among the different-diet fed animals ($P > 0.05$). Daily live weight loss was significantly highest ($P < 0.05$) in the treatment supplemented with Cowpea compared to the commercial goat feed, Silverleaf desmodium and Velvet bean.

Cowpea showed higher effective (58.6 %) rumen CP degradability value compared to Silverleaf (46.1 %) and Velvet bean (38 %) at 0.06/h outflow rate. Silverleaf produced (15.5 g/day) higher digestible microbial true protein compared to Cowpea (13.8 g/day) and Velvet bean (13.2 g/day). There was no significant difference in efficiency of microbial N synthesis 2.21, 2.01 and 2.06 for Velvet bean, Silverleaf and Cowpea respectively. The higher amount of digestible microbial true protein from the degradability of Silverleaf and lower values of degradability (higher undegradability) CP caused the legume to have higher efficiency of utilisation in comparison to Cowpea and Velvet bean. Higher DM intake values were observed in animals on Cowpea compared to animals on Silverleaf and Velvet bean. Nitrogen retention was higher on animals on Cowpea (0.84 g/day) compared to animals on Silverleaf (-0.46 g/day) and Velvet bean 9-1.29 g/day). Milk production was not significantly different amongst the treatments 405 g/day, 425 g/day and 447 g/day in Velvet bean, Silverleaf and Cowpea respectively. Fat, protein, lactose and ash in the milk were not different between the animals of the four treatments.

Among the legume forages Silverleaf desmodium had significantly ($P < 0.05$) the lowest efficiencies of utilisation of histidine, lysine and tyrosine, whereas, Velvet bean had significantly the highest efficiency of utilisation of phenylalanine ($P < 0.05$) compared to Silverleaf desmodium and Cowpea. Cowpea had the highest efficiency of utilisation of aspartic acid ($P < 0.05$) for milk production.

The efficiency of utilisation of nitrogen in the supplements could have been limited by metabolisable energy in the diets. Supplementation of poor quality veld hay with forage legumes could improve utilisation especially in the dry season. The levels of milk production attained in this study imply that some conventional commercial goat feeds can be substituted with forage legumes without significantly affecting milk yield and composition in goats.

Key words: legume forages, degradability, digestion, utilisation, milk production, goats

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PUBLICATIONS

Parts of the work described in this thesis have been published or presented in conference meetings.

1. **Katsande S.**, J. J. Baloyi, F. V. Nherera-Chokuda, N. T. Ngongoni, G. Matope. P. I. Zvinorova and J. Gusha. 2015. Apparent Digestibility and Microbial Protein Yield of *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* forage legumes in goats. African Journal of Range and Forage Science, http://dx.doi.org/10.2989/10220119.2015.1043646.
2. **Katsande, S.**, J.J. Baloyi, F.V. Nherera-Chokuda, N.T. Ngongoni and G. Matope. 2015. *In Vitro* Degradability of Forage Legumes Using the Ankom^{RF} Gas Technique. Indian Journal of Animal Research 48(1).
3. **Katsande, S.**, J.J. Baloyi, F.V. Nherera-Chokuda, N.T. Ngongoni and G. Matope. 2015. Rumen Degradability of *Desmodium uncinatum*, *Mucuna pruriens* and *Vigna unguiculata* forage legumes using the *in vitro* Daisy^{II} technique. African Journal of Agriculture Research Accepted.
4. **Katsande S.**, J. J. Baloyi, F. V. Nherera-Chokuda, N. T. Ngongoni, G. Matope. 2014. *In vitro* degradability of *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* forages using the Gas Technique. Proceedings of 49th GSSA Congress Advancing Rangeland Ecology and Pasture Management in Africa. 21-25 July 2014. Phillip Sanders Resort and Conference Centre Bloemfontein.

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LIST OF ABBREVIATIONS

Ad lib	Ad libitum
ADF	Acid Detergent Fibre
ADF-N	Acid Detergent Fibre Nitrogen
ADIN	Acid Detergent Insoluble Nitrogen
BCP	Bacterial Crude Protein
BV	Biological Value
CF	Crude Fibre
CHO	Carbohydrate
CP	Crude Protein
DE	Digestible Energy
DIP	Degradable Intake Protein
DM	Dry Matter
EAA	Essential Amino Acid
EE	Ether Extract
EFA	Essential Fatty Acid
FAO	Food and Agriculture Organisation
FAO-STAT	Food and Agriculture Organisation Statistics
GE	Gross Energy
ME	Metabolisable Energy
MP	Metabolisable Protein
N	Nitrogen
NAN	Non-ammonia nitrogen
NDF	Neutral Detergent Fibre
NE	Net Energy
NFE	Nitrogen Free Extract
NIRS	Near Infrared Reflectance Spectroscopy
NPN	Non-protein Nitrogen
NPN	Non-protein Nitrogen
NRC	National Research Council
TDN	Total Digestible Nutrients
UIP	Undegradable Intake Protein
VFA	Volatile Fatty Acid
WHO	World Health Organisation

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Small ruminants represent the third largest livestock sector in the world, with 8.6% of an estimated one billion tonnes of biomass (Squires, 2011). Furthermore, they comprise 10% of the total mass of domestic herbivores and 11% of the mass of ruminants (Pulina *et al.*, 2013). They make an important contribution to the subsistence sub-sector of the economy of many countries in Africa, Asia and in the Middle East (Dikshit *et al.*, 2010; Squires, 2011; Datta, 2013).

Approximately 90% of animal keepers in southern Africa are smallholders, while 75% of all farm animals are kept by rural communities (Maphosa, 2009). In the smallholder sector that operates under conditions of resource constraints, they usually pursue diverse livelihood strategies of which livestock forms a major risk alleviating component (Kohler-Rollefson, 2004; Zvinorova *et al.*, 2013). Smallholder production systems of small ruminants is characterised by low productivity due to poor breeds, feed unavailability, and diseases. The current reality impedes economic growth and it is difficult to attain expected livelihood gains required by the people. This is due to technical inefficiencies resulting in failure in designing ruminant feeding systems which are relevant to specific production environments. Earlier studies (Moyo, 1996; Mapiye *et al.*, 2007; Mapekula *et al.*, 2009) have shown that in the smallholder sector, production is largely dependent on indigenous animals and their crosses with exotic breeds. Indigenous breeds are reported to be more resistant to nematodes (Ndlovu *et al.*, 2007), ticks and tick-borne diseases (Norval *et al.*, 1996; Muchenje *et al.*, 2008), have high feed utilisation efficiency at low feeding levels and have the ability to select high quality diets when grazing coarse forages on rangelands (Collins-Luswet, 2000). Nevertheless, Ngongoni *et al.*, (2006) and Muchenje *et al.*, (2007) reported that local breeds are failing to produce to their full genetic potential and have high mortality rates under low levels of management.

At that juncture, sub-Saharan Africa is experiencing a mutual falling of meat and milk consumption as poverty is increasing due to low productivity. Poor nutrition is the major limitation to livestock productivity in sub-Saharan Africa (Teferedegne, 2000). This is because animals thrive largely on high-fibre feeds (stovers, straws, and native pasture hay) which are deficient in nutrients (nitrogen, phosphorus, calcium and sulphur) essential for microbial fermentation. Therefore, the ingestion and digestibility of digestible nutrients are inevitably low.

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These deficiencies may, to a certain extent, be alleviated by supplementing roughage diets with forage legumes (Gusha *et al.*, 2014) containing the deficient nutrients.

Protein is a limiting nutrient in the diet of ruminants (Sarwar *et al.*, 2002) and deficiencies in livestock can be corrected by supplementation with high-density feeds such as oilseed cakes and proteins of animal origin. Sufficient crude protein supply is important when dry matter intake is relatively low and crude protein (CP) and energy requirements are high (Shahzad *et al.*, 2008). According to Bodine *et al.*, (1999), when diet quantity or quality limits animal performance, supplementation with either CP or energy may be required to increase or maintain the level of production. Crude protein requirements are dependent on body weight (BW), sex, breed, production levels, body condition, daily gain, compensatory growth, environmental difference, microbial yield effects and forage availability (NRC, 1996). However, protein sources such as oilseed cakes are beyond the economic reach of most smallholder farmers (Teferedegne, 2000). Therefore, potential strategies for increasing the quality and availability of feeds for smallholder ruminant animals in the dry season may be through the use of fodder trees and shrub forages (Allam *et al.*, 2012). Addition of better forage, for example legume fodder, to cereal crop residues has been shown to increase voluntary intake or digestibility of the rations (Baloyi *et al.*, 2001). Therefore, legume forages could be used as non-conventional animal feed because of their availability, low cost and also because they are non-human feed.

Forage legumes such as *Leucena leucocephala*, *Vigna unguiculata*, *Medicago sativa*, *Mucuna pruriens*, *Desmodium incinatum* and *Trifolium repens* have been reported to contain reasonable high amounts of nitrogen and have been identified as potential supplements to poor-quality grasses (Ranjbar, 2007; Ravhuhali *et al.*, 2010; Gwanzura *et al.*, 2012; Baloyi *et al.*, 2014). However, in ruminants, a large portion of the feed protein is transformed into microbial protein and is ultimately digested along with rumen undegradable dietary protein in the intestines. The absorbable amino acids originating from feed and microbial protein represent metabolisable protein (MP), which is in essence utilised by the animal for maintenance, growth, and milk production (Das *et al.*, 2014). A better understanding of protein digestion, absorption, and metabolism by goats is an essential element in an attempt to improve growth rate, milk production and feed efficiency in this species.

Degradability, digestion and N retention of forage legumes by ruminants is superior to most grass species (Archimède *et al.*, 2000; Sandoval Castro *et al.*, 2003a; Sandoval Castro *et al.*, 2003b; Mendoza-Castillo *et al.*, 2003; Ayala-Burgos *et al.*, 2003). Several studies reported superior digestibility and N retention of various forage legumes and silages in ruminants

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(Chikagwa-Malunga *et al.*, 2009; Loyra-Tzab *et al.*, 2011; García-Galvan *et al.*, 2012). Even though positive N balances were observed some authors (Baloyi *et al.*, 2008; Chikagwa-Malunga *et al.*, 2009; Loyra-Tzab *et al.*, 2013) reported lower N balance at higher legume forage inclusion levels and this could be due to uncoupled fermentation that results in N loss as urea in urine. The same as with any other protein supplements, efficiency of N utilisation may also be influenced by diet presentation (anti-nutritional factors, particle size) (Chay-Canul *et al.*, 2009). Moreover, legumes generally improve milk production when fed either as forages or silages (Broderick *et al.*, 2001; Mendoza-Castillo *et al.*, 2003; Dewhurst *et al.*, 2003; Fraser *et al.*, 2004).

1.2 Statement of the problem

Development of smallholder goat production is a possibility to increase food security and income for resource-poor farmers (Ngongoni *et al.*, 2007; Pulina *et al.*; 2013). A study done by Shackleton *et al.*, (2000) showed that meat and cash sale provide approximately 25% of the annual benefits from livestock, while milk, draught power, transport and manure account for more than 75%. However, livestock production in sub-Saharan Africa is predominantly based on natural pastures as well as crop residues (Rebero and Mupenzi, 2012). The quantity and quality of these feed resources vary with season, herbage type and geographical location (Mupenzi *et al.*, 2009). The challenge is to develop feed supplementation strategies that improve ruminant production without compromising the exceptional qualities attainable under favourable conditions, such as healthy animals, low production costs and ecological stability.

Moreover, the predicament is not always a factor of low crude protein (CP) from forages, but rather poor efficiency of CP utilisation (Spiekers, 2011). Amino acids absorbed from the small intestine of a ruminant animal are utilised for protein synthesis with different efficiencies (Löest, 2006). The efficiency of amino acid utilisation will depend on the type of amino acid, and whether it is limited or supplied in excess (Löest; 2006). The efficiency with which animals utilise absorbable amino acids for protein synthesis is also affected by demands for amino acids in metabolic processes that do not support protein synthesis directly (Owens and Pettigrew, 1989). Essential quantitative results on the efficiency of utilisation of protein in different forage legumes by goats are limited.

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1.3 Justification of the study

The goat is believed to be the most important domestic animal to man in the tropics (Debele *et al.*, 2011) because it has constantly confirmed to be of enormous advantage to resource poor and landless farmers in Africa. However, inadequate availability of feed is the biggest problem in livestock production in the sub-Saharan African countries (Agyemang, 2000) because systems in the communal areas are predominantly based on pastoralism and agro-pastoralism. Consequently, several authors have reported kid mortality of 10-30% (Turkson 2003; Snyman, 2010a; Debele 2011) and this negatively affects goat production.

Relatively low birth weight, slow growth rate and insufficient milk production by does, were identified as the foremost limitations directly linked with elevated kid mortality and results in significant decline in total productivity (Husain 1993; Snyman, 2010a; 2010b). Snyman, (2010b) ranked the combination of udder problems and too little milk as the second important cause of kid mortalities in raring goats in South Africa. Therefore, potential feeding strategies aimed at increasing milk production through the use of high quality, available and affordable forages for smallholder goats are crucial.

According to Argüello (2011), modern nutrition is the basis for improving goat production in developing countries using locally available feedstuff or feed. These feed resources may improve nutrition, thus bringing improvements in animal production to meet anticipated demand. The development of accurate feed composition information for the tropics that can be used to develop accurate feeding recommendations is extremely important for livestock production (Tedeschi *et al.*, 2002) to develop feeding systems that optimize use of available forages.

Animal productivity could be increased through the introduction of low-cost technologies that would improve the current systems of management. Acceptable and successful feeding systems are methods that are practical, simple, consistently reproducible and within the limits of the farmer's resources (Douthwaite, 2002). This approach underscores the importance of dual-purpose crops that bring N into the farming system through fixation (FAO, 2009) and feed (residues) in meeting household needs under the current and foreseeable future scenarios (Delgado *et al.*, 1999). Going beyond their importance as food and feed, legumes can be regarded as a pivot of sustainable farming in regions characterized by farming systems that make limited use of purchased inputs (Anele *et al.*, 2011). This research focused on efficiency of utilisation of selected forage legumes in goats and also identifying utilisation that suits diverse needs of farmers with affordable forage legumes.

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1.4 Research objectives

The main objective of the study was to determine the utilisation of protein for goat milk production supplemented with selected forage legumes.

The specific objectives of this study are to:

1. Determine rumen degradability patterns of *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata (L.)* Walp forage legumes;
2. Investigate rumen disappearance of dry matter, crude protein, calcium and phosphorus in *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* (L.) Walp forage legumes;
3. Determine rumen degradability of amino acids in *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* (L.) Walp forage legumes;
4. Establish apparent digestibility and microbial protein yield of *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* (L.) Walp forage legumes in goats;
5. Determine the effects of *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata (L.)* Walp as protein supplements on milk production in goats;
6. Determine the utilisation efficiency of amino acids for milk production in goat supplemented with *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata (L.)* Walp forage legumes.

1.4.1 Research Hypotheses

The general hypothesis of the study is that the degradability, digestion and milk utilisation efficiency of CP in different forage legumes is the same.

1. The different forage legumes *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata (L.)* Walp have the same rumen degradability pattern.
2. Rumen disappearance of dry matter, crude protein, calcium and phosphorus in *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* (L.) Walp forage legumes are the same;
3. There are no significant differences in rumen degradability of amino acids in *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* (L.) Walp forage legumes;
4. Apparent digestibility and microbial protein yield of *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* (L.) Walp forage legumes in goats are the same;

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5. Milk production of goats using *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata (L.)* Walp as protein supplements are similar; and
6. Utilisation of amino acids for milk production from *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata (L.)* Walp are similar.

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CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

In Sub-Saharan Africa, accessibility of feed to livestock is dictated by rainfall patterns and drought is common hence, high-quality green feed is normally scarce and fluctuating (Ajay *et al.* 2005; Asaolu *et al.* 2011) in availability. Supplementation is required to increase the total quantity of protein as well as satisfy essential amino acid requirements (WHO, 2007 Mustafa and Alamini, 2012). However, supplementation with conventional protein sources (cotton seed meal, soya bean meal and fish meal) becomes essential, but it is neither cost effective nor environmentally friendly due to excess nitrogen excretion. The importance of tropical forage legumes and browse legumes as sources of extraordinary quality protein for animal production in the tropics especially ruminant (Baloyi *et al.* 2008), has a role in small ruminant production. Studies have shown that legumes have high CP values (12-38 %), for that reason, they have the potential to improve productivity of ruminants fed low quality roughages (Hess *et al.*, 2008; Gwanzura *et al.*, 2012). The use of leguminous crops as supplements is extremely essential in sub-Saharan African countries where extensive livestock production pre-dominates.

2.2 Legumes in ruminant nutrition

Herbaceous forage legumes such as *Vigna unguiculata, Desmodium uncinatum, Medicago sativa, Stylosanthes scabra and Mucuna pruriens* and browse legumes such as *Leucaena leucocephala, Gliricidia sepium* and *Acacia angustissima* contain protein, mineral elements and vitamins, which are in limited quantities in mature natural veld pastures, particularly during the mid-dry season (Baloyi *et al.*, 2008). The elevated protein levels of these legumes show that they have great prospective for use as protein supplements in ruminant feeding. Mgheni *et al.*, (1993) reported effective nitrogen degradability (at a passage rate of 0.02) that ranges between 32 and 80 percent for some tropical herbaceous legumes (*Neanotonia wightii, Mucuna pruriens, Pueraria phaseoloides* and *Leucaena leucocephala).* In this study *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* forage legumes were selected because of their prevalence and adaptability in sub-Saharan Africa.

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Desmodium uncinatum (Silverleaf desmodium)

Desmodium uncinatum is a perennial legume with stems that may grow several metres long and trail over surrounding vegetation. These angular stems are covered with short, hooked hairs that are sticky and the stems can produce roots at the nodes when in contact with moist soil. The trifoliate leaves have pointed leaflets (up to 10 cm long x 5 cm wide) and the upper side is dark green with an irregular generally pyriform silvering about the midrib, the lower side is lighter green and uniform in colour, both covered with whitish hairs. Flowering stems may be up to 1 m high, ending in fairly open racemes on a long peduncle with paired pink to bluish flowers. Curved pods may break into up to 8 segments when mature.

Silverleaf desmodium is sown in permanent grazing pastures, but is also used for green or conserved feed, for ground cover and in cropping for intercropping and mulch. Crude protein value is high 11.5 to 20.8 % DM, however, tannin levels can exceed 3% and these can protect the protein increasing the 'undegradable dietary' protein and the efficiency of digestion (Baloyi *et al.,* 2001). The high tannin levels reduce palatability until livestock acquire the taste for it and there is no toxicity that has been recorded (Frutos *et al.*, 2004). Tannins are capable of decreasing protein degradability due to their ability to form a complex with protein in the feed and have a potential of reducing the rate of activity of rumen bacteria and to interact with carbohydrates and proteins (Baloyi *et al.*, 2008). Legume yields of 4–7 t/ha and legume/grass yields of 15 t/ha DM have been recorded, with increases of 90–150 kg/ha in soil nitrogen.

Mucuna pruriens (Velvet bean)

Most *Mucuna* species exhibit reasonable tolerance to a number of abiotic stress, including drought, low soil fertility, and high soil acidity, although they grow poorly in cold, wet soils and are sensitive to frost (Campbell-Jones, 2012). Levels of above ground biomass range from 5 to more than 12 tonnes (t) of dry matter (DM)/hectare (ha); below ground, more than 1 t of dried roots per ha may be produced (Campbell-Jones, 2012). In many tropical countries, *Mucuna pruriens* is used as an important forage, fallow and green manure crop. It is a legume and hence, it fixes nitrogen and fertilizes the soil. Velvet bean (*Mucuna* spp.) is a crop that has been increasingly adopted in the tropics to improve the fertility of agricultural soils (Ayala-Burgos *et al.,* 2003). The whole plant is fed to animals as silage, dried hay or dried seeds. *M. pruriens* silage contains 11-23% crude protein, 35-40% crude fibre, and the dried beans 20-35% crude protein. However, Velvet bean grains contain several antinutritive compounds (Buckles *et al.*, 1999). In particular, the content of L-Dopa (3-4-dihydroxy-Lphenylalanine) is high 2.23 to 5.36 % (Ayala-Burgos *et al.,* 2003) in the dry seeds.

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Vigna unguiculata (Cowpea)

Cowpea legume has the potential to function as a key integrating factor in intensifying agricultural systems through supplying protein in human diets and fodder for livestock, as well as bringing N into the farming system through fixation (FAO, 2009). Going beyond its importance for food and feed, Cowpea can be regarded as a fulcrum of sustainable farming in regions characterized by farming systems that make limited use of purchased inputs (Anele *et al.*, 2011). Cowpea is grown widely in sixteen African countries with the continent producing more than three-quarters of the world total with the bulk coming from smallholder farmers in semiarid zones of the region (Anele, *et al.*, 2011). Cowpea proved to be a forage source of high quality for ruminant production, enhancements in milk and meat production could be recognised (Baloyi *et al.*, 2008) if Cowpea forage is supplemented to poor quality hay diets.

2.3 Nutritional composition of legumes

Forage legumes are superior in protein and mineral content (particularly calcium, magnesium, copper and cobalt.) even though their nutritive value falls slightly with age (Baloyi *et al.*, 1997; Dewhurst *et al.*, 2009).

Velvet bean seeds contain 250–350 g crude protein (CP)/kg dry matter (DM) and the whole plant contains 140–190 g CP/kgDM (Topps and Oliver, 1993; Maasdorp and Titterton, 1997; Nyambati, 2002). However, Velvet bean seeds contain secondary compounds such as tannins (3–61 g/kg DM that can reduce DM digestibility and CP utilisation (Makkar *et al.*, 1988; Mangan, 1988). Velvet bean contains l-dopa (23–92 g/kg DM), which can be lethal to monogastrics (Szabo and Tebbett, 2002; Del Carmen *et al.*, 2002). Undesirable symptoms of ingesting l-dopa include weight loss, reduced feed intake and feed conversion efficiency in monogastrics (Carew *et al.*, 2002; Del Carmen *et al.*, 2002), and diarrhoea, skin lesions, vomiting and confusion in humans (Szabo and Tebbett, 2002). However, l-dopa is degraded in the rumen (Chikagwa-Malunga *et al.*, 2008) and those effects have not been observed in ruminants (Sandoval-Castro *et al.*, 2003). Nevertheless, the microbes that degrade l-dopa are yet to be identified.

Maturity influences the nutritive value and secondary compound concentration in seeds, leaves, stems and pods of forage legumes (Maasdorp and Titterton, 1997). Little is known of the maturity stage at which the nutritive value of most of the legumes is optimised. Forage legumes are normally harvested between 110 and 270 days after planting (DAP) (Eilitta *et al.*,

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2002), depending on location, but when grown for forage, plants are usually harvested between 90 and 120 days (Eilitta *et al.*, 2002) after planting.

2.4 Degradability and digestion of forage legumes

Ruminant dietary protein supplies are determined by the requirements of the rumen microorganisms and by the needs of the host animal (Loest, 2006). However, dietary protein needs for the host animal are complex, because ruminal microorganisms alter the quantity and quality of ingested protein prior to reaching the small intestine to be utilised by the animal. In ruminant nutrition there is interest in protein sources that are slowly degraded in the rumen. These relatively slowly rumen degradable protein sources can have special value for lactating ruminants in early lactation and for young growing ruminants (Santos *et al.*, 1984) whose protein requirements are relatively high.

Protein digestion by ruminants falls into two phases namely the microbial action in the reticulorumen and the second involves post-ruminal digestion that occurs principally in the small intestine (van't Klooster and Boekhoh, 1972). Due to dietary protein degradation by rumen microbes and the synthesis of amino acids by rumen microbes from dietary protein sources, specific dietary amino acid requirements are of little importance in ruminant animals (Titgemeyer, 2003).Protein requirements are expressed as crude protein (CP), either in amounts or as a percentage of the dietary dry matter. Crude protein is determined by multiplying the nitrogen content in a feed by the factor 6.25 (feed protein averages 16 percent nitrogen) (Van Soest, 1987). Non-protein nitrogen (NPN) compounds, such as alkaloids, nitrogenous glucosides, lipids or ammonium salts, are considered to have a CP value, but supply no amino acids directly. Micro-organisms in the rumen convert nitrogen from NPN sources into amino acids for their use. Van Soest, (1987) mentioned that digestion of microbial protein in the small intestine releases amino acids for absorption and utilisation, the same as amino acids released from the digestion of true proteins (composed of amino acids) in feeds.

Approximately 60 percent of the CP in a typical ruminant diet is broken down by microbial digestion to ammonia (Volden *et al.*, 2002). The rumen microbes must convert the ammonia to microbial protein if the animal is to receive any benefit. Fermentable energy must be available for the micro-organisms to grow and synthesise the necessary amino acids. If rumen ammonia levels are excessively high, the ammonia is absorbed into the blood and recycled (Oltjen and Beckett 1996) or excreted in urine as urea.

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Feed proteins are hydrolysed into peptides and amino acids by rumen micro-organisms. Most amino acids are rapidly degraded to organic acids, ammonia and carbon dioxide (Oh *et al.*, 2008). The ammonia produced is the primary nitrogenous nutrient for bacterial growth (Kamalak *et al*, 2005). Some species of ruminal bacteria use peptides directly for synthesis of microbial protein. Chalupa (1975) indicated that normally as little as 40 per cent or as high as 80 per cent of dietary protein might be degraded in the rumen and converted into microbial protein. Rumen microbial protein production requires energy (that is derived from simple and structural carbohydrates) and the amount of dietary protein changed into microbial protein must be a critical phase of nitrogen economy of the ruminant (Choi *et al.*, 2002) and should be an important factor in determining whether synchronisation by artificial procedures is necessary.

While amino acids are rapidly deaminated in the rumen in real conditions, the rumen microbial populations derive 25-50 per cent of their nitrogen from sources other than ammonia (Kamalak *et al*, 2005). Oldham, (1981) reported that unaltered amino acids or peptides originate directly from feed protein, from recycled nitrogen to the rumen or from turnover of bacterial and protozoal protein within the rumen.

Ruminal degradation of feed can be reduced by decreasing retention times in the rumen. Factors known to influence this include the level of feed intake, specific gravity, particle size of the diet, concentrate to roughage ratio and rate of rumen digestion (Kamalak *et al*, 2005). The breakdown of proteins by microorganisms, as mentioned earlier, gives rise also to intermediate products such as free amino acids in the rumen. The low concentration of free amino acids in the rumen usually suggest rapid utilisation, but increased concentration after feeding imply that proteolysis occurs faster than does subsequent utilisation of free amino acids (Chalupa, 1975; Choi *et al.*, 2003).

Many rumen micro-organisms require ammonia (breakdown product of protein) for growth and synthesis of microbial protein. Ammonia also may be provided from non-protein nitrogen (NPN) sources such as nitrogenous glucosides, ammonium salts, alkaloids, and other compounds (Chanjula *et al.*, 2004). Rumen microbes convert the ammonia and organic acids into amino acids that are assembled into microbial protein. Excess ammonia is mostly absorbed from the rumen into the blood stream (where it is converted by the liver to urea for excretion or recycled back through the saliva or rumen epithelial wall), but small amounts may pass into the lower digestive tract and be absorbed (Promkot and Wanapat, 2005). Feed protein (that escapes breakdown in the rumen) and microbial protein pass to the abomasum and small intestine for digestion and absorption. The two systems are mutually supportive and

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efficient rumen fermentation ensures that the host animal receives the maximum amount of digestible nutrients from a given feed. The rumen microbes need protein (amino acids, peptides), glucose and minerals, in particular sulphur, potassium and phosphorous, as pre-cursors.

Maximum efficiency will occur when a continuous supply of fermentable carbohydrate is matched with the correct amount of ammonia and amino acids so that microbial protein is formed without waste of the major substrates (microbial cells contain 40 to 60 percent protein on a dry matter basis (Chalupa, 1975; Wanapat and Khampa, 2007).

2.4.1 In sacco and in vitro degradability

The ordinary way to estimate protein degradability in the rumen is to take samples of the feed and put them in porous nylon-bags and place them in the rumen of an animal (sheep, goat, cow or buffalo) that has a rumen cannular (Ørskov and McDonald 1979). The bags are taken out at different times, and the amount of protein that is left in the feed that is still left in the bag is measured. From the amount of protein that is left in the bag at different times, the rate (and extent) of protein degradation can be calculated. Ruminant feed nutritive value is a factor of the concentrations of the chemical components, as well as their rate and extent of digestion (McDonald, 1981; Getachew *et al.*, 2004).

However, the *in vivo* nylon-bag technique of determining digestibility of feeds is laborious, expensive, requires large quantities of feed, and is basically inappropriate for single feedstuffs thus making it not fitting for regular feed evaluation. Furthermore, the *in vivo* method is also open to errors associated with the use of digesta-flow-rate markers, microbial markers, and inherent animal variation (Stern *et al.*, 1997). Therefore, *in vitro* methods can provide less expensive and more rapid alternatives (Lizárraga Sánchez, 2000) in this situation.

There are a number of *in vitro* methods available for evaluating the nutritive value of feeds at affordable cost. The use of *in vitro* gas production methodology to estimate digestion of feeds depend on on measured associations among the *in vivo* digestibility of feeds and *in vitro* gas production, in relative to the feed's chemical composition (Menke and Steingass, 1988). *In vitro* gas methods primarily measure digestion of soluble and insoluble carbohydrates, and the total gas produced from a feed on incubation reveals production of volatile fatty acids (VFA), which are the main source of energy for ruminants (Menke and Steingass, 1988). The gas produced is from microbial degradation of feeds, and indirectly from buffering of acids generated (Getachew *et al.* 2004) as a result of fermentation.

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2.4.2 *In vitro* Daisy^{II} technique

In vitro procedure developed by Tilley and Terry (1963) has extensively existed as an accurate *in vitro* technique for predicting diet digestibility (Stern *et al.*, 1997; Earing *et al.* 2010). However, a more efficient alternative to the Tilley and Terry method has been developed using the Ankom Daisy^{II} incubator (Ankom Technology Corp., Fairport, NY, USA). Studies done by Wilman and Adesogan (2000) evaluated the two techniques and established that the Daisy^{II} system presented faintly less accurate estimation of ruminant *in vivo* digestibility. Moreover, Holden (1999) established that the two systems are comparable. Daisy equipment involves enclosing the feed sample in a filter bag placed in a common rumen fermenter which can digest up to 100 samples at one time compared with 40 in the Tilley and Terry systems. The Daisy^{II} equipment permits bunch handing, removes the filtration step and retains uniform heating with regular shaking. Earing *et al.* (2010) elucidated that rumen digesta is shaken continuously (Daisy), compared with twice daily and the buffer preparation and the rumen fluid: buffer ratio differs for each method.

2.4.3 *In vitro gas technique*

The *in vitro* rumen gas technique is capable of being used to study the nutritional value of feed ingredients at the same time on mixed rations (Moss, 2002). The system was effectively used to measure the impact of grain processing on starch fermentability, the influence of lipids and fatty acids on microbial fermentation, to evaluate empirical equations to estimate the ME content of feeds and to measure methane production from diets fed to lactating cows (Getachew *et al.*, 2005). Sandoval-Castro *et al.*, (2000a) pointed out that the *in vitro* rumen gas technique offers a unique tool for researchers to address a wide range of nutritional issues in ruminants.

Menke *et al.* (1979) introduced the method of assessing *in vitro* degradability of forages by the gas technique. Moreover, Menke and Steingass (1988) demonstrated that gas production of forages during *in vitro* fermentation with rumen micro-organisms, is an indirect measure of their digestibility and metabolisable energy content. Later on Theodorou *et al.* (1994) initiated an uncomplicated gas production technique (PTT), using a pressure transducer, to measure the fermentation kinetics of forages incubated *in vitro* with rumen microorganisms and this method has a potential to evaluate the rumen fermentation characteristics of large numbers of samples at a given time.

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In the gas technique strong correlation was reported between digestibilities measured *in vivo* and that predicted from an *in vitro* rumen gas production technique in combination with chemical composition (Menke *et al.*, 1979). However, a significant amount of research has used *in vitro* rumen gas techniques to estimate digestibility of feeds, study effects of secondary compounds on rumen microbial activity, assess kinetics of fermentation, study associative effects of various types of feeds, examine influences of feed additives on rumen fermentation, study partitioning of fermented substrates into fermentation products and assess the composition of gases from fermentation of various feeds ((Sandoval Castro *et al.*, 2000b; Getachew *et al.*, 2005).

The technique used for the *in vitro* rumen gas production is equivalent in theory, but there are close expected differences associated with the path of implementing the procedure by any laboratory (Parr, WH. 1988). These are associated with the differences of the species of the rumen fluid donor animal, and diet composition, which can manipulate the degree of microbial production in the rumen fluid used for inoculating the *in vitro* system (Getachew, 2002). Above all, Lizárraga Sánchez, (2000) observed that the gas technique permits the evaluation of the effects of feed additives and feed supplements on digestibility of feed fractions and rumen microbial activity.

2. 5 Microbial protein yield and purine derivatives technique

Microbial production in ruminant livestock is usually estimated by the amount of urinary purine derivatives (PD) excretion (Liang *et al.*, 1994). Urinary purine metabolites (allantoin, oxypurines: hypoxanthine, xanthine and uric acid) have been used as markers of microbial protein synthesis (Czauderna *et al.*, 2004). The theory behind this microbial marker is that duodenal purine bases (PB), are efficiently absorbed in the gut and the bulk of the purine derivatives are excreted through the kidney. The technique is an alternative, non-invasive method based on the principle that purine metabolites are derived mainly from the nucleic acids of microorganisms flowing out from the rumen (Gonzalez-Ronquillo *et al.*, 2003; Kowalczyk *et al.*, 2003). However, the relationship between the urinary excretion of allantoin and duodenal supply of nucleic acids is usually obscured by an endogenous fraction coming from the turnover of nucleic acid in tissues and incomplete urinary recovery of purines (Orellana-Boero *et al.*, 2001). Therefore, the amount of PD can be used to predict microbial protein flow because the proportion of PD in urine directly reveals microbial protein flow (Belenguer *et al.*, 2002). Nevertheless, the purine derivative and the flow of purine bases between urinary sample differs among species hence response models are species specific

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(Chen *et al.*, 1990). There are different models used to estimate rumen microbial protein synthesis among different species due to the differences in the enzyme activities of uricase and xanthine oxidase involved in the purine metabolism in the intestinal mucous, in the liver and plasma (Carro *et al.*, 2008). It is advantageous to extend the purine derivative technique to other species like the goat (*Capra hircus*) (Stangassinger *et al.*, 1995) which is a livestock of economic significance.

Mota *et al.*, 2008 in an experiment using both goats and sheep reported no differences between animal species in total PD excretion, although goats showed a greater excretion of allantoin and decreased excretion of xanthine and hypoxanthine compared with sheep. Moreover, differences between goats and sheep were more marked in the medium-quality diet contrasted to those of high-quality diet.

Duodenal input and urinary output

Gonza´lez-Ronquillo *et al.*, (2004) observed a positive correlation between milk yield and urinary PD excretion. The increase in rumen microbial yield observed implied an increase in the nutrient supply to the host animal and subsequently an increase in milk yield. Belenguer *et al.*, (2002), reported a correlation between milk yield and duodenal PB flow to have a precise estimation of the variation in the duodenal PB flow when RNA was infused into the duodenum. Moreover, a similar relationship was observed between duodenal PB and urinary PD in goats and in sheep in a study done by Belenguer *et al.*, (2002).

A linear association between duodenal input and urinary output has been observed in several studies using different species of ruminants, milking cows (Gonza´ lez-Ronquillo *et al.*, 2004, dry goats (Belenguer *et al.*, 2002), steers (Orellana-Boero *et al.*, 2001) and sheep (Balcells *et al.*, 1991). Verbic *et al.*, (1990) and Orellana-Boero *et al.*, (2001) reported a urinary recovery of PD in lactating goats which was in the range observed in cattle (0.75 to 0.8). Chen *et al.*, (1990) and Balcells *et al.*, (1991) observed urinary recovery of PD in sheep to be in the range 0.7 to 0.8. Guerouali *et al.*, (2004) reported urinary recovery of PD in camels to be 0.72 whilst Pimpa *et al.*, (2003) observed the recovery in buffaloes to be 0.11.

Studies have reported similar recoveries in lactating goats and in dry goats (0.74; Belenguer *et al.*, 2002); thus, the metabolic changes that occur during lactation will not alter the metabolism of exogenous PB. Therefore, in goats a common coefficient could be used regardless of the physiological state of the animals. Distinctively in cattle, lactation has effects

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on the recovery of duodenal PB as the recovery proportions is significantly lower during peak lactation compared to late lactation (Gonza´lez-Ronquillo *et al.*, 2004).

2.6 Efficiency of utilisation

The term efficiency implies a ratio of outputs to inputs. Live weight gain and daily dry matter feed intake are typically used to measure ratio based feed efficiency traits like gross feed efficiency (NRC, 2001). However, little emphasis has been placed on assessing efficiency of utilisation of forage legumes on milk production in goats which is defined in broad sense as the relationship between the daily volume of produced milk and the daily dry matter intake (de Oliveira *et al.*, 2014). Decreasing the cost of feeding depends upon improvements in the conversion of the feed to milk nutrients as observed by Linn, (2006).

Determination of the efficiency of utilisation of feeds (in particular protein) in ruminants is more complicated compared to monogastrics. This is because amino acid profiles available for absorption in the small intestine (metabolisable protein) reflect mainly the combination of microbial protein, ruminally undegradable protein, and endogenous proteins (Löest, 2006). A study by Chen *et al.*, (1987) investigated the effect of dietary proteins on the peptide flow from the rumen of lactating dairy cows. When the cows were fed a forage diet with a soybean meal supplement, the estimated peptide flow from the rumen was over 200 g/d, implying that at this rate of flow, peptides escaping ruminal degradation could have accounted for more than 25 per cent of the milk protein synthesised by the cows.

Broderick and Wallace (1988) indicated that the free amino acids and peptides can account for only 1.2 to 2.4 per cent of the total NAN flow to the duodenum. However, discrepancy in diets, animals, and the analytical techniques used could be the reasons for the above divergence in the duodenal flux of peptides and amino acids. However, the effect of diet on the amount and quality of peptides and free amino acids flowing out of the rumen is yet to be established.

Free amino acids are the major form in which nitrogen is absorbed in ruminants (NRC, 1985). The distal section of the small intestines (ileum) is the principal site of amino acid absorption in ruminants (Webb and Matthews, 1994). Cook *et al.* (1965), using both *in vitro* and *in vivo* procedures, demonstrated the ability of the ruminant fore stomach epithelial tissue to absorb free amino acids. The study demonstrate that the glycine can be absorbed through the fore stomach epithelial tissue.

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Cook *et al.* (1965) also conducted *in vivo* experiments to investigate absorption of amino acids across the rumen using two catheterised goats and a steer. When the goats were either force fed ground shelled corn or when glycine was infused into the rumen, the blood glycine concentration in the ruminal vein increased markedly and remained higher for a considerable period of time. In the goat that was infused with glycine into the rumen, the blood glycine concentration in the ruminal vein exceeded that of the jugular vein after feeding. A clear difference was evident after 2h but was no longer so after 3h. Correspondingly, when a mixture of amino acids (serine, aspartic acid, methionine, threonine, isoleucine, glutamine, asparagine and leucine) was infused in the rumen of a steer, the concentrations of these amino acids in the ruminal vein increased to different levels. Consequently individual amino acids appear to be absorbed across the rumen in a different manner. However, the study was criticized, as the original concentrations of amino acids used may not occur under normal physiological conditions in the rumen.

When Leibholz (1971) investigated the absorption of histidine across ruminal epithelial tissues (*in vitro*), a greater uptake of L-histidine than D-histidine across ruminal epithelium suggested that the absorption was associated with factors other than simple diffusion (Korhonen *et al.*, 2000; Huhtanen *et al.*, 2002;). The increase in histidine uptake in the presence of glucose or volatile fatty acids (VFA) and the inhibition in histidine uptake in the presence of iodoacetate and 2-4-dinitrophenol implied that energy was required for this process. In addition, the transfer of L-histidine across the ruminal epithelium was reduced by 10 to 50 per cent in the presence of other amino acids, signifying that a common mediated transport mechanism could be involved (McLaughlin, 2002) in the absorption of these amino acids.

Methionine and Lysine have been identified repeatedly as the two most limiting amino acids for lactating dairy cows (Noftsger and St-Pierre, 2003; Noftsger *et al.*, 2005; Socha *et al.*, 2005; St-Pierre and Sylvester, 2005; Ordway *et al.*, 2009; Doepel and Lapierre, 2010 and 2011; Appuhamy *et al.*, 2011; Chen *et al.*, 2011). Matthews and Webb (1995) studied the absorption of methionine and methionylglycine via ruminal and omasal epithelia of sheep using parabiotic chambers. Serosal appearance and tissue accumulation of 35S-methionine and 35S-methionylglycine was quantified over a period of 240 minutes. The quantity of methionine transferred was linearly dependent on time, being greater across omasal than ruminal epithelia, and was greater than methionylglycine. Transepithelial passage of methionine was non-saturable between substrate concentrations of 0.375 to 12 m*M*. Hence they assumed that the absorption of methionine across ruminal and omasal epithelia occurred by non-mediated mechanisms.

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Sloan, (2005), Schwab *et al.* (2009) and Whitehouse *et al.* (2009) showed that lysine is a limiting amino acid in milk production in dairy animals. McCollum (1996) investigated the absorption of lysine across ruminal and omasal epithelia of wethers using parabiotic chambers. The appearance of lysine (using 3H-Llysine as a representative marker) in the serosal buffers increased linearly with time for both tissues during a 60 minute incubation. When the absorption was expressed on a tissue dry weight basis, more lysine absorption was observed in omasal tissues than in ruminal tissues. The tissues responded differently to increases in the mucosal concentrations of lysine. The serosal appearance of lysine across ruminal epithelia increased proportionally as the lysine concentration increased in the mucosal buffers.

The efficiencies of essential amino acid utilisation for non-ruminants vary from 64 % (tryptophan) to 96% (lysine) (Fuller, 1994). Oldham and Alderman (1982) calculated the efficiencies of metabolisable protein utilisation for growth and the results range from 27 to 75%. Furthermore, Lobley (1992) reported that efficiency of amino acid utilisation for growth may vary from 40 to 80%, while Gerrits *et al.* (1998) reported efficiencies of amino acid use for protein deposition in preruminant Holstein calves that ranged from 13% for methionine to 51% for arginine. The efficiency with which amino acids are utilised for tissue deposition varies depending on whether that amino acid is first-limiting and whether there is a demand for that specific amino acid in various metabolic processes. Therefore, most of the efficiency values reported for a specific amino acid are dependent on the most limiting nutrient (energy or another amino acid). An amino acid is utilised less efficiently when a nutrient other than that amino acid limits protein synthesis (Löest, 2006) compared to when that amino acid is first limiting.

Santos *et al.* (1984) used diets containing soybean meal (SBM), corn gluten meal (CGM), wet brewers grains (WBG) and distillers dried grains with solubles (DDG) and concluded that diets containing CGM, WBG or DDG will generally supply more total amino acids to the intestine than a diet containing SBM. Net amounts of amino acids available for absorption in the intestine were also higher for these diets because protein availability in the intestine was not impaired even though protein was more resistant to microbial breakdown in the reticulorumen.

It is also essential to note that retention of N into animal products (milk, meat and wool) is generally low in small ruminants, ranging between 5% and 20 % (Decandia *et al.*, 2012).

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2.7 Milk production

Numerous trials had been undertaken to improve utilisation of dietary nutrients by feed supplementation with various additives from different sources (Abd El-Latif *et al.*, 2004; Ahmed *et al.*, 2008; Ordway *et al.*, 2009; Doepel and Lapierre, 2010). However, these studies have revealed that the use of tree and shrub forages as supplements to different basal diets lead to improved ruminant livestock performance in terms of milk production, growth rate and reproductive efficiency (Ondiek *et al.,* 2000; Baloyi *et al.*, 2008). Legumes (Leuceana, Alfalfa, Clitoria and Velvet bean) are recommended for soil fertility improvement (Saha *et al.*, 1997), due to their high nitrogen content. They can also be used as nitrogen supplements in ruminant rations (Dewhurst *et al.,* 2009). Castillo *et al.*, (2001) postulated that supplementation of readily degraded energy will enhance the utilisation of available N in the rumen and further improve the productivity of goats through an increased efficiency of utilisation of ammonia-N for microbial protein synthesis. Moreover, Nyambati *et al.*, (2003) showed that milk production can be increased by supplementing with Velvet bean, highlighting the importance of integrating legumes into the low-input, mixed cropping systems in the tropics. Legumes can be supplemented to ruminants on low quality forages such as maize stover (Muinga *et al.*, 2003) and tropical grass hays.

Lactating dairy goats require forage to maintain a normal milk fat content. In addition to the quantity of forage consumed, the particle size, type of forage, fermentation rate, and surface exchange properties influence rumination, milk yield, and milk fat content. Milk fat decrease can be observed in high producing dairy goats during early lactation. A high forage ration results in higher chewing activity which increases salivation and favours the growth of cellulolytic microbes and production of acetic acid. When acetate to propionate ratio increases in the rumen it promotes the synthesis of milk fat, as acetate is the major precursor of milk fat.

Requirement of forage with high fibre to maintain milk fat content was confirmed by feeding diets rich in starch (15 vs. 43 % NDF or 6 vs. 25% ADF) that resulted in milk fat decrease in multiparous Alpine and Sannen goats (Schmidely *et al.*, 1999). However, Sanz Sampelayo *et al.*, (1998) in a research using Granadina goats in lactation, reported that the physical form of feed (pelleted alfalfa vs long hay) showed no effect on milk production (1.31 vs 1.35 kg/day) and milk fat content (6.7 vs. 6.3 %). There were no relationships among dietary forage intake, chewing activities and milk fat content. Santini *et al.*, (1991) in a study using large subjects of multiparous does illustrated an apparent dietary fibre intake effect on milk fat content, accompanied by a linear surge from 4.85 to 5.40% as the intake of dietary acid detergent was increased from 396 to 839 g/d.

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2.8 Digestion Kinetics

In lactating ruminants, dry matter intake (DMI) is limited by physiological parameters during high concentrate feeding and by physical factors during high forage feeding. Increase in dietary fibre content reduces DMI in goats. Increase in forage as to concentrate ratio from 45:55 to 45:55 decreased total daily DMI from 84.3 to 59.8 g/kg $BW^{0.75}$ (Kawas *et al.*, 1991). Santini *et al.*, (1991) observed that DM intake peaked when goats were fed a diet containing 18% ADF with 2.30 Mcal/kg ME and there was a slight reduction in DMI when dietary ADF was higher than 18%. Moreover, when dietary ADF was lower than 18%, there was a decrease in DMI. There was a linear decrease in DMI with increasing dietary NDF (52.4 to 62.1 %) in Boer goats and crosses (Luginbuhl *et al.*, 2000). High fibrous forages ferment and pass from the reticulorumen more slowly than other dietary components (Allen, 1996); they have greater filling effect over time.

In lactating goats physiological DMI regulation happens due to dietary energy density. Illius and Jessop, (1996) observed that microbial and ammonia recycling and the hind-gut fermentation contribution decrease the asynchrony in the balance of nutrients absorbed into the bloodstream. Lu *et al.*, (1991) used a stepwise regression analysis and reported dietary energy density, body weight and milk yield as the highest three variables which accurately estimate dry matter intake.

Measurements of digestion kinetics are useful for the clarification of the effect of diet on intake and digestibility of nutrients. However, data on goats are to some extent limited.

2.9 Nutrient utilisation kinetics

A lot of ruminant nutrition systems are currently being used to predict feed biological values (National Research Council's nutrient requirements of small ruminants (NRC, 2007); Agricultural and Food Research Council (ARC, 1980; AFRC, 1998); Institut National de la Recherche Agronomique (INRA, 1989, 2007). The calculated feed biological values aid in the evaluation of efficiency of different feeds under specific utilisation (Herrero *et al.*, 2000). In contrast, mechanistic models can provide more accurate generalizations, based on biologically appropriate contributory relationships. In dynamic models, these associations consist of flows and feedback loops linking biological compartments.

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Small Ruminant Nutrition System (SRNS) is the result of a joint collaboration among Sassari University (Italy), Texas A&M University, and Cornell University. A Small Ruminant Nutrition System is used to predict feed biological values based on carbohydrate and protein fractions and their ruminal degradation rates, concentrate, forage and liquid passage rates, physically effective fibre and microbial growth (Cannas and Van Soest, 2000; Cannas *et al.*, 2007a). The system was mathematically designed to predict feed biological value when feeding sheep and goats and it uses animal and environmental factors to predict energy, protein, calcium and phosphorus requirements (Tedeschi *et al.*, 2005). Cornell Net Carbohydrate and Protein System for sheep (CNCPS-S) structure (Cannas *et al.*, 2004; 2006) was used to develop the Small Ruminant Nutrition System model. The Small ruminant nutrition system mechanistics were tailored to explain protein and energy requirements of goats and sheep reared under different practical conditions (Cannas *et al.*, 2000; 2008a).

Accuracy of the SRNS was evaluated by Cannas *et al.*, (2007a) and observed that the system is able to predict accurately and precisely the daily MEI of goats (root of the mean squared error of prediction, RMSEP, =0.24 Mcal/d; r^2 =0.99; concordance correlation coefficient, CCC, =0.99). However, the SRNS weakness is on under prediction of energy balance of lactating goats (RMSPE of 0.40 Mcal/d; mean bias of -0.31 Mcal/d and r^2 of 0.79). Moreover, Cannas *et al.* (2006) reported that the SRNS under predicted growth in growing lambs. Conversely, mean and the systematic bias were very small when SRNS were evaluated for growing kids Cannas *et al.* (2007b) with goat breeds for milk, meat, and indigenous.

According to Pulina *et al.*, (2013), based on thermodynamic view, it is clear that it is more convenient to raise small ruminants for milk production than for meat. However, milk production is very difficult or even impractical in many areas of the world where the environment is severe or the forage quality is poor and availability variable.

Small ruminants are normally fed *ad libitum* whether they are reared intensively, with the amount of feed available controlled directly by the farmer, or extensively, with the availability of herbage from pasture or foliage from bushes varying according to geographic and climatic conditions. Voluntary feed intake can be limited due to supply and the animals cannot ingest enough feed to meet their nutritional requirements. In this case, production and reproduction levels can be maintained only by using supplements such as hay, silages and concentrates.

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2.10 Summary

Transformations of nitrogenous feed components occurring in the reticulo-rumen and their digestibility have been widely studied. However, no quantitative results are available on the coefficients of utilisation of legumes in the resource-poor goat production sector. Most research on protein utilisation has focused on intensive livestock farming systems, based on dairy and beef cattle, whereas sheep and goats have received little attention, despite the fact that they constitute around 52% of the world population of domesticated ruminants (FAO-STAT, 2011). There is the need to evaluate the nutritive value of selected legumes in order to predict animal performance in terms of N retention and milk production using indigenous goats. Therefore, this study is aimed at testing the hypothesis that degradability, digestion and utilisation of CP in different forage legumes may exhibit the same efficiencies.

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RUMEN DEGRADABILITY PATTERNS OF THE SELECTED LEGUMES USING THE ANKOM^{RF} GAS TECHNIQUE

3.1 Abstract

The Ankom^{RF} gas production technique was used to assess rumen degradability patterns of *Mucuna pruriens* (Velvet bean), *Vigna unguiculata Walp (L)* (Cowpea) and *Desmodium uncinatum* (Silverleaf desmodium) forage legumes. Forage samples of 1.0 g DM^{-1} were placed into 250 ml glass jar and incubated at 39 ^{0}C over 72 hours. Rumen liquor that was taken from 2 fistulated Friesian cows on a dairy ration was mixed with saliva in the ratio of 4:1. Gas production was measured using the Ankom^{RF} gas technique. Most but not all of the gas in the 3 forage legumes was produced within the first 48 hours post-incubation. The volumes of gases produced were significantly different (P <0.05) and ranged from 61.4 to 70.4 ml, with an average of 64.2 ml, whilst the fractional rate of gas production varied between 4.6 and 5.6% h^{-1} with a mean of 5% h^{-1}. Cowpea produced significantly the highest amount of gas followed by Velvet bean (P <0.05). Therefore, the selected forage legumes are degradable in the rumen.

Keywords: Ankom^{RF} gas production; *Desmodium uncinatum; Mucuna pruriens; Vigna unguiculata Walp (L);* Rumen degradability.

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3.2 Introduction

Protein and energy are considered as basic feed components that are expensive ingredients of ruminant feed due to the continuous increase in their price. However, protein supplements are integral component of animal diets and are required to increase the total quantity of protein as well as satisfy essential amino acid requirements (Mustafa and Alamin, 2012). Understanding the rate and extent of ruminal degradation of various protein sources is important for formulating diets to supply enough rumen degradable protein to optimize rumen microbial growth and microbial protein to support the host. In order to improve protein utilisation in ruminant feed, protein should be divided to its components to distinguish, the quantities of protein degraded in the rumen and the amount undegraded from the rumen and absorbed in the gut (Rezai *et al.,* 2012).

The gas method was selected for this study because it allows study of the effects of feed additives and feed supplements on digestibility of feed fractions and rumen microbial activity (Getachew *et al.,* 2001). Fermentation kinetic parameters may be derived using mathematical functions to estimate the fermentation rate, with the major assumption that the rate at which gas is produced is directly proportional to the rate at which substrate is degraded (France *et al.,* 2000). The gas-production technique has proved to be efficient in determining the nutritive value of feeds containing anti-nutritive factors, positively related to intake, microbial protein synthesis and *in vivo* digestibility (Ndlovu and Nherera, 1997). Both *in vitro* gas production and the ANKOM devices can be used as rapid evaluation tools to assess nutritional quality of feeds. Therefore, this study was designed to estimate rumen degradability patterns of tropical forages *Mucuna pruriens* (Velvet beans), *Vigna unguiculata* Walp (L), (Cowpea) and *Desmodium uncinatum* (Silverleaf desmodium) using the Ankom^{RF} gas production technique.

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3.3 Materials and methods

3.3.1 Forages

Velvet bean, Cowpea and Silverleaf desmodium legumes were grown in 2012/13 summer at the University of Venda Experimental farm (22° 56' 60S and 30° 28' 60E; altitude 709 m); (752 mm of rainfall per year, with most rainfall occurring mainly during mid-summer; average minimum temperature 7.5°C and maximum 30.3°C). During the rainy season land of approximately 3.0 hectares was ploughed and disked for sowing the Velvet bean, Cowpea and Silverleaf desmodium legumes.

Pure stands of the legumes were planted between 10th and 28th January 2013 on red clay soils. Each legume was sown on about 1.0 ha of land. The inter-row spacing for Silverleaf desmodium was 60 cm at a seeding rate of 1.5 kg ha^{-1}. Cowpea was planted at a seeding rate of 30 kg ha^{-1} with the inter-row spacing of 50 cm. Velvet bean was planted at 20 kg ha^{-1} at an inter-row and intra-row spacing of 60 x 60 cm. There was no fertilization applied and weeding was done twice by hand hoes.

Velvet bean and Cowpea were harvested when about 70 per cent of the pods had ripened, whilst Silverleaf desmodium was harvested in May after flowering. The legumes were harvested during the same range of days after planting. The whole plant was harvested and air dried under shade. The forages were later milled through a 25 mm screen and stored in hessian bags. The legumes were further milled to pass through a 2 mm sieve before carrying out the Ankom^{RF} gas technique.

3.3.2 Animals and feeding

Two mature Holstein cows (mean weight 600kg) fitted with rumen cannulae of 8 cm internal diameter were used to determine *in sacco* Nylon-Bag and *in vitro* Daisy^{II} degradability of Velvet bean, Cowpea and Silverleaf desmodium. The two cows were fed a milk ration (19% CP dairy meal and maize silage) 30 days prior to the beginning of the degradability trials.

3.3.3 Experimental procedure

The Ankom^{RF} gas production technique was used for the incubation (Ankom^{RF}, 2008) at Agricultural Research Council Irene in South Africa. Rumen liquor was collected from two cannulated cows and forage samples of 1.0 g/DM of milled Velvet bean, Cowpea and

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Silverleaf desmodium were placed into 250 ml glass jar and incubated at 39°C over 72 hours. The buffer (saliva) and the rumen liquor were mixed in the ration 4:1 respectively under anaerobic conditions. The ratio of rumen liquor and saliva was chosen such that microbial activity is adequately high that it does not have an effect on rate and level of fermentation. Gas productions were corrected for blank gas production using the three blanks (rumen fluid + buffer solution) that were incubated at the same time.

In the gas technique every experimental component consisted of a 250 ml glass jar with attached module top. The Ankom^{RF} module tops used contained the communication system of a radio frequency wireless system that monitored gas pressure, allowed the discharge of gas formed for the period of the fermentation and sustained a low positive pressure in the bottle headspace, thereby avoiding the entrance of air (Tagliapietra *et al.,* 2011). However, the gas that accumulated in the headspace of bottles was automatically released when the pressure inside the units reached 6.9 kPa above ambient pressure. The monitor measured pressure every 5 minutes.

Gas volume was calculated in ml at 39°C with pressure measured in psi (Ankom^{RF}, 2008):

$V_x = V_j P_{psi} \times 0.068004084$

Where:

V_x= gas volume at 39°C in ml;

V_j= headspace of digestion jar (Glass Bottle) in ml;

P_{psi} = cumulative pressure recorded by Gas Monitor System Software.

Time specific gas production was calculated using the equation of McDonald (1981) with the modification of Wulf and Südekum (2005), which assumes that no degradation occurs during the lag phase as done by Edmunds *et al.,* (2012):

Effective gas production (ml/g DM) = $a + (bc/(c + Kp))\ e^{-KpL}$ (3)

Where:

Kp is the rate of gas production,

a is the soluble fraction,

b is the insoluble but potentially rumen degradable fraction

(calculated as *d* - *a*, where is the potentially ruminal degradable fraction),

c is the rate constant of disappearance of fraction *b* and *L* is the lag phase.

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Effective gas production were estimated at assumed passage rates (Kp) of 0.02, 0.04 and 0.06/h (Kp2, 4, 6) to represent low, medium and high feeding amounts and typical rates of ruminal solid outflow for forages.

3.3.4 Chemical analyses

The dried materials were ashed at 550 ^{0}C for 6 h in a muffle furnace to measure ash content. Crude protein content was determined by the Kjeldahl procedure (AOAC 2000). Modified acid detergent fibre and crude fibre were analysed using the procedure outlined by AOAC, (2000). Calcium (Ca) and phosphorous (P) content were determined using the spectrophotometry (Danovaro 2009) and EDTA (Kaur 2007). Condensed tannins were analysed as tannic acid equivalent in the three forage legumes according to the method of Terrill *et al.*, (1992).

3.3.5 Statistical analysis

Proc NLIN of SAS (2013) was used to calculate the gas production parameters. Analysis of variance was performed on gas production kinetics with forage species and incubation period as the sources of variation. The statistical significance of the differences between means was tested using the PDIFF. The General Linear Model of SAS (2013) was used:

$Y_{ijk} = ì + B_i + I_j + (B \times I)_{ij} + e_{ijk}$

Where:

Y_{ijk} = gas production

ì = overall mean;

B_j = legume effect;

I_j= incubation period

$(B \times I)_{ij}$ = interaction between incubation and legume;

e_{ijk} = random residual error.

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3.4 Results

Chemical composition of the three legumes (Table 3.1) showed that Cowpea had significantly the highest amount of crude protein ($P < 0.05$) followed by Silverleaf desmodium then Velvet bean. However, Silverleaf desmodium contained significantly the highest levels of DM and tannins whilst Velvet bean had the least DM and the Cowpea with the lowest tannins level. Velvet bean had the highest level of MADF and Cowpea with the lowest. Velvet bean had significantly the highest level of ash compared to the rest of the legumes. Silverleaf had the highest amount of calcium whilst Cowpea had the highest amount of phosphorus compared to the rest of the legumes ($P < 0.05$) Gas production from the three legumes are summarised in Figure 3.1. A common feature of the gas accumulation profiles was the presence of a gas production curve trend which provided the use of a simple exponential form by Ørskov and McDonald (1979) suitable for their description. Most but not all of the gas in the 3 forage legumes was produced within the first 48 h post-incubation. The volumes of gases produced ranged from 61.2 to 70.4 ml, with an average of 64.2 ml, whilst the fractional rate of gas production varied between 4.6 and 5.6 % h^{-1} with a mean of 5 % h^{-1} (Figure 3.1). Velvet bean, Cowpea produced the highest amount of gas followed by Velvet bean. However, Silverleaf desmodium produced gas at a higher rate than Velvet bean up to 48 hours of incubation. Effective gas production, (a), (b) and (c) were significantly different for the three legumes ($P < 0.05$) Table 3.2.

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Table 3.1: Chemical composition, g/kg dry matter (DM) of *Mucuna pruriens* (Velvet bean), *Desmodium uncinatum* (Silverleaf desmodium), and *Vigna unguiculata* (L.), Walp (Cowpea)

Feed	DM	CP	P	Ca	ASH	CF	MADF	Tannins
	g kg^{-1}							
M. pruriens	804^{c}	148^{c}	2.0^{c}	2.4^{c}	121^{a}	253^{a}	390^{a}	4.4^{b}
D. uncinatum	964^{a}	163^{b}	3.0^{b}	4.8^{a}	46.1^{c}	228^{b}	270^{b}	11.2^{a}
V. unguiculata	855^{b}	180^{a}	3.2^{a}	3.8^{b}	88.1^{b}	204^{c}	225^{c}	1.2^{c}
SEM	1.1	0.79	0.06	0.11	0.72	0.45	3.36	0.09

DM = dry matter, CF = crude fibre, MADF =modified acid detergent fibre, P= phosphorus, Ca= calcium; Upper case letter within the same column indicate significant difference ($P < 0.05$); SEM standard error of the mean

Table 3.2: Equation terms for gas production of *Mucuna pruriens* (Velvet bean), *Desmodium uncinatum* (Silverleaf desmodium), and *Vigna unguiculata* (L.), Walp (Cowpea) in ml g^{-1} DM incubated

	Degradability constants			Effective degradability		
Sample	a	b	c	0.02	0.04	0.06
M. pruriens	1.75^{c}	59.2^{b}	0.046^{c}	42.8^{c}	33.2^{b}	27.2^{c}
D. uncinatum	3.13^{a}	55.81^{c}	0.056^{b}	48.46^{a}	30.0^{c}	31.6^{a}
V. unguiculata	2.45^{b}	64.8^{a}	0.049^{a}	44.2^{b}	35.6^{a}	30.0^{b}
SEM	1.03	3.02	0.006	1.88	1.8	1.32

Superscript letter within the same column indicate significant difference ($P < 0.05$) standard deviation SEM standard error of the mean

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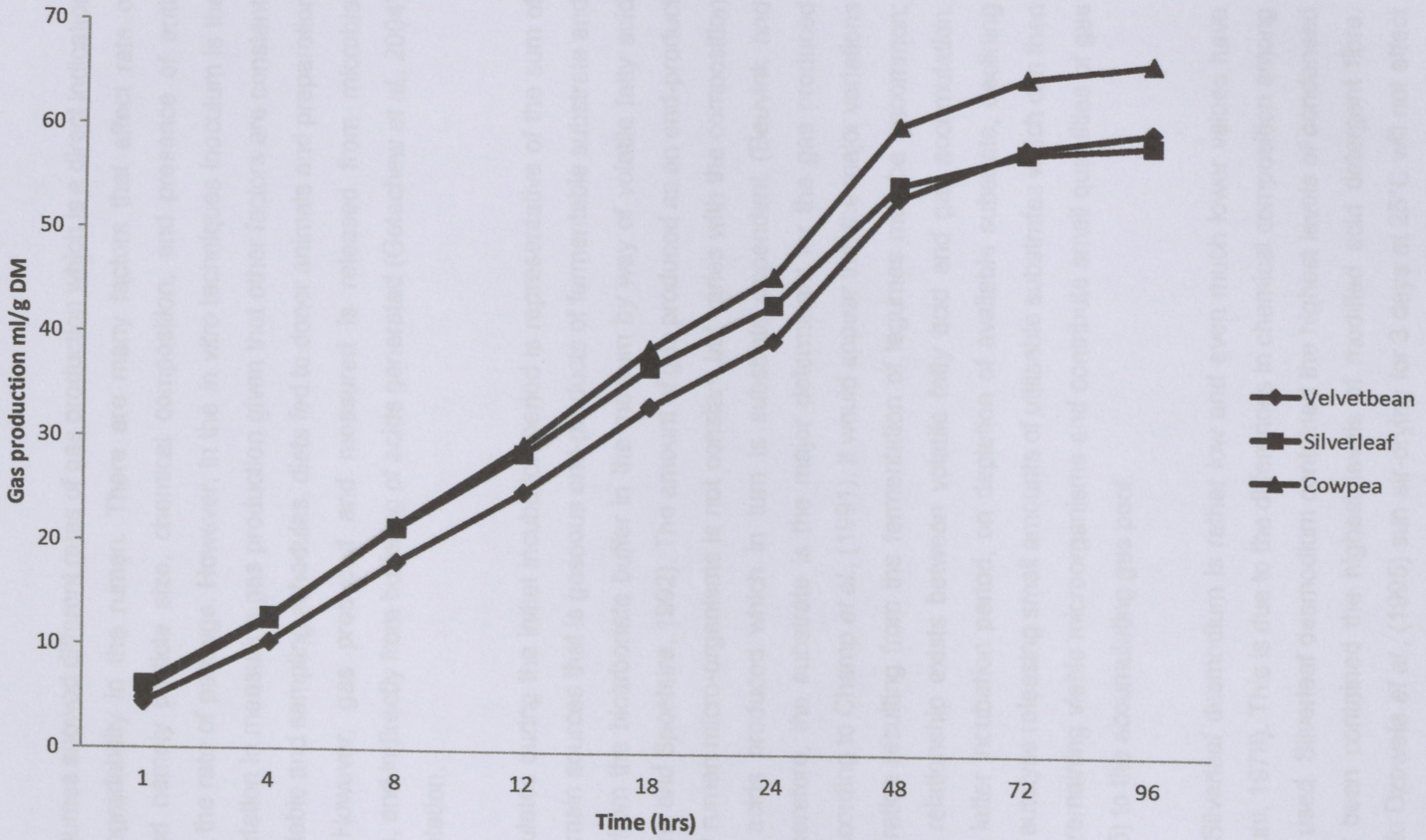

Figure 3.1: *In vitro* gas production characteristics (ml/g DM incubated) for Mucuna pruriens (Velvet bean), Vigna unguiculata (Cowpea) and Desmodium uncinatum (Silverleaf desmodium)

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3.5 Discussion

The three forage legumes showed different rates of gas production which is a direct indication of their rates of degradability in the rumen. There are many factors that affect rate of degradability of feed namely particle size, chemical composition, and presence of anti-nutritional factors to the rate of passage. However, in the *in vitro* techniques inoculum is the largest source of variation in measuring gas production given that other factors are constant, as rumen fluid is variable and sampling schedules, diets fed to donor animals and preparation of the inoculums. However, gas produced and measured is released from microbial degradation of feeds, and indirectly from buffering of acids generated (Getachew *et al.,* 2004) as a result of fermentation.

The gas that accumulates during the initial incubation period is representative of the sum of gas output from two main sources that is gaseous end-products of fermentable substrate and the release of CO_2, from the bicarbonate buffer in the medium by way of volatile fatty acid neutralisation (Beuvink and Spoelstra, 1992). The amount of gas produced as an end-product from fermentation by rumen micro-organisms is not constant but varies with the composition of the volatile fatty acids produced which in turn is substrate dependent (Beuvink and Spoelstra, 1992). Therefore, the substrate is the major determinant for the gas produced during incubation. According to Champ *et al.,* (1991), it would appear that the major variations in volatile fatty acid ratios resulting from the fermentation of legumes may be uncommon; indeed a very close relationship exists between volatile fatty acid and gas accumulation. However, during the latter incubation period, on depletion of available substrate, declining microorganisms may autolyse releasing small amounts of utilisable substrates which can then be fermented by the remaining viable microorganisms and contribute small quantities of gas (Longland *et al.,* 1995) to the accumulating gas pool.

The degradability of Silverleaf desmodium is rather low and even much lower values have been recorded (Mtimuni, 1978). This is due to the differences in chemical composition among all the three legumes used. Silverleaf desmodium contained the highest levels of condensed tannins whilst Velvet bean contained the highest levels of modified acid detergent fibre. Moreover, according to Dzowela *et al.,* (1995) sun air-drying for 3 days at 25°C will not affect the quantity of phenolics in leaves but it reduce *in vitro* degradability. Ndlovu and Nherera (1997) reported that the formation of artifacts cannot be completely ruled out when sun air-drying forages. However, the drying method used in the present experiment is unlikely to have affected the chemical composition of the forage legumes.

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In vitro gas techniques are routinely used to evaluate the nutritional quality of feeds due to their convenience, adaptability and efficiency (Getachew *et al*, 2005). The gas production technique has proved to be a potentially useful technique for feed evaluation as it is capable of measuring the rate and extent of nutrient degradation (Chai *et al.*, 2004). In addition, the technique has some vital advantages for example, it is a visibly insidious technique and therefore, it is possible for it to be gradually accepted on animal welfare basis. Moreover, the technique is able to evaluate bulky numbers of samples per given time as in selection of forages in a feeding programme. In principle, the *in vitro* gas method measures digestion of soluble and insoluble carbohydrates and the amount of gas produced from a feed on incubation reflects production of volatile fatty acids (Getachew *et al.* 2004). Cumulative gas produced at different incubation times can be measured on a single and small sample, providing kinetic data from which extent and rate of feed degradation can be estimated (Lopez *et al.*, 2007).

3.6 Conclusion

Cowpea produced the highest amount of gas followed by Velvet bean. However, Silverleaf desmodium produced gas at a higher rate than Velvet bean up to 48 hours of incubation. Effective gas production, (a), (b) and (c) were significantly different for the three legumes. Silverleaf desmodium had the least gas production levels and this could be due to the high tannin content. Therefore, the selected forage legumes can be utilised by ruminants as they are degradable in the rumen.

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CHAPTER 4

Rumen dry matter and crude protein degradability of selected forage legumes

4.1 Abstract

The experiments were carried out at ARC Pretoria (Irene) to assess *in sacco* and *in vitro* rumen disappearance of dry matter (DM), crude protein (CP), phosphorus (P) and calcium (Ca) of *Desmodium uncinatum* (Silverleaf), *Mucuna pruriens* (Velvet bean) and *Vigna unguiculata* (L.) Walp (Cowpea) forage legumes. Two cows fitted with 10 cm diameter rumen cannulae on a complete dairy feed ration were used. The forages were shade dried and milled through a 2 mm sieve. Five grams of sample was weighed into each nylon-bag and incubated into the rumen cannulated cows using the Nylon-Bag technique. The *in vitro* Daisy^{II} experiment was carried out using 0.70 g of sample in each Daisy^{II} bag. Rumen liquor was taken from a fistulated Friesian cow on dairy ration and the samples were incubated for 2, 4, 8, 12, 18, 24, 48, 72 and 96 hours. Rumen liquor that was taken from two fistulated Friesian cows on a dairy ration was mixed with saliva in the ratio of 4:1. Dry matter, CP and P effective degradability of Cowpea were the highest and significantly ($P < 0.05$) different from Velvet bean and Silverleaf. Dry matter effective degradability of Velvet bean was higher than that of Silverleaf ($P < 0.05$). However, the CP effective degradability of Silverleaf was significantly ($P < 0.05$) higher than Velvet bean. Significant ($P < 0.001$) dry matter degradability linear correlations 0.93, 0.72 and 0.82 for Velvet bean, Silverleaf desmodium and Cowpeas respectively were observed between the *in sacco* and *in vitro* Daisy^{II} techniques.

Key words: rumen degradability; dry matter; crude protein; calcium; phosphorous

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4.2 Introduction

Protein supplements are integral components of animal diets and are required to increase the total quantity of protein as well as satisfy essential amino acid requirements (Mustafa and Alamin, 2012) for ruminants. Understanding the rate and extent of ruminal degradation of various protein sources is important for formulating diets to supply enough rumen degradable protein to optimize rumen microbial growth and microbial protein to support the host. In order to improve protein utilisation in ruminant feed, protein should be divided into its components to distinguish the quantities of protein degraded in the rumen and the amount undegraded from the rumen (Rezai *et al.,* 2012) and absorbed in the gut.

The extent of protein degradation in the rumen gives a measure of the available nitrogen to microorganisms and undegradable dietary protein to the small intestine (Promkot and Wanapat, 2003). Protein quality can be determined through the use of rumen degradability characteristics of the protein particularly the proportion of soluble degradable protein compared to rumen undegradable protein (Crawford *et al.,* 1978). Degradability of dietary crude protein depends on the type of feedstuff and dry matter intake. In case of high producing ruminants, microbial protein only may be insufficient to meet the protein requirements without rumen undegradable protein supply. Concurrently, determination of Ca and P bioavailability is also important because the minerals are required for both host animal and rumen microbes.

Major minerals such as calcium (Ca) and phosphorus (P) need to be investigated. Coupling of Ca and P and metabolic interaction with vitamin D for bone metabolism, and the requirements of these nutrients are dependent on the concentrations of each other. These minerals are also required for metabolic functions in growth, productivity, and reproduction. Deficiencies of these minerals can limit animal performance and result in various health problems. Therefore, in depth study on rumen disappearance of these minerals is vital.

Determination of the amount of protein degraded in the rumen and that is undegraded from the rumen and absorbed in the gut is essential (Rezai *et al.*, 2012). The information on the amino acid composition of the undegraded dietary protein, which is currently limited, may assist in the improvement of amino acid evaluation of feeds (González *et al.*, 2009). This means that the crude protein degradation kinetics is an appropriate estimate to predict the degradation kinetics of the individual nutrients and, therefore, the supply of the nutrients to the small intestine (Miranda *et al.*, 2012).

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In sacco nylon bag technique is one of the most popular ways of evaluating the extent and pattern of degradability of feed protein in the rumen (Ørskov and McDonald, 1979; Chumpawadee *et al.* 2005; Edwards *et al.*, 2012). *In sacco* techniques mimic *in vivo* conditions and are widely adopted as the standard method for analysing degradability of forages as well as providing reference values against which *in vitro* techniques are correlated. However, the correlation between the *in sacco* Nylon Bag and Daisy^{II} techniques on the degradability of specific forage legumes is not available.

The objective of this study was to determine *in vitro* and *in sacco* rumen disappearance of dry matter (DM) crude protein (CP) phosphorus (P) and calcium (Ca) of the selected legume forages.

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4.3 Materials and methods

4.3.1 Forages

Desmodium uncinatum, Mucuna pruriens and Vigna unguiculata forage legumes were grown and harvested at the University of Venda experimental farm as described in Chapter 3. The shade dried forages were milled through a 2 mm sieve.

4.3.2 Animals and feeding

Two mature Holstein cows (mean weight 600kg) fitted with rumen cannulae of 8 cm internal diameter were used to determine *in sacco* Nylon-Bag and *in vitro* Daisy^{II} degradability of Velvet bean, Cowpea and Silverleaf desmodium. The two cows were fed a milk ration (19% CP dairy meal and maize silage) 30 days prior to the beginning of the degradability trials.

4.3.3 Experimental procedure

a) Rumen degradability of Desmodium uncinatum, Mucuna pruriens and Vigna unguiculata forage legumes using the In vitro Daisy^{II} technique

The *in vitro* Daisy^{II} experiment was carried out as done by De Figueiredo *et al.,* (2000) at ARC Pretoria (Irene). An amount of 0.70±0.05 g of *Desmodium uncinatum, Mucuna pruriens and Vigna unguiculata* forage legume samples were weighed into the Daisy^{II} bags. Rumen liquor was taken from a fistulated cows and kept in a thermos bottle preheated with 39 °C water. The rumen inoculum was blended at high speed for 30 seconds in a blender container whilst flushing with CO_2. The blended digesta was filtered through four layers of cheesecloth and continually flushed with CO_2. A ratio of buffer to rumen fluid 5:1 was used for the Daisy^{II} procedure. The 1600 ml buffer/rumen mixture was added to each digestion jar of Daisy^{II}. The digestion containers were purged for 30 seconds, the caps secured and the samples were digested for 2, 4, 8, 12, 18, 24, 48, 72, and 96 h. After incubation the jars were removed and the fluid drained. The bags were immersed for 5 minutes in acetone and then rinsed thoroughly with cold water until the water was clear using a minimum of agitation.

b) Rumen degradability of Desmodium uncinatum, Mucuna pruriens and Vigna unguiculata (L) Walp *forage legumes using the In sacco Nylon-Bag technique*

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Triplicate bags per sample were incubated simultaneously in the ventral rumen of the two cows with the number or replications depending on incubation time, expected degradability, and amount of residual material required for post incubation analyses. Nylon bags (45-µm pore size 5cm x 5cm bag size) containing 5.0g ± 0.5 of legume forage were incubated in the rumen of each animal. Plastic ties were used to seal the bags and then attached them to a cylindrical metal weight. The weight was attached to the inside of the fistula with a 70 cm strand to allow free movement within the rumen. All incubations began at 0700, an hour before the morning feed. Incubation periods of forage legumes were 0, 2, 4, 8, 18, 24, 48, 72 and 96 hours. After recovery from the rumen, the bags were washed with running tap water and stored at -20°C. After thawing, all the bags were washed three times for 5 minutes in a manually operated stomacher. The same procedure was applied to a series of bags to obtain the 0 hour value.

4.3.4 Chemical analyses

All the samples were analysed as illustrated in Chapter 3.

4.3.5 Data and statistical analysis

Disappearance of DM, CP, P and Ca of the three legumes samples were calculated from the loss of DM, CP, P and Ca from the bags after washing (zero samples) and incubation into the rumen. The degradability characteristics were calculated according to the models:

$P = a+b\ (1-e^{-ct})$ Ørskov and McDonald, (1979) (1)

$P = a+b\ (1-e^{-c\ (t-tl)})$ McDonald, (1981) (2)

Time specific degradation and disappearance of CP, DM, P and Ca was calculated using the equation of McDonald, (1981) with the modification of Wulf and Südekum, (2005), which assumes that no degradation occurs during the lag phase as done by Edmunds *et al.*, (2012):

Effective degradability = $a + (bcl\ (c + K_p))e^{-KpL}$ (3)

Where:

K_p is the rate of passage through the rumen;

a is the soluble fraction;

b is the insoluble but potentially rumen degradable fraction (calculated as *d* - *a*, where *d* is the potentially ruminal degradable fraction);

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c is the rate constant of disappearance of fraction *b* and

L is the lag phase.

Effective degradability were estimated at assumed passage rates (Kp) of 0.02, 0.04 and 0.06 h^{-1} (K_p2, 4, 6) to represent low, medium and high feeding amounts and typical rates of ruminal solid outflow for forages.

The model used for the degradation and disappearance values to determine the effects of the legume on degradation values were determined using the following model:

$$Y_{ijkh} = \mu + A_i + B_j + I_h + (A \times B \times I)_{ijh} + e_{ijkh}$$

Where:

Y_{ijkh} = DM, N, Ca or P disappearance constants a, b and c or disappearance of the DM, N, Ca and P;

μ = overall mean;

A_i = fixed effect of animal;

B_j = legume effect;

I_h= incubation period

$(A \times B \times I)_{ijh}$ = interaction between animal and legume;

e_{ijkh} = random residual error.

Disappearance values were generated using Proc NLIN and analysis of variance (ANOVA) test was carried out on the disappearance of DM, CP, P and Ca values using GLM of Statistical Analysis System (SAS, 2013). The statistical differences between means were tested using the PDIFF. Linear regression (95% confidence interval) was used to estimate the relationship between the *in sacco* Nylon-Bag and *in vitro* Daisy^{II} estimates for dry matter degradability.

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4.4 Results

a) Rumen degradability of Desmodium uncinatum Mucuna pruriens and Vigna unguiculata forage legumes using the In vitro Daisy[II] technique

Generally, dry matter, crude protein, phosphorous and calcium disappearance of Cowpea were the highest at 96 hours of incubation (Figure 4.1, 4.2, 4.3 and 4.4). Dry matter, CP and P effective degradability of Cowpea were the highest and significantly different from Velvet bean and Silverleaf desmodium ($P < 0.05$) as shown inTables 4.1 and 4.2. Dry matter effective degradability of Velvet bean was significantly higher than Silverleaf desmodium. However the CP effective degradability of Silverleaf desmodium was significantly higher than Velvet bean. Effective disappearance of Ca was significantly highest in Velvet bean forage followed by Cowpea ($P < 0.05$).

b) *Rumen degradability of Desmodium uncinatum, Mucuna pruriens and Vigna unguiculata (L)* Walp *forage legumes using the In sacco Nylon-Bag technique*

The *in sacco* DM and CP disappearance values of the legumes are shown in Tables 4.3. Cowpea forage legume had the highest disappearance values from 3 to 96 hours followed by Velvet bean and lastly Silverleaf desmodium. However, on Ca disappearance, Silverleaf desmodium had higher disappearance values from 3 to 72 hours than Velvet bean. Means for DM, CP, P and Ca effective disappearance and standard deviations of the three forage legumes are shown in Table 4.4 and 4.5. Forage of Cowpea had the highest (a) and (b) value on DM, Ca and P disappearance and were statistically significant ($P < 0.05$). However, Velvet bean had the highest values of (a) and (b) on CP degradability and were not significantly different ($P > 0.05$) from Cowpea, while Silverleaf desmodium had significantly the lowest values ($P < 0.05$). Similar results were observed on effective disappearance where Cowpea had the highest values at p= 0.02, 0.04 and 0.06 outflow rates on DM, Ca and P and they were statistically significant ($P < 0.05$). Nevertheless, Velvet bean had the highest value of effective degradability on CP degradability which was not significantly different from Cowpea while Silverleaf desmodium had significantly the lowest values ($P < 0.05$).

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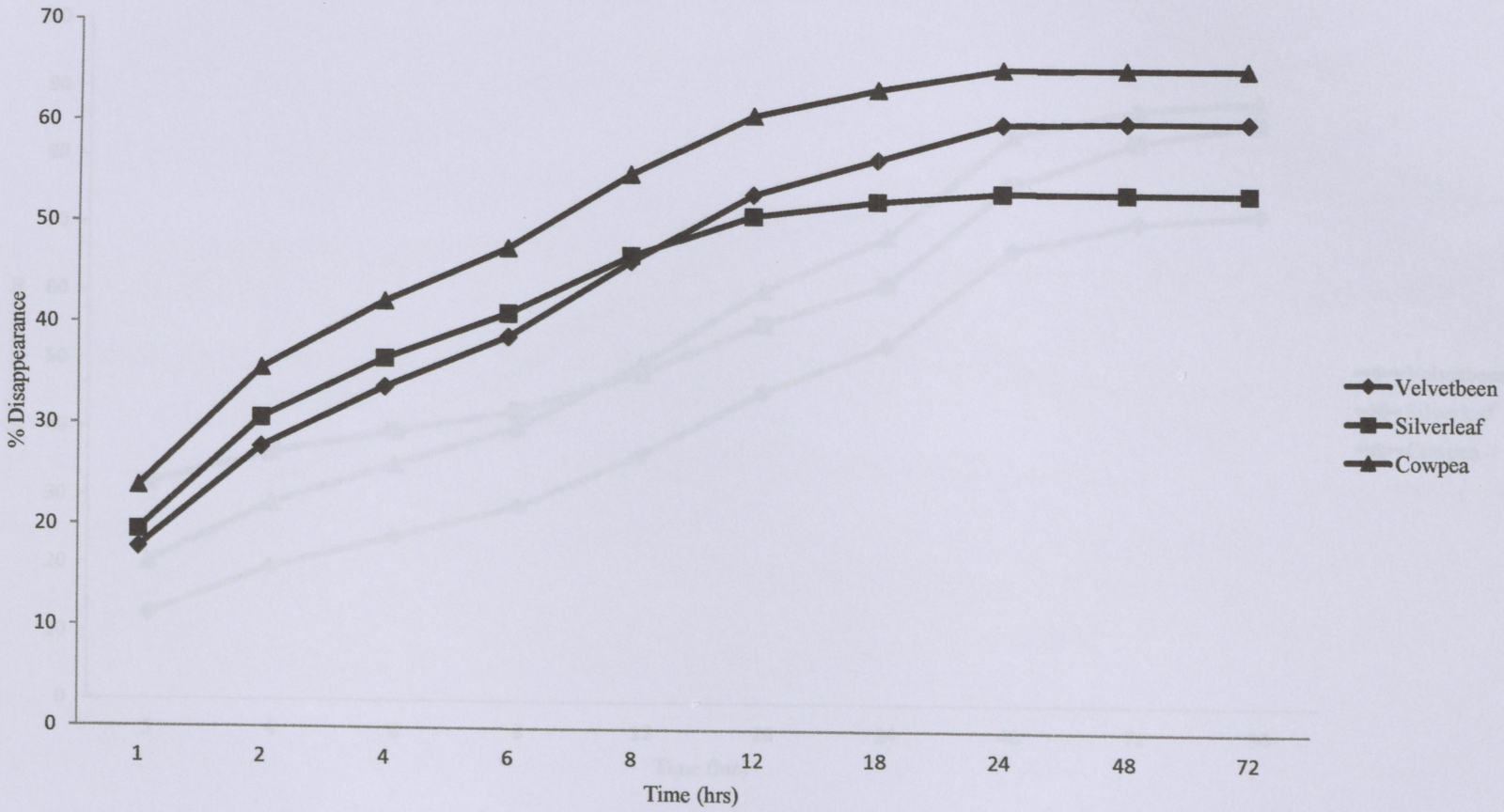

Figure 4.1: *In vitro* dry matter degradability (disappearance %) of *Desmodium uncinatum* (Silverleaf desmodium) *Mucuna pruriens* (Velvet bean) and *Vigna unguiculata* (L.) Walp (Cowpea) by Daisy[II]

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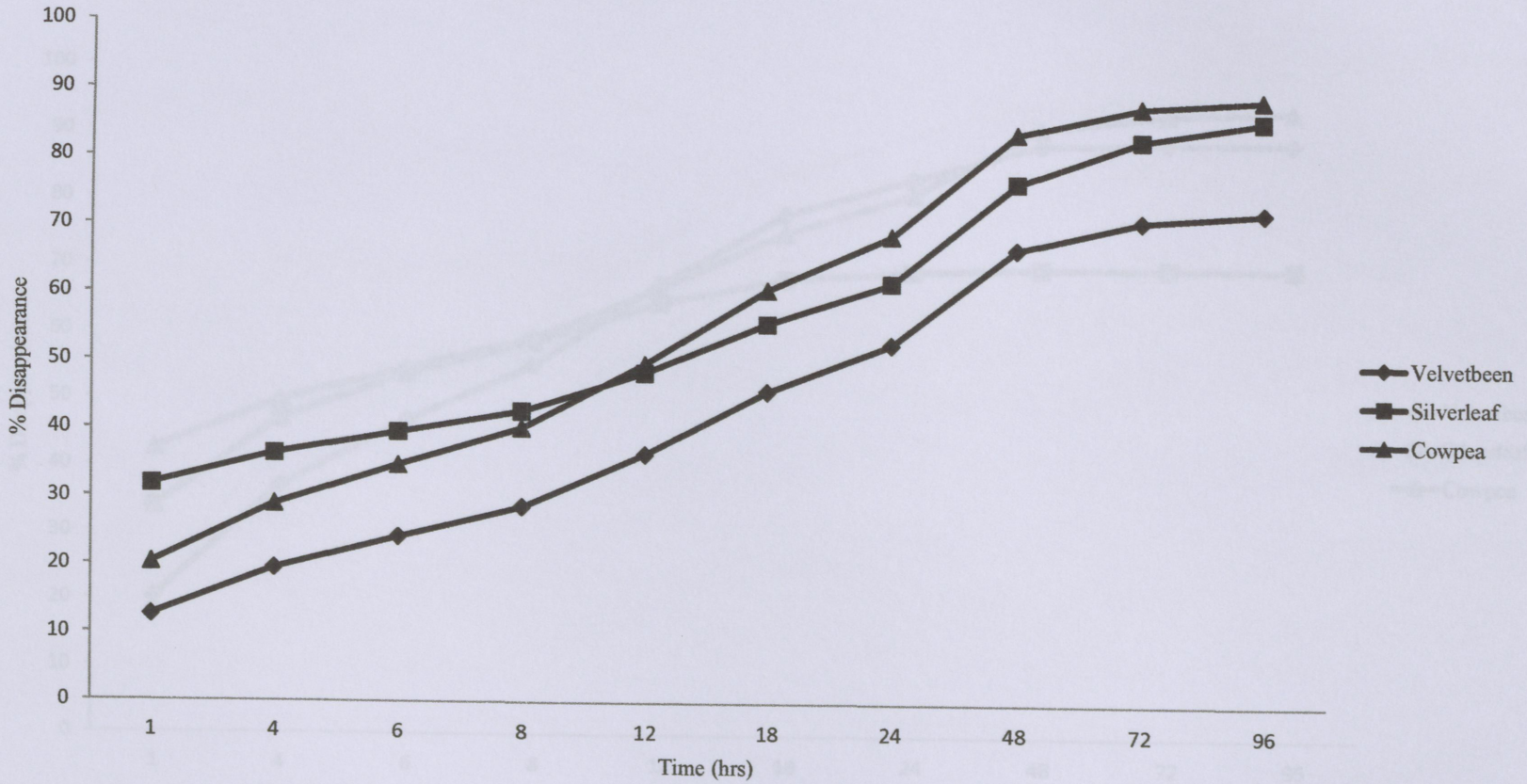

Figure 4.2: *In vitro* crude protein degradability (disappearance %) of *Desmodium uncinatum* (Silverleaf desmodium) *Mucuna pruriens* (Velvet bean) and *Vigna unguiculata* (L.) Walp (Cowpea) by Daisy[II]

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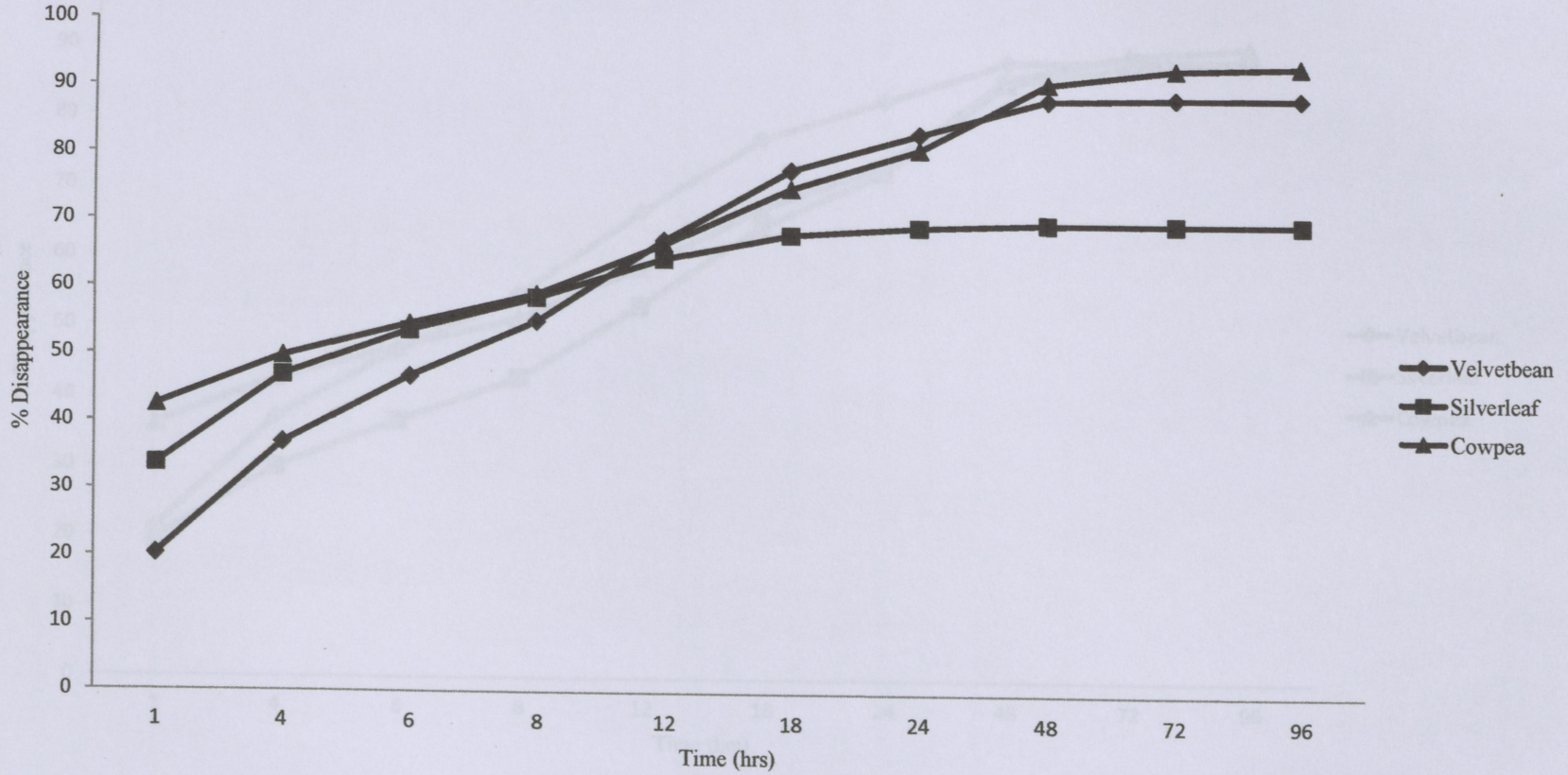

Figure 4.3: *In vitro* phosphorus disappearance (%) of *Desmodium uncinatum* (Silverleaf desmodium) *Mucuna pruriens* (Velvet bean) and *Vigna unguiculata* (L.) Walp (Cowpea) by Daisy[II]

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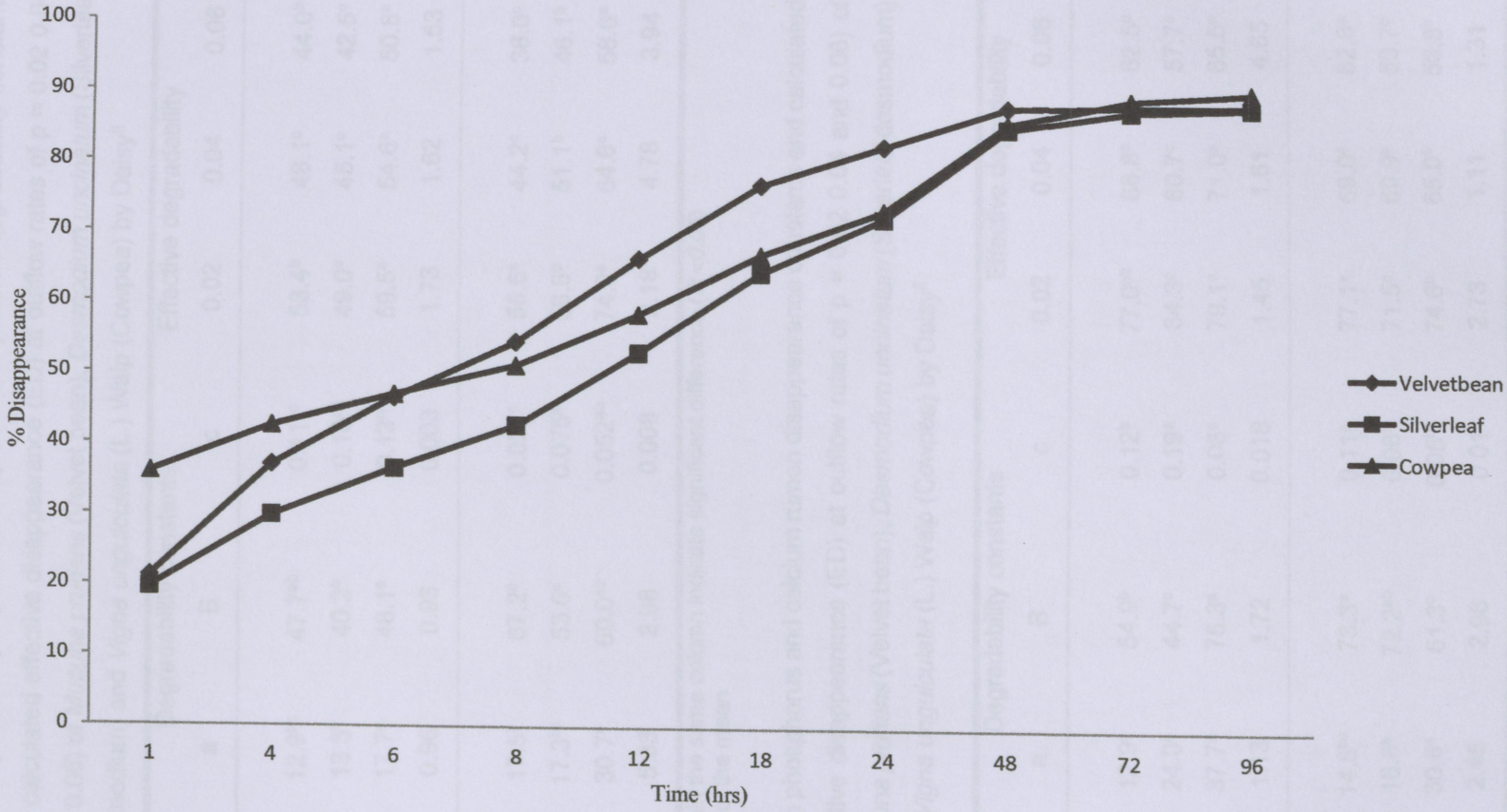

Figure 4.4: *In vitro* calcium disappearance (%) of *Desmodium uncinatum* (Silverleaf desmodium) *Mucuna pruriens* (Velvet bean) and *Vigna unguiculata* (L.) Walp (Cowpea) by Daisy[II]

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Table 4.1: Mean dry matter (DM) and crude protein (CP) rumen degradability constants and calculated effective disappearance (ED) at outflow rates of p = 0.02 0.04 and 0.06) of *Mucuna pruriens* (Velvet bean), *Desmodium uncinatum* (Silverleaf desmodium) and *Vigna unguiculata* (L.) Walp (Cowpea) by Daisy[II]

	Degradability constants			Effective degradability		
Sample	a	B	c	0.02	0.04	0.06
Dry matter						
M. pruriens	12.9^{bc}	47.7^{ab}	0.11^{bc}	53.4^{b}	48.1^{b}	44.0^{b}
D. uncinatum	13.3^{b}	40.2^{b}	0.16^{a}	49.0^{c}	45.1^{c}	42.5^{c}
V. unguiculata	17.7^{a}	48.1^{a}	0.13^{b}	59.5^{a}	54.6^{a}	50.8^{a}
SEM	0.96	0.93	0.003	1.73	1.62	1.53
Crude Protein						
M. pruriens	17.5^{b}	67.2^{a}	0.027^{c}	55.6^{c}	44.2^{c}	38.0^{c}
D. uncinatum	17.3^{bc}	53.6^{c}	0.075^{a}	58.6^{b}	51.1^{b}	46.1^{b}
V. unguiculata	30.7^{a}	60.0^{ab}	0.052^{ab}	74.0^{a}	64.6^{a}	58.6^{a}
SEM	5.33	2.98	0.008	2.18	4.78	3.94

Upper case letter within the same column indicate significant difference ($P < 0.05$)
SEM =standard error of the mean

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Table 4.2: Mean phosphorus and calcium rumen disappearance constants and calculated effective disappearance (ED) at outflow rates of p = 0.02 0.04 and 0.06) of *Mucuna pruriens* (Velvet bean), *Desmodium uncinatum* (Silverleaf desmodium) and *Vigna unguiculata* (L.) Walp (Cowpea) by Daisy[II]

	Degradability constants			Effective degradability		
Sample	a	B	c	0.02	0.04	0.06
Phosphorus						
M. pruriens	12.9^{c}	54.9^{b}	0.12^{b}	77.0^{ab}	68.8^{b}	62.5^{b}
D. uncinatum	24.0^{b}	44.7^{c}	0.19^{a}	64.3^{c}	60.7^{c}	57.7^{c}
V. unguiculata	37.7^{a}	75.3^{a}	0.06^{c}	79.1^{a}	71.0^{a}	65.5^{a}
SEM	1.13	1.72	0.018	1.45	1.61	4.65
Calcium						
M. pruriens	14.8^{bc}	73.3^{a}	0.11^{a}	77.1^{a}	69.0^{a}	62.8^{a}
D. uncinatum	16.6^{b}	72.2^{ab}	0.06^{b}	71.5^{c}	60.9^{c}	53.7^{c}
V. unguiculata	30.6^{a}	61.3^{c}	0.05^{bc}	74.6^{b}	65.0^{b}	58.8^{b}
SEM	2.45	2.96	0.01	2.73	1.11	1.31

Upper case letter within the same column indicate significant difference ($P < 0.05$)
SEM = standard error of the mean

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Table 4.3: *In sacco* dry matter and crude protein degradability (% disappearance) of *Desmodium uncinatum* (Silverleaf desmodium), *Mucuna pruriens* (Velvet bean) and *Vigna unguiculata* (L.), Walp (Cowpea)

	Time (hours)					
Feed	3	6	12	24	48	72
Dry matter						
M. pruriens	23.2	30.8	42.2	54.9	60.1	61.0
D. uncinatum	19.1	23.8	32.1	44.2	56.5	61.1
V. unguiculata	28.0	36.8	50.0	63.3	70.1	71.0
Crude protein						
M. pruriens	22.4	30.2	44.2	64.6	85.5	93.5
D. uncinatum	23.5	28.2	37.0	51.0	68.0	76.3
V. unguiculata	24.3	32.0	45.6	65.6	86.1	94.1

Table 4.4: Mean dry matter (DM) and crude protein rumen degradability constants and calculated effective degradability (ED) at outflow rates of p = 0.02, 0.04 and 0.06) of *Mucuna pruriens* (Velvet bean) *Desmodium uncinatum* (Silverleaf desmodium), and *Vigna unguiculata* (L.), Walp (Cowpea)

	Degradability constants			Effective degradability		
Sample	a	b	c	0.02	0.04	0.06
Dry matter						
M. pruriens	14.4^{c}	46.8^{c}	0.08^{ab}	53.4^{b}	48.1^{b}	44.0^{b}
D. uncinatum	14.2^{bc}	49.7^{b}	0.04^{c}	49.0^{c}	45.1^{c}	42.5^{c}
V. unguiculata	17.7^{a}	53.4^{a}	0.09^{a}	59.5^{a}	54.6^{a}	50.8^{a}
SEM	3.17	4.54	0.006	1.70	1.61	1.72
Crude Protein						
M. pruriens	9.12^{c}	84.3^{a}	0.04^{a}	59.3^{b}	49.7^{b}	43.4^{b}
D. uncinatum	18.7^{a}	65.7^{c}	0.03^{b}	53.5^{c}	46.1^{c}	41.5^{c}
V. unguiculata	16.4^{ab}	82.7^{ab}	0.04^{a}	64.8^{a}	55.4^{a}	49.4^{a}
SEM	2.17	2.46	0.002	6.76	2.49	2.91

Superscript letter within the same column indicate significant difference (*P <0.05*) SEM standard error of the mean

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Table 4.5: Mean phosphorus and calcium rumen disappearance constants and calculated effective degradability (ED) at outflow rates of p = 0.02, 0.04 and 0.06) of Desmodium uncinatum (Silverleaf desmodium), Mucuna pruriens (Velvet bean) and Vigna unguiculata (L.), Walp (Cowpea)

				Effective degradability		
Sample	a	b	c	0.02	0.04	0.06
Phosphorus						
M. pruriens	21.9^{b}	59.1^{c}	0.10^{a}	62.0^{b}	54.0^{a}	49.3^{b}
D. uncinatum	11.2^{c}	60.9^{b}	0.09^{b}	61.1^{c}	53.4^{ab}	47.8^{c}
V. unguiculata	24.1^{a}	62.4^{a}	0.05^{c}	67.5^{a}	57.3^{a}	51.1^{a}
SEM	0.85	0.61	0.001	2.97	3.90	4.83
Calcium						
M. pruriens	12.3^{c}	63.3^{b}	0.05^{b}	58.5^{c}	48.7^{c}	42.4^{c}
D. uncinatum	26.9^{a}	61.1^{c}	0.03^{c}	62.4^{b}	51.6^{b}	45.4^{b}
V. unguiculata	20.4^{b}	69.3^{a}	0.06^{a}	72.2^{a}	61.8^{a}	54.8^{a}
SEM	4.96	5.33	0.004	1.97	3.64	4.96

Superscript letter within the same column indicate significant difference ($P < 0.05$), SEM standard error of the mean

c) Relationship between *in sacco* and *in vitro* Daisy^{II} dry matter rumen degradability of the legume forage

When both *in sacco* and *in vitro* techniques were compared for dry matter degradability of the three forage legumes, a significant relationship was obtained at P<0.001 (Figures 4.5, 4.6 and 4.7). When the values for the legume dry matter degradability were considered, there were significant ($P < 0.05$) correlations 0.93, 0.72 and 0.82 for Velvet bean, Silverleaf desmodium and Cowpeas respectively. The effective degradability values of both techniques were numerically within the same ranges.

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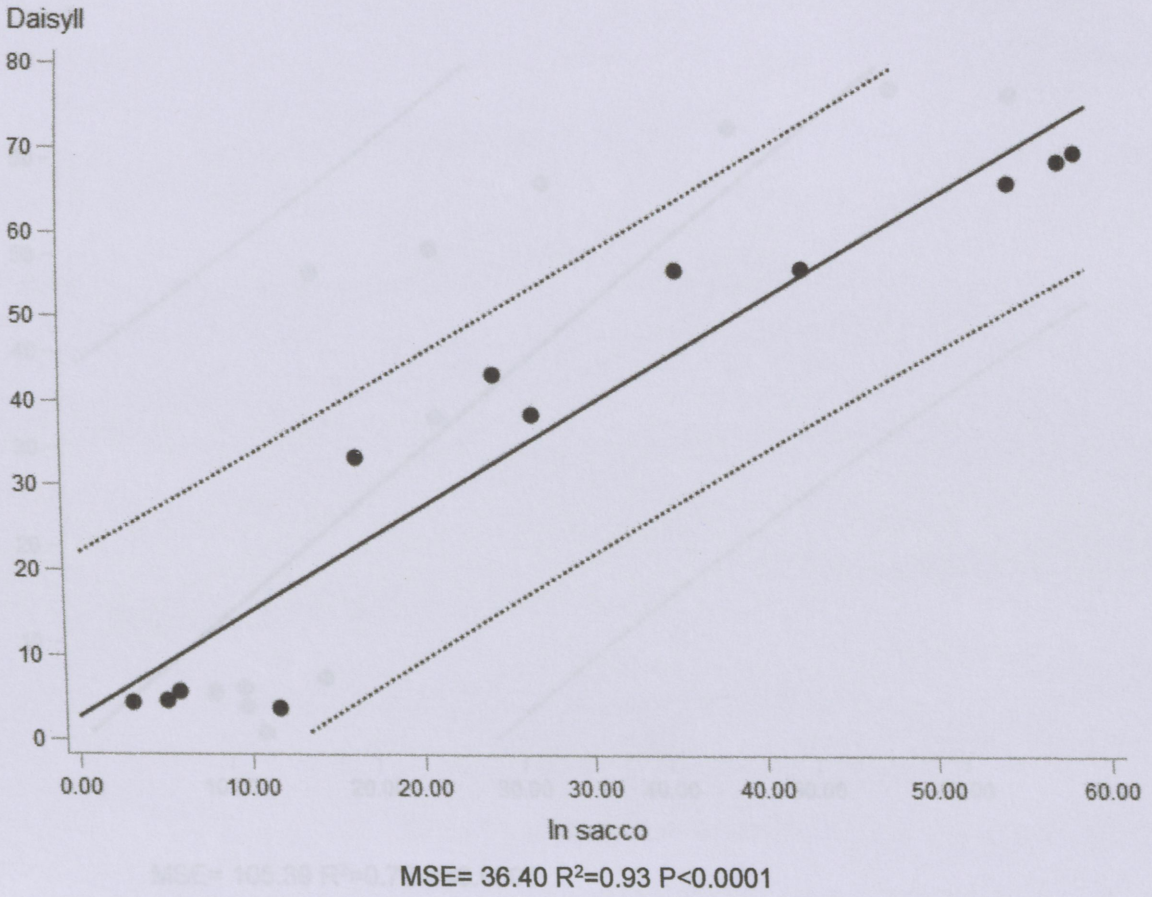

Figure 4.5: Relationship and linear regression equation between *in sacco* and *in vitro* Daisy[II] dry matter rumen degradability of *Mucuna pruriens* (Velvet bean) forage.

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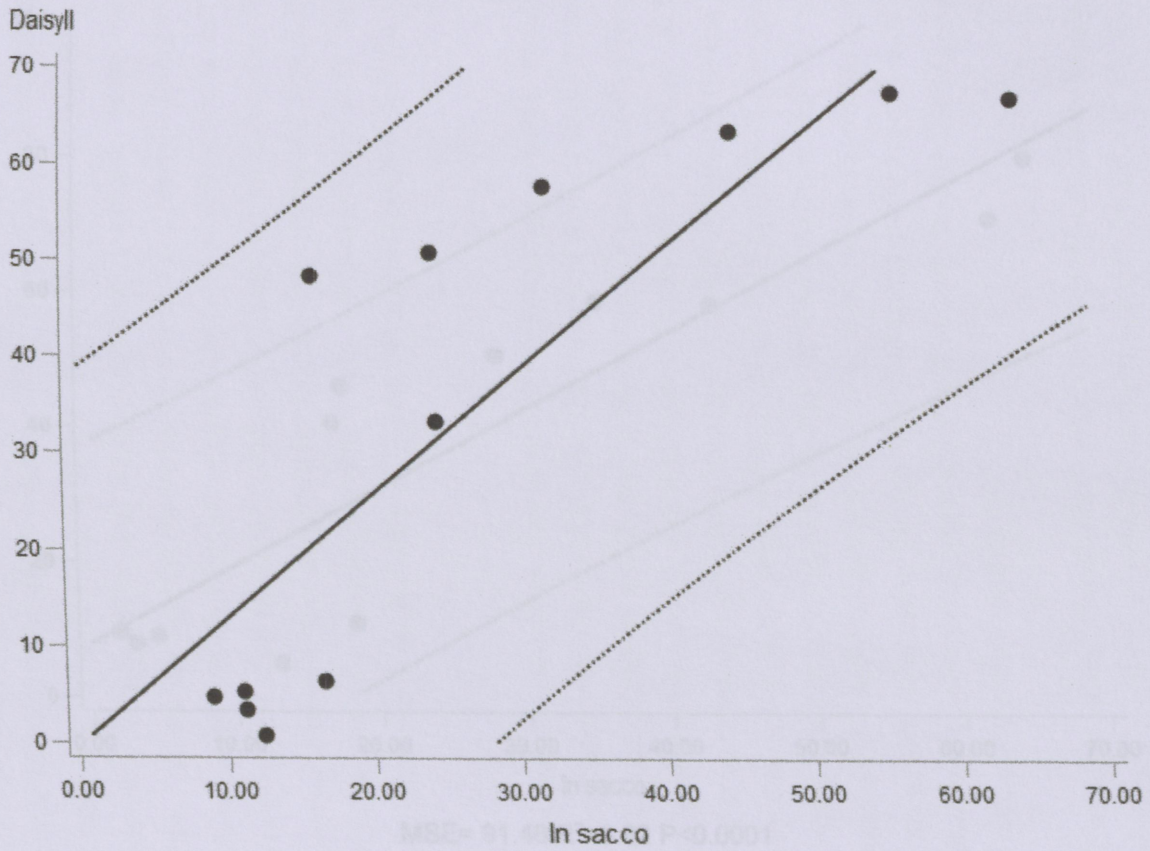

Figure 4.6: Relationship and linear regression equation between *in sacco* and *in vitro* Daisy^{II} dry matter rumen degradability of *Desmodium uncinatum* (Silverleaf desmodium) forage.

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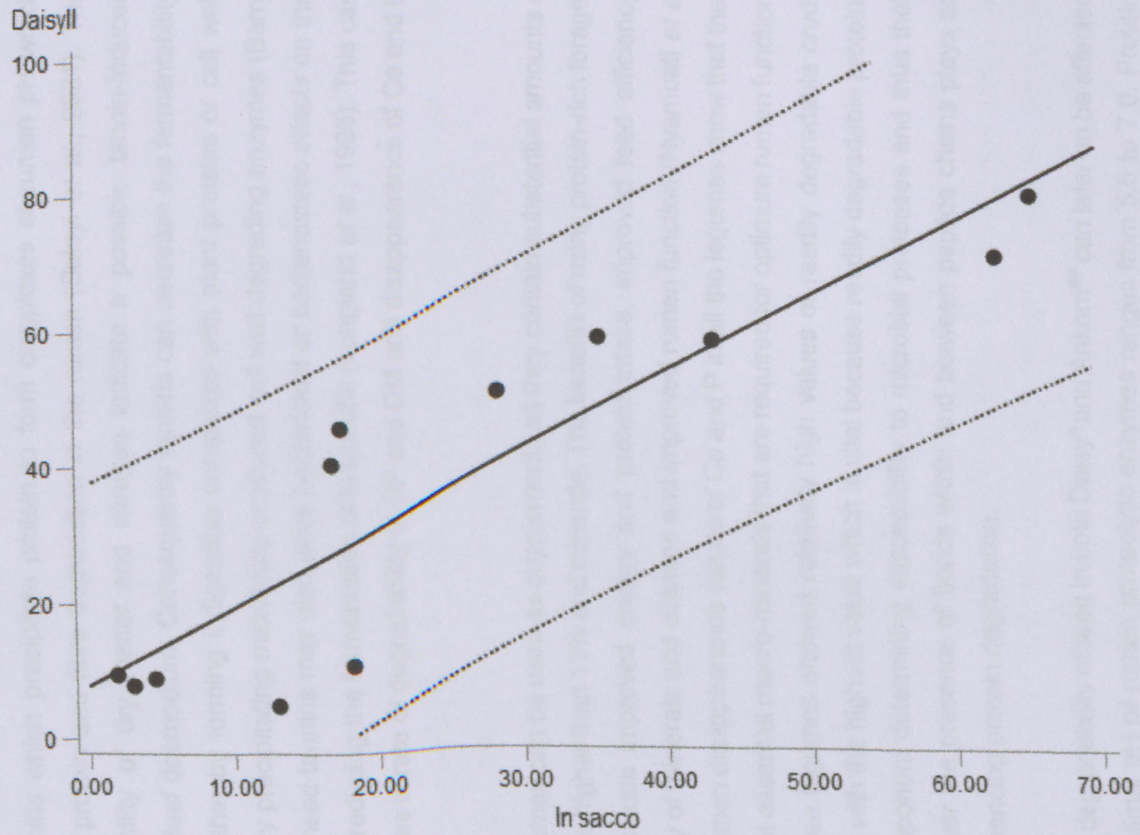

Figure 4.7 Relationship and linear regression equation between *in sacco* and *in vitro* Daisy^{II} dry matter rumen degradability of *Vigna unguiculata* (Cowpea) forage.

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4.5 Discussion

a) *Rumen degradability of Desmodium uncinatum Mucuna pruriens and Vigna unguiculata forage legumes using the In vitro Daisy^{II} technique*

The degradability of Silverleaf desmodium was low in this study. Silverleaf desmodium legume forage contains tannins which precipitate protein to form complexes at rumen pH which negatively influence protein and fibre degradation in the rumen (Baloyi *et al.*, 2001). The values on degradability of dry matter and nitrogen indicate a possible tannin-induced depression for Silverleaf desmodium. Consequently tannins can decrease the fermentability of plant cell walls either by forming indigestible complexes with plant proteins or cell wall polysaccharides or by precipitating microbially-produced cell wall degrading enzymes (Barry *et al.*, 1986). Condensed tannins may also have bactericidal or bacteriostatic effects on the rumen bacteria thus reducing the fermentation of feedstuffs (Longland *et al.*, 1995). This can explain the differences in rate of degradability of CP and DM and disappearance of Ca and P observed.

However, these legumes can be used as supplements as they contain substantial amounts of crude protein that are higher than 12% and minerals. The benefits of using protein-rich forages as a supplement include improved energy and protein intake, improved feed efficiency, increased availability of minerals and vitamins and improved rumen function (Nyambati *et al.* 2003). The higher rumen disappearance values of Ca and P in all the legumes show that they can provide sufficient essential macro-minerals that are required for optimum rumen function. In this study the three legumes showed relatively high values of readily degradable crude protein with Cowpea with the highest value which is vital because readily degradable proteins is a major feed component determining susceptibility to microbial proteases and thus their degradability. However the presence of bonds within and between peptide chains plays an important role in influencing protein degradation.

In vitro crude protein degradability of feed in both Daisy^{II} and Ankom^{RF} can later on be affected by low pH as the optimal pH of rumen proteolytic enzymes ranges from 5.5 to 7.0. Protein degradation is reduced at the lower end of the pH environment (Cardozo *et al.*, 2002). Moreover the effect of pH and the substrate being fermented may affect the principal microbial inhabitants and alter protein degradation due to interactions among nutrients. Therefore, that reduction in cellullolytic bacteria as an outcome of low pH directs to a decrease in fibre degradation plummeting access of proteolytic bacteria to proteins ultimately reducing protein

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degradation (Stern *et al.,* 2006). However, the buffers to inoculum ratio used in this study were formulated to buffer the pH of the media over the incubation period.

b) *Rumen degradability of Desmodium uncinatum, Mucuna pruriens and Vigna unguiculata (L)* Walp *forage legumes using the In sacco Nylon-Bag technique*

All the three legumes showed high disappearance values of DM, CP, P and Ca although they were significantly different. Effective disappearance values of Cowpea were the highest and this might be due to low levels of tannins and high levels of CF. Cowpea forage is a source of protein and is quite digestible for ruminants (Cook *et al.,* 2005; Anele *et al.,* 2011). However, DM degradability of the whole plant falls within the cited values but was reported to differs greatly among leaves (60-75%) and stems (50-60%) so that the proportions of leaves and stems in the haulms affects directly their nutritional value (Singh *et al.,* 2010). The forage of Cowpea was used as whole plant and some deviations from other experiments carried out are due to the crop age or with changes in environment as supported by Mullen, (1999). The high degradability values reflected by the Cowpea agree with Ravhuhali *et al.*, (2011) that cultivars with low amounts of total tannins do not exert negative effects on digestibility.

All the legumes contain significant levels of CP, Ca and P which were highly degradable and this is recommended in animal production because the benefits of using protein-rich forages as a supplement include improved energy and protein intake, improved feed efficiency, increased availability of minerals and vitamins, improved rumen function and generally, enhanced animal performance (Nyambati *et al.,* 2003). Velvet bean had the highest degradability levels of CP although not significantly higher than Cowpea. The high levels of CP degradability in Velvet bean can be explained by the lower amount of total tannins compared to Silverleaf desmodium. Therefore, both meat and milk production can benefit from including legumes such as Velvet bean forage and pods in feeds (Pengelly *et al.,* 2004). High levels of CP degradability in Velvet bean explain the reason dairy cows have shown that supplementation of grass-based diets with Velvet bean forage can increase production (compared to non-supplemented diets) or maintain it (compared to other protein supplements) (Mureithi *et al.,* 2003; Muinga *et al.,* 2003; Murungweni *et al.,* 2004; Juma *et al.,* 2006). Moreover, Velvet bean forage was found to be an interesting feed for ruminants even in areas with low fertility and a short rainy season (Sidibé-Anago *et al.,* 2009). It proved to be a cost-effective forage legume for growing cattle on natural rangeland (veld) and in pen-fattened animals also as a substitute for the commercial concentrate (Murungweni *et al.,* 2004).
Silverleaf desmodium had the least disappearance levels of DM, CP, P and Ca but highest level on total tannins. The high fibre content of Silverleaf desmodium could be an artifact of

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the high tannin content, as it was observed in other legume species (Reed, 1986).The degradability of Silverleaf desmodium is rather low and even much lower values have been recorded (Mtimuni, 1978). The tannins bind with protein and increase 'undegradable dietary' protein increasing the efficiency of digestion, but also slows nitrogen cycling. However, according to Baloyi *et al.,*(2009) the true intestinal digestibility of DM, nitrogen and amino acids was higher for Silverleaf desmodium than for *Cowpea*, and the undegraded protein supplied post-ruminally could be used for productive processes such as milk production and growth.

Owing to its high tannin content, Silverleaf desmodium would be better fed only at low proportions in the diet. High levels of degradability of Silverleaf desmodium forage are due to the drying effect because after drying the amount of condensed tannins will decrease (Bayoli *et al.,* 2001). There are also seasonal differences in the quality of haulms so attention must be given to handling of haulms to minimize the amount of leaves lost during the harvesting season (Anele *et al.,* 2012).

c) Relationship between *in sacco* and *in vitro* Daisy^{II} dry matter rumen degradability of the legume forage

Strong positive correlation coeffients of the *in sacco* and the Daisy^{II} techniques obtained in this study were higher than the values obtained by Earing *et al.*, (2010). Even higher values were where obtained on dry matter degradability of Velvet bean legume forage. Wilman and Adesogan (2000) evaluated the 2 techniques and reported that the Daisy^{II} system provided faintly less accurate prediction of ruminant *in sacco* digestibility, whereas Holden (1999) found the 2 systems to be similar. Lattimer *et al.*, (2007) observed that the Daisy^{II} can be applied to predict valid estimates of dry matter digestibility (DMD) of high quality diets when equine faeces were used as the *in vitro* inoculum source. *In vitro* Daisy^{II} degradability estimates may well be obtained in a shorter period of time with a reduced amount of effort than required by the *in sacco* Nylon-Bag technique. The results obtained in the current study agreed well with those of Lattimer *et al.* (2007) in that both studies validated the potential of the Daisy^{II} to predict *in sacco* degradability estimates.

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4.6 Conclusion

Forage of Cowpea had the highest (a) and (b) values on DM, Ca and P disappearance and were statistically significant (*P <0.05*). Velvet bean had the highest values of (a) and (b) of CP disappearance and were not significantly different from Cowpea. Similar results were observed on effective degradability where Cowpea had the highest values at the outflow rates p= 0.02, 0.04 and 0.06 on DM, Ca and P disappearance and they were statistically significant (*P<0.05*). Therefore, the selected forage legumes can be degraded in the rumen in the synthesis of high quality microbial protein. However, this also shows high alterations of the N content in the feed and can result in N losses as urea if the diet is not synchronised to couple N and energy levels.

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CHAPTER 5

Amino acids degradability in *Mucuna pruriens*, *Vigna unguiculata* and *Desmodium incunatum* forage legumes using the *in sacco* Nylon-Bag and the *in vitro* Daisy^{II} techniques

5.1 Abstract

Protein is the most limiting nutrient in ruminants relying on natural rangelands due to seasonal fluctuation in quality fodder in response to seasonality of rainfall. Farmers resort to the use of non-conventional protein sources such as forage and browse legumes. Rumen degradation of amino acids of the forages is yet to be fully evaluated. This study conducted at Agriculture Research Centre (ARC) Irene was aimed at determining amino acid profiles and rumen degradability of amino acids in Velvet bean (*Mucuna pruriens*), Cowpea (*Vigna unguiculata* Walp *(L)* and Silverleaf desmodium (*Desmodium uncinatum*) using the *in sacco* Nylon-Bag and *in vitro* Daisy^{II} techniques. Two Friesian cows fitted with 10 cm diameter rumen cannulae on a complete dairy feed ration were used as rumen liquor donors for the *in vitro* and for the incubation of the Nylon-Bags for the *in sacco* for 2, 4, 8, 12, 18, 24, 48, 72, 96 hours. Silverleaf desmodium had significantly higher concentration of all amino acid than the other legumes ($P < 0.05$). Aspartic had the highest concentration in all legumes yet cysteine had the lowest concentration in all the studied legumes. Comparisons of the two techniques show some significant differences in rate of disappearance of amino acids ($P < 0.05$). Legume forage of Cowpea showed significantly the highest level of effective degradability of all amino acids (at outflow rates 2%; 4%; 6%) followed by Silverleaf desmodium and then Velvet bean in both techniques ($P < 0.05$). When both the *in sacco* and *in vitro* techniques were compared for overall degradability of amino acids in all the three forage legumes using correlation, a significant relationship was obtained at $P<0.001$.

Key words: Amino Acids Degradability; Forage legumes; Nylon-Bag; Daisy^{II}

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5.2 Introduction

Protein is regarded as one of the major limiting nutrients in the nutrition of ruminants (Sarwar *et al.*, 2002), especially those relying on natural pastures. Natural pastures have dry standing hay with low crude protein levels from April to November due to rainfall seasonality in the subtropical region. Deficiencies can be corrected by supplementation with high-density feeds such as oilseed cakes and proteins of animal origin. However, protein sources such as oilseed cakes are beyond the economic reach of most farmers (Teferedegne, 2000). Therefore, potential strategies for increasing the quality and availability of feeds for smallholder ruminant animals in the dry season may be through the use of fodder trees and shrubs (Allam *et al.*, 2012) forages.

Significant transformations of nitrogenous feed components that in the reticulorumen. Therefore, to improve protein utilisation in ruminant feed, protein should be separated into its components of rumen degradable and undegraded from the rumen and absorbed in the gut (Rezai *et al.*, 2012). The information on the amino acid composition of the degraded feed protein, which is scarce at the moment, may help to improve the protein evaluation of feeds (González *et al.*, 2009). Therefore, the crude protein degradability kinetics is a suitable evaluation to estimate the degradability kinetics of the individual amino acids and, hence, the supply of amino acids to the post-ruminal sites (Miranda *et al.*, 2012). Rumen degradability of amino acids is one of the most important variables in protein evaluation systems for ruminants (Weisbjerg *et al.*, 1996). The objectives of the study were to determine amino acid profiles and rumen degradability of amino acids in *Mucuna pruriens, Vigna unguiculata* Walp *(L)* and *Desmodium uncinatum* using the *in sacco* Nylon-Bag and *in vitro* Daisy^{II} techniques.

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5.3 Materials and method

5.3.1 Forages

Mucuna pruriens, Vigna unguiculata Walp *(L)* and *Desmodium uncinatum* legumes were harvested, dried and milled as described in Chapter 3.

5.3.2 Experimental procedure

In vitro Daisy^{II}

The *in vitro* Daisy^{II} experiment was carried out as described in Chapter 4.

In sacco Nylon-Bag technique

In sacco Nylon-Bag technique of Ørskov and McDonald (1979) was also done as described in Chapter 4.

5.3.3 Chemical analysis

The chemicals were analysed as outlined in Chapter 3. The amino acid composition of the original forage legumes and residues was determined by High Performance Liquid Chromatography (HPLC), following hydrolysis as outlined by AOAC (1990). Approximately 50 -75 mg sample was accurately weighed into a screw capped Pyrex glass hydrolysis tube. Acid hydrolysis was carried out to determine all amino acids except methionine, cysteine and tryptophan. 5 ml of 6 M HCL was added to each tube, mixed in an ultrasonic bath for 15 minutes, flushed with oxygen-free nitrogen for 1 minute and cap closed. Hydrolyse was done for 24 h in a heating block pre-heated to 110°C. The product of hydrolysis was allowed to cool to room temperature and transferred into a 50 ml volumetric flask before being diluted to volume.

The hydrolysates were filtered through Whatman No. 4 filter paper. An aliquot of filtrate based on protein content of the sample was placed into a 50 ml quickfit round bottom flask. A 1 ml of internal standard was added, mixed and dried under a vacuum. The dried residue was dissolved in 2.5 ml acetic acid (25 mM). A working standard was also prepared at this point. The solution was then filtered through a Miller LCR 0.5 (m syringe filter and stored at -4°C. A Waters C18 HPLC was used for the analysis and amino acid standard solution from Sigma-

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Aldrich USA (AA-S-18) was used.

5.3.4 Statistical analysis

The degradability parameters were calculated using the equations described in Chapter 4. Analysis of variance (ANOVA) test was carried out on the disappearance of amino acids value using Statistical Analysis System (SAS, 2013). The mean values obtained were fitted to the equation of McDonald and Ørskov (1979) using SAS (2013). Furthermore, linear regression (95% confidence interval) was used to estimate the linear relationship between the *in sacco* Nylon-Bag and *in vitro* Daisy^{II} estimates for the amino acids degradability.

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5.4 Results

Amino acid concentration is presented in Table 5.1. Silverleaf desmodium had significantly higher concentration of all amino acid than the other legumes (P <0.05). Aspartic had the highest concentration in all legumes and cysteine had the lowest concentration in all the studied legumes. There was a considerable variation in amino acid content of the three legumes. Silverleaf desmodium contained significantly (P <0.05) the most amino acids compared to Cowpea and Velvet bean. On essential amino acids, Silverleaf desmodium contained significantly (P <0.05) highest levels followed by Cowpea then Velvet bean. However, Velvet bean contained significantly (P <0.05) more isoleucine compared to Cowpea but not significantly (P >0.05) different levels of leucine and methionine.

The readily degradable component of amino acids when using the Daisy^{II} technique was higher in Cowpea compared to Velvet bean as shown in table 5.1. Cowpea had higher levels of slowly degradable amino acids than Velvet bean except histidine, isoleucine and leucine which were higher in Velvet bean when using Daisy^{II}. Whilst, in the *in sacco* Nylon-Bag technique the readily and slowly degradable component in all amino acids where highest in Cowpeas followed by Silverleaf desmodium and then Velvet bean (Table 5.2). However, Silverleaf desmodium had highest readily and slowly degradable aspartic acid when using the *in sacco* method.

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Table 5.1: Mean amino acid composition (g/kg CP) of the forage legumes *Mucuna pruriens, Desmodium uncinatum* and *Vigna unguiculata* (L) Walp

Amino acid	*M. pruriens*	*D. uncinatum*	*V. unguiculata*	SEM
Glycine	41.2^{b}	47.9^{a}	39.4^{c}	0.24
Alanine	44.1^{b}	59.5^{a}	43.5^{bc}	0.23
Arginine	38.0^{c}	52.4^{a}	47.6^{b}	0.26
Aspartic Acid	80.5^{c}	175.9^{a}	90.5^{b}	0.39
Cysteine	6.28^{abc}	7.4^{a}	6.5^{ab}	0.23
Glutamic acid	83.8^{c}	108.9^{a}	104.4^{b}	0.28
Histidine	14.6^{c}	24.4^{a}	16.5^{b}	0.24
Isoleucine	36.5^{b}	47.2^{a}	33.6^{c}	0.32
Leucine	59.4^{bc}	81.0^{a}	59.7^{b}	0.31
Lysine	39.0^{c}	67.5^{a}	42.8^{b}	0.24
Methionine	13.3^{bc}	14.6^{a}	13.5^{ab}	0.23
Phenylalanine	40.6^{c}	59.8^{a}	52.4^{b}	0.28
Proline	37.6^{c}	52.5^{a}	44.6^{b}	0.26
Serine	33.0^{c}	54.6^{a}	39.6^{b}	0.17
Threonine	32.4^{c}	54.9^{a}	40.8^{b}	0.34
Tyrosine	22.2^{c}	30.5^{a}	24.7^{b}	0.19
Valine	43.5^{c}	60.9^{a}	46.6^{b}	0.26

Different superscripts in the same row means significantly different at $P < 0.05$, SEM=standard error of the mean

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Table 5.2 shows the results of *in vitro* Daisy^{II} method and Table 5.3 shows the result obtained when the *in sacco* nylon bag technique was used respectively. Moderate rate of passage 0.04 was used as the recommended out flow rate in semi-intensive production system. Comparisons of the two techniques show some significant differences in the rate of disappearance of amino acids. The results of the comparisons are shown in Table 5.4. The *in sacco* Nylon-Bag techniques showed a significantly higher level of amino acid disappearance (readily and slowly degradable) and effective degradability (at outflow rates p=0.02; 0.04; 0.06/h) compared to the Daisy^{II} technique. Cowpea showed the highest level of effective degradability of amino acid (at outflow rates p=0.02; 0.04; 0.06/h) followed by Silverleaf desmodium and then Velvet bean in both techniques. The *in vitro* Daisy^{II} Silverleaf desmodium values failed to converge to proc NLIN of SAS (2013).

When both *in sacco* and *in vitro* techniques were compared for overall degradability of amino acids in all the three forage legumes a significant relationship was obtained at P<0.001 (Figures 5.1, 5.2 and 5.3). When the values for all the legume amino acids degradability were considered, there were significant correlations 0.99, 0.84 and 0.98 for Velvet bean, Silverleaf desmodium and Cowpeas respectively.

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Table 5.2: Degradability and effective degradability coefficients for amino acids in forage legumes measured in g/kg DM using the *In vitro* Daisy[II]

Amino acid	Legume	a	b	Rumen fractional outflow rate 0.04/h
Glycine	Velvet bean	1.81	12.5	14.3
Glycine	Cowpea	2.46	13.8	16.3
Alanine	Velvet bean	5.63	38.9	44.6
Alanine	Cowpea	6.61	37.3	44.0
Arginine	Velvet bean	4.7	32.5	37.2
Arginine	Cowpea	7.27	41	48.3
Aspartic Acid	Velvet bean	10.2	70.3	80.6
Aspartic Acid	Cowpea	13.7	77.3	91.1
Cysteine	Velvet bean	0.84	5.85	6.73
Cysteine	Cowpea	0.96	5.38	6.38
Glutamic acid	Velvet bean	10.7	74.1	84.9
Glutamic acid	Cowpea	15.8	89.1	105
Histidine	Velvet bean	1.81	12.5	14.3
Histidine	Cowpea	2.46	13.9	16.4
Isoleucine	Velvet bean	4.56	31.6	36.2
Isoleucine	Cowpea	5.15	29.1	34.3
Leucine	Velvet bean	7.52	52.0	59.6
Leucine	Cowpea	9.09	51.3	60.4
Lysine	Velvet bean	4.82	33.3	38.2
Lysine	Cowpea	6.45	36.5	43.0
Methionine	Velvet bean	1.69	11.6	13.3
Methionine	Cowpea	2.08	11.8	13.9
Phenylalanine	Velvet bean	5.15	35.6	40.8
Phenylalanine	Cowpea	7.91	44.6	52.5
Proline	Velvet bean	4.73	32.7	37.5
Proline	Cowpea	6.79	38.3	45.1
Serine	Velvet bean	4.09	28.3	32.4
Serine	Cowpea	6	33.9	39.9
Threonine	Velvet bean	4.09	28.3	32.4
Threonine	Cowpea	6.18	34.8	41.1
Tyrosine	Velvet bean	2.73	18.9	21.7
Tyrosine	Cowpea	3.77	21.3	25.1
Valine	Velvet bean	5.45	37.7	43.2
Valine	Cowpea	7.08	39.9	47.0

SAS (2013) could not produce the effect of amino acid*legume and dependent variable degradability and SEMs because of non-estimable least-squares means.

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Table 5.3: Degradability and effective degradability coefficients for amino acids in forage legumes measured in g/kg DM using the *in sacco* Nylon-Bag technique

Amino acid	Legume	a	b	Rumen fractional outflow rate 0.04/h
Glycine	Velvet bean	0.2	8.76	9
Glycine	Silverleaf	0.31	14.9	15.3
Glycine	Cowpea	0.35	11.4	11.8
Alanine	Velvet bean	0.61	27.3	27.9
Alanine	Silverleaf	0.75	35.8	36.6
Alanine	Cowpea	0.94	30.7	31.7
Arginine	Velvet bean	0.51	22.8	23.3
Arginine	Silverleaf	0.66	31.5	32.2
Arginine	Cowpea	1.03	33.8	34.8
Aspartic Acid	Velvet bean	1.09	49.3	50.5
Aspartic Acid	Silverleaf	2.22	106	108.7
Aspartic Acid	Cowpea	1.95	63.8	65.8
Cysteine	Velvet bean	0.09	4.1	4.23
Cysteine	Silverleaf	0.09	4.28	4.41
Cysteine	Cowpea	0.14	4.44	4.62
Glutamic acid	Velvet bean	1.15	52	53.1
Glutamic acid	Silverleaf	1.37	65.9	67.3
Glutamic acid	Cowpea	2.24	73.4	75.7
Histidine	Velvet bean	0.2	8.76	9
Histidine	Silverleaf	0.31	14.9	15.3
Histidine	Cowpea	0.35	11.4	11.8
Isoleucine	Velvet bean	0.49	22.2	22.7
Isoleucine	Silverleaf	0.59	28.2	28.9
Isoleucine	Cowpea	0.73	23.9	24.7
Leucine	Velvet bean	0.81	36.5	37.3
Leucine	Silverleaf	1.02	48.9	50
Leucine	Cowpea	1.3	42.2	43.6
Lysine	Velvet bean	0.52	23.4	23.9
Lysine	Silverleaf	0.85	40.6	41.5
Lysine	Cowpea	0.92	30.0	31
Methionine	Velvet bean	0.18	8.14	8.36
Methionine	Silverleaf	0.18	8.76	8.98
Methionine	Cowpea	0.3	9.68	10.0
Phenylalanine	Velvet bean	0.55	25.0	25.6
Phenylalanine	Silverleaf	0.75	36.0	36.8
Phenylalanine	Cowpea	1.12	36.7	37.9
Proline	Velvet bean	0.05	23.0	23.0
Proline	Silverleaf	0.66	31.7	32.4
Proline	Cowpea	0.95	31.5	32.5
Serine	Velvet bean	0.44	19.8	20.3
Serine	Silverleaf	0.67	32.7	33.4
Serine	Cowpea	0.85	27.9	28.8
Threonine	Velvet bean	0.44	19.8	20.3
Threonine	Silverleaf	0.7	33.2	33.9
Threonine	Cowpea	0.87	28.7	29.6
Tyrosine	Velvet bean	0.3	13.3	13.6
Tyrosine	Silverleaf	0.38	18.3	18.7
Tyrosine	Cowpea	0.54	17.5	18.1
Valine	Velvet bean	0.58	26.4	27.1
Valine	Silverleaf	0.77	36.7	37.6
Valine	Cowpea	1.0	32.8	33.9

SAS (2013) could not produce the effect of amino acid*legume and dependent variable degradability and SEMs because of non-estimable least-squares means.

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Table 5.4: Mean degradability and effective degradability coefficients for forage amino acids

				Rumen fractional outflow rate		
Legume	a	b	c	0.02/h	0.04/h	0.06/h
In sacco Nylon	4.66[a]	30.7[a]	0.05	35.4[a]	35.4[a]	34.5[a]
In vitro Daisy[II]	0.58[b]	23.9[b]	0.05	24.6[b]	24.6[b]	24.6[b]
SEM	0.28	2.14	0	2.34	2.24	2.33
Velvet bean	2.19[b]	23.4[b]	0.06	25.5[a]	25.6[a]	25.6[a]
Silverleaf	0.58[c]	31.2[a]	0.05	33.8[a]	33.8[a]	33.8[a]
Cowpea	3.05[a]	27.5[ab]	0.04	30.5[a]	30.6[a]	30.6[a]
SEM	0.3	2.59	0.009	2.83	2.83	2.82

Different small letters and superscript letters in the same column mean significant different at $P < 0.05$, SEM standard error of the mean

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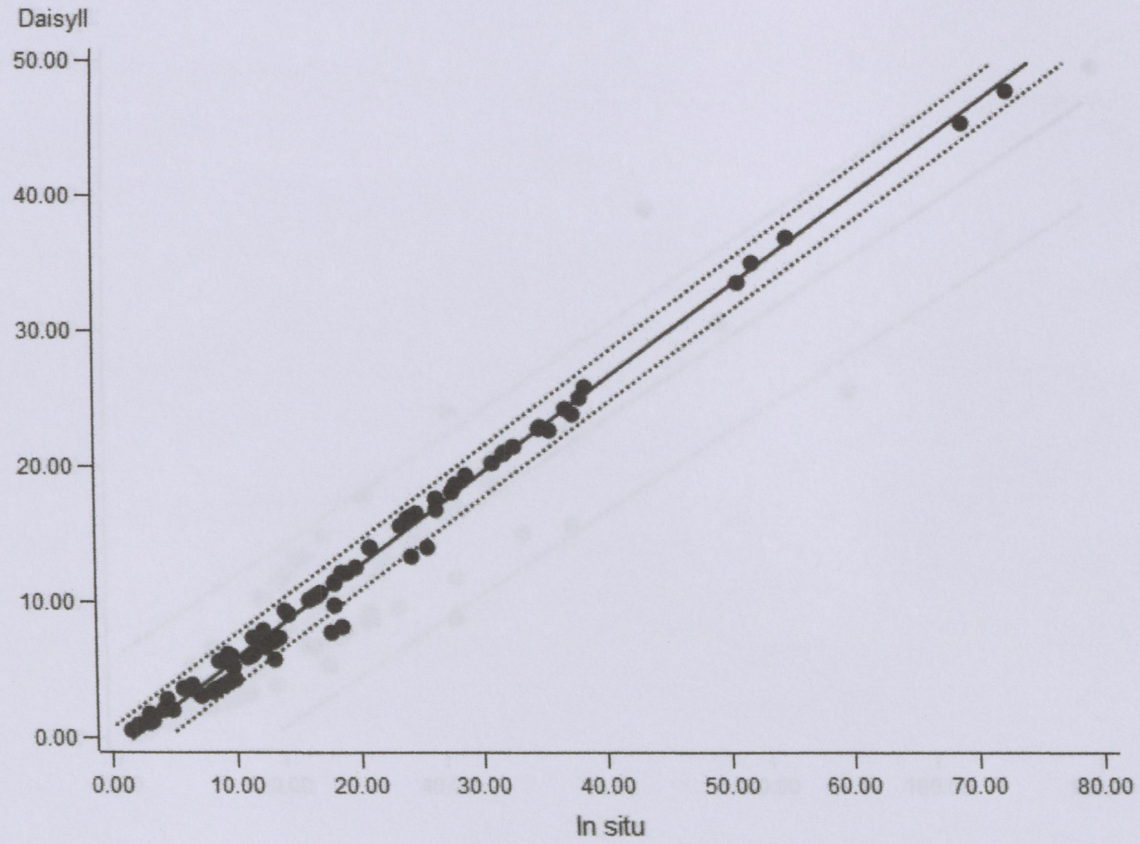

MSE= 1.35 R^2= 0.99 P <0. 0001

Figure 5.1 Relationship and linear regression equation between *in sacco* and *in vitro* amino acids degradability of *Mucuna pruriens* (Velvet bean) forage.

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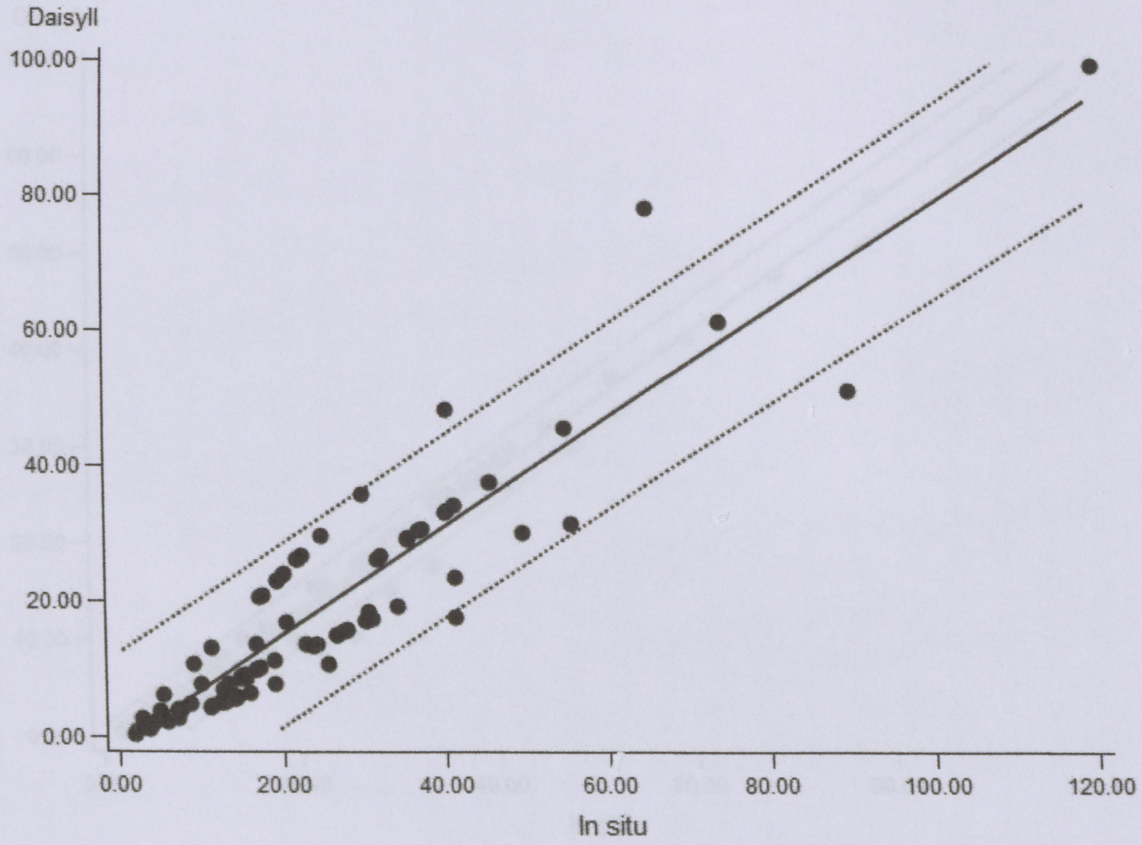

MSE= 7.68 R^2 =0.84P<0.0001

Figure 5.2: Relationship and linear regression equation between *in sacco* and *in vitro* rumen amino acids degradability of *Desmodium uncinatum* (Silverleaf desmodium) forage.

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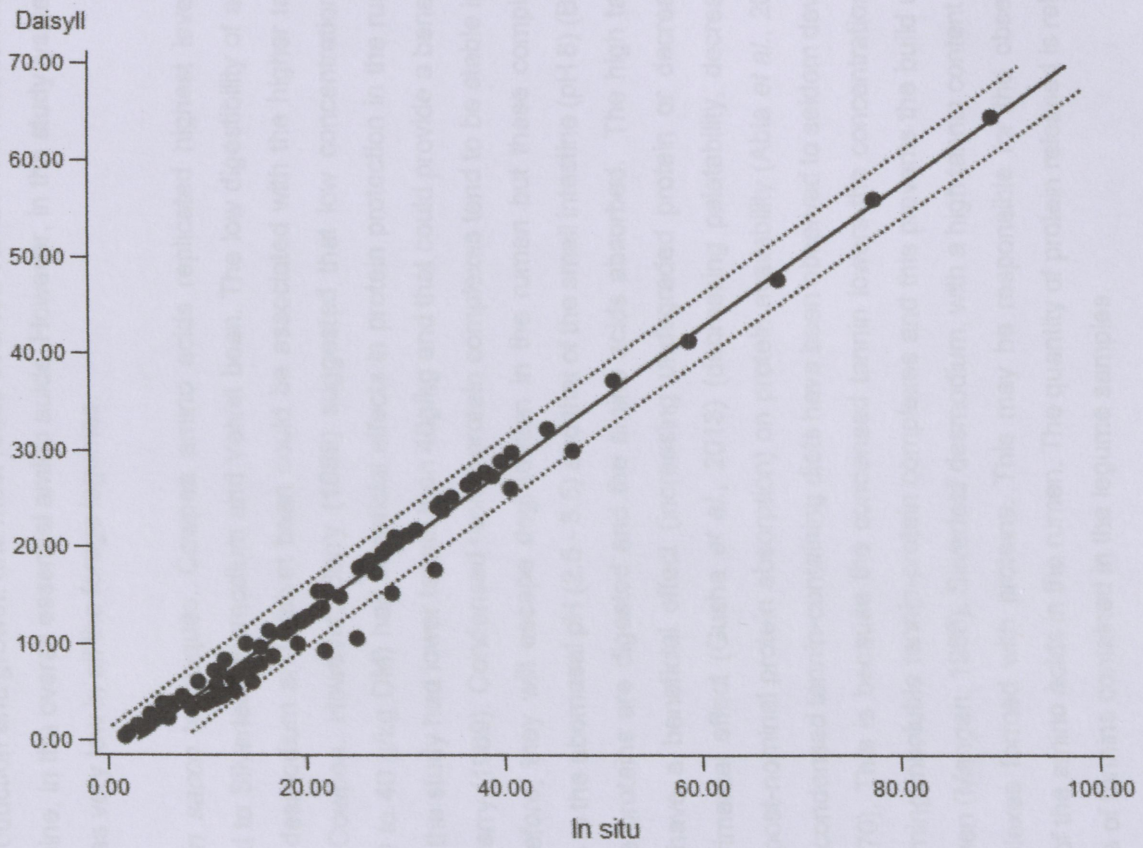

MSE= 2.18 R^2 =0.98 P<0.0001

Figure 5.3: Relationship and linear regression equation between *in sacco* and *in vitro* rumen amino acids degradability of *Vigna unguiculata* (Cowpea) forage.

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5.5 Discussion

The amounts of total essential amino acids in the forage legumes were very similar in particular lysine and methionine concentrations which are the same as reported by NRC (2001) in tropical forages. Miranda *et al.* (2012) reported that methionine and lysine are the two essential limiting amino acids for milk production and growth, and most feeds sources contain low methionine and lysine levels mostly lysine, in the overall essential amino acids. However, in the study lysine was high but methionine was very low in all the forage legumes.

In both *in vitro* and *in sacco* techniques, Cowpea amino acids replicated highest levels of degradability compared to Silverleaf desmodium and Velvet bean. The low digestibility of amino acids in the Silverleaf desmodium and Velvet bean could be associated with the higher tannin content compared to Cowpea. However, Barry (1989) suggested that low concentrations of condensed tannins (20 to 40 g/kg DM) had beneficial effects in protein protection in the rumen. All the forages used in the study had lower tannin than 40g/kg and that could provide a beneficial effect as reported by Barry (1989). Condensed tannin-protein complexes tend to be stable in the rumen pH (4-7). Therefore, they will escape degradation in the rumen but these complexes dissociate on exposure to the abomasal pH (2.5 - 3.5) and that of the small intestine (pH 8) (Baloyi *et al.*, 2009) where the proteins are digested and the amino acids absorbed. The high tannin levels may therefore have a beneficial effect (increasing undegraded protein or decreasing ammonia loss) or detrimental effect (Gusha *et al.*, 2013) (depressing palatability, decreasing ammonia, decreasing post-nominal protein absorption) on protein availability (Abia *et al.*, 2006). Moreover, animals fed condensed tannin-containing diets have been observed to seldom develop bloat (Jones *et al.*, 1970). This is because the condensed tannin lowers the concentrations of soluble proteins by forming insoluble tannin-protein complexes and this prevents the build up of stable foams in the rumen (Mangan, 1988). Silverleaf desmodium, with a high tannin content may thus have more complexes formed with proteins. This may be responsible for the observed reduced degradability of the amino acids in the rumen. The quantity of protein released is related to the quantity and type of tannins contained in the legume samples.

Cowpea had the highest value of total amino acid degradability which is vital because readily degradable protein is a major aspect determining susceptibility to microbial proteases and thus their degradability. Nevertheless, the presence of bonds within and between peptide chains plays an important role in influencing protein degradation. Schwingel and Bates (1996) showed that

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acidic subunit glycinin (with strong disulfide bonds) the basic glycine and several Leu- containing peptides in the N-terminal group in feed are fairly resistant to degradation. Furthermore, some peptide bonds are more resistant to degradation by ruminal microbes than others as demonstrated by Yang and Russell (1992) that dipeptides formed of Lys-Pro are hydrolysed in the rumen 5-fold slower than the dipeptide Lys-Ala and dipeptides formed of Pro-Met are degraded 2.5-fold slower than dipeptides formed of Met-Ala.

Velvet bean showed the least amino acid degradability in both techniques and this might be due to high levels of MADF. Van Soest (1980) reported that neutral detergent and acid detergent fibres were significantly and negatively correlated to degradability of forages. The justification was that ADF and NDF are critical products with nutritional properties that define forage components that are less soluble in particular systems and are moderately less digestible compared to starch. Moreover, Linn and Kuehn (1993) reported that high ADF and NDF fibre levels decreased degradability of forages and can be used to calculate net energy contents of forages in ruminants. In this study forages legumes of different chemical composition gave different degradability. However, on the contrary, Ng'ambi (1995) observed that chemical quantities of forages provided no suggestion of forage degradability.

The widely used rumen *in sacco* technique to measure degradation may overestimate its degradability in dried and ground leaves because small particles pass through the bag pores without being degraded (Mota *et al.,* 2005). The determination of rumen degradability *in sacco* is too expensive, time-consuming and labour-intensive for routine analysis and animal welfare is questionable (Stern *et al.,* 1997). Therefore, *in vitro* Daisy^{II} technique mimics *in vivo* conditions and are widely adopted as a method for analysing rumen degradability of forages. Studies done by Wilman and Adesogan (2000) evaluated the 2 techniques and established that the Daisy^{II} system presented faintly less accurate estimation of ruminant *in vivo* degradability. Moreover Holden (1999) established that the *in vivo* and *in vitro* Daisy^{II} systems are comparable.

Both techniques gave particular variation in the total amino acid degradation in the rumen of different forage legumes. Variability in the total amino acid degradability kinetics relative to that of legume forage species, and amongst individual amino acids, has been observed (Skórko-Sajko *et al.*, 1994; Dakowski *et al.*, 1996). Moreover, there was a considerable variation in the soluble amino acid fraction of the forage legumes, which reinforces the proposal of using the systematic

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model that endeavours to evade the exploitation of empiric units that are commonly associated with inaccurate estimates and present partial inference possibility (Van Soest, 1994).

The *in sacco* Nylon-Bag technique demonstrated significantly higher level of amino acid disappearance and effective degradability compared to the *in vitro* Daisy^{II} technique. However, strong positive relationships were observed between the two techniques. Borucki *et al.* (2007) reported that amino acid digestibility in *in vitro* were lower than *in sacco;* therefore, this may sustain that *in sacco* amino acid degradability was higher compared to *in vitro* Daisy^{II}. In both techniques the pattern of ruminal degradation was characterised by low degradation of glycine, whereas methionine and the branched-chain amino acids were degraded to the least extent which was also elucidated by Borucki *et al.* (2007). However, Holden (1999) concluded that the Daisy^{II} technique can be used to improve labour efficiency when estimating rumen degradability of forages.

5.6 Conclusion

The results of the present study confirmed rumen degradability of Silverleaf desmodium, Cowpea and Velvet bean forage legumes amino acids. Cowpea is highly degradable compared to Silverleaf desmodium and Velvet bean. The levels of condensed tannins were lower than the levels that cause some detrimental effects but are sufficient to promote increase in undegraded protein. Interpretation of the achieved results is that in developing countries, Silverleaf desmodium, Cowpea and Velvet bean forage legumes are used as supplements to well degradable diets. When the values for all the legume amino acids degradability were considered, there were significant correlations 0.99, 0.84 and 0.98 for Velvet bean, Silverleaf desmodium and Cowpeas respectively. Therefore, any of the two methods can be used to determine rumen degradability of amino acids.

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Apparent digestibility, microbial protein supply and nutrient supply kinetics of selected forage legumes in goats

6.1 Abstract

The study examined the effects of feeding forage legumes as protein supplements to poor quality natural pasture (veld) hay offered to goats on microbial protein yield and nitrogen metabolism. Four indigenous Nguni type goats were used in a 4 x 4 Latin cross-over design experiment. The goats were randomly allocated to four dietary treatments comprising commercial goat feed alone (CF), veld hay supplemented with either Cowpea (CW), Velvet bean (VB) or Silverleaf desmodium (SD). Microbial protein yield was determined using the purine derivatives technique and nitrogen retention was calculated from the difference between nitrogen intake and nitrogen excreted. Total nitrogen (N) intake was significantly highest in the CF followed by CW, SD and VB at 14.17, 12.6, 11.6 and 11.1 g/d respectively ($P < 0.05$). Microbial protein supply, the calculated microbial true protein and the digestible microbial true protein were all affected ($P < 0.05$) by legume supplementation. Animals on poor quality forages tended to show the most efficient microbial protein synthesis 2.21, 2.06, 2.01 and 1.95 for VB, CP, SD and CF respectively. However, supplementation of poor quality veld hay failed to meet the maintenance requirements of the animal as evidenced by negative nitrogen balances in VB and SD, 1.2 and 0.46 g/d respectively. The efficiency of utilisation of nitrogen in the supplements could have been limited by unavailable fermentable metabolisable energy in the diets. Therefore, poor quality veld hay can be supplemented with forage legumes to improve utilisation and these forage legumes could help the communal farmers provide feed for their goats mainly in the dry season.

Key words: Apparent digestibility; microbial protein; nutrient supply kinetics; forage legumes

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6.2 Introduction

Goat production in the communal areas of South Africa are based on pastoralism and agro-pastoralism. The majority of the production systems are subsistence-based with limited use of technology and external inputs (Palmer and Ainslie, 2006). In these goat rearing systems rangelands are the sole source of forage. Rangelands in the Limpopo province are dominated by *Terminalia prunioides, Boscia foetida, Gynura bicolour, Adansonia digitata* and *Colophospermum mopane* trees. The most common veld grasses are *Aristida* species, *Stipagrostis uniplumis*, *Enneapogon cenchroides* and *Brassica deflexa* which are low in protein content (Acocks, 1988). Therefore, forage legumes like *Vigna unguiculata* (Cowpea), *Desmodium uncinatum* (Silverleaf) and *Mucuna pruriens* (Velvet bean) are important feed resources as they are high in protein, total ash and phosphorus (Maasdorp *et al.*, 1999; Hove *et al.*, 2001; Baloyi *et al.*, 2008). These tropical legume forages are made up of leaves with high proportions of readily digestible thin-walled non-lignified mesophyll tissue and low cell wall contents which results in superior degradation (Norton and Poppi, 1995; Baloyi *et al.*, 2008).

Cowpeas and Silverleaf have high potential degradability, indicating that they might need to stay in the rumen for a longer time (Molina-Alcaide *et al.*, 1996) thus increasing microbial protein yield. Microbial protein is important because it contributes about two thirds of the amino acids absorbed by ruminants (Pathak 2008). Protein supplements which have proper levels of rumen undegradable protein provide growth limiting amino acids like lysine and methionine (Rezai *et al.*, 2012). Nyambati *et al.* (2003) showed that milk production can be increased by supplementing with Velvet bean, highlighting the importance of integrating legumes into the low-input, mixed cropping systems in the tropics.

Forage legumes also tend to be more digestible than grasses and crop residues (Matizha *et al.*, 1997; Ranjbar, 2007; Njidda and Olatunji, 2012). Moreover, studies have shown that legume supplements will increase fibre digestion in the rumen by promoting the growth of cellulolytic microbes (Ndlovu and Buchanan-Smith, 1985). Feeding of legume fodder to ruminants on poor diet has been shown to increase voluntary feed intake or digestibility of the rations (Baloyi *et al.*, 2001). It is thus necessary to evaluate the actual contribution of these legumes in the goat as protein supplements for suitability in small-holder systems to counteract negative effects. Therefore a study was designed to determine apparent digestibility, nitrogen retention and total microbial protein yield from *Vigna unguiculata* (Cowpea), *Desmodium uncinatum* (Silverleaf) and

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Mucuna pruriens (Velvet bean) forage legumes in goats. The study aimed at contributing useful empirical data on apparent digestibility of selected forage legumes in indigenous goats.

However, there is also a need to calculate nutrition utilisation kinetics of the forage legumes using a nutrition model. A software model to predict specific nutrient requirements and feed biological values for sheep and goat was developed, based on the structure of the Cornell Net Carbohydrate and Protein System for Small Ruminants (Cannas *et al.*, 2004; 2006; 2007). Biological values of feeds are estimated based on protein and carbohydrate fractions and their ruminal degradation rates, rates of passage of concentrate and liquid forage, microbial production, and physically effective fibre (Cannas *et al.*, 2007).

Therefore a study was designed to determine microbial protein supply, apparent digestibility and nutrient supply kinetics of *Vigna unguiculata* (Cowpea), *Desmodium uncinatum* (Silverleaf desmodium) and *Mucuna pruriens* (Velvet bean) forage legumes in goats.

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6.3 Materials and methods

6.3.1 Feed

Forage legume materials were harvested from legume plantations and dried as described in Chapter 3. The dried samples were then milled through a 25mm sieve before feeding.

6.3.2 Animals and housing

The trial was carried out at the University of Venda experimental farm. The ethical and Higher Degrees Committees of the University of Venda approved the use of animals and all protocols and procedures in this study. Four indigenous Nguni type male goats (29±0.5 kg) were used in a 4x4 Latin square Experimental procedure. The animals were vaccinated against enterotoxaemia (pulpy kidney) and injected with a broad spectrum anthelmintic just before the start of the experiment and then placed in individual metabolism crates. All the animals were allocated to each treatment and moved into metabolism crates for the total collection of faeces and urine for apparent digestibility and nitrogen metabolism measurements.

The metabolism crates were constructed from metal and had wire gauze floors, which allowed urine to fall through. The faeces were caught using collection harness bags on the back of the animal. Urine was collected separately by means of metal tray attached below the metal gauze and gravitated into a plastic bucket with 10% H_2SO_4 (to preserve the N in the urine) placed at the rear of the crate.

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6.3.3 Experimental procedure

The diets were offered in a 4x4 Latin-square design, with forage and feeding periods as factors. Goats were fed legume forage at 300 $g.kg^{-1}$ of the total dry matter (DM) intake measured daily at 3 % of BW (adjusted daily). This amount of legumes (300 $g.kg^{-1}$ of the total DM intake) in supplements is close to the legume content in reinforced pastures in the tropics (Baloyi *et al.*, 2014). The dietary treatments consisted of (1) commercial goat feed (complete feed with 16.29% crude protein) (CF) *ad libitum*; (2) Velvet bean (VB); (3) Silverleaf (SD) and (4) Cowpea (CW). Hay (*Aristida* species, *Stipagrostis uniplumis*, *Enneapogon cenchroides* and *Brassica deflexa*

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grasses) was given *ad libitum* after consumption of protein feed. Fresh water was available *ad libitum* during the experimental period.

Each goat was fed each treatment for 15 days (10 days of adaptation and 5 days of measurement), before being switched to the next treatment. The total experiment lasted 60 days. Samples of diets were collected daily during each collection period and composited. Samples of feed refusals (sealed plastics) and faeces (stored in a freezer) were also obtained and composited for each period.

6.3.4 Chemical analysis

Feed, urinary and feacal chemical composition was analysed as described in Chapter 3. The dried materials were ashed at 550 ^{0}C for 6 h in a muffle furnace to measure organic matter (OM) content.

Calculations

Microbial protein yield was determined using the purine derivatives technique as described by Chen and Gomes (1992). The urine samples were analysed for allantoin alone (Chen and Gomes, 1992). Feed biological values were predicted based on carbohydrate and protein fractions and their ruminal degradation rates, forage, concentrate and liquid passage rates, microbial growth, and physically effective fibre (Cannas *et al.*, 2007).

Nitrogen retention

(i) Nitrogen retention was calculated from dietary N intake (NI) less total nitrogen output (faecal N (FN) and urinary N (UN) losses).

Nitrogen retention (g/d) = NI - (FN + UN)/NI (1)

Apparent digestibility *(g/d) = NI - (FN + UN)/ NI* (2)

(ii) Digestible organic matter intake (DOMI) was calculated from the organic matter intake less faecal organic matter.

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DOMI (g/d) = organic matter intake – faecal organic matter (3)

(iii) Metabolisable energy (ME) intake and digestible organic matter in the rumen (DOMR) were calculated from the DOMI content according to AFRC (1993).

Metabolisable energy (ME) intake (MJ/d) = 0.0157 x DOMI (4)

(iv) *Digestible organic matter in the rumen (DOMR) (g/d) = 0.65 x DOMI* (5)

Microbial protein yield

(i) The microbial protein yield was calculated from daily urinary purine derivative, allantoin, excretion using the equations of Chen and Gomes (1992) and Chen *et al.*, (1990).

$$Y = 0.84X + (0.15W^{0.75}e^{-0.25X}) \quad (6)$$

Where:

Y = purine derivative excretion in urine (mmol/d),

X = concentration of microbial purines absorbed after duodenal and intestinal digestion (mmol/d).

The calculation of X was performed by means of the Newton-Raphson iterative process until it approached a constant value, which is the answer (Chen and Gomes, 1992), $W^{0.75}$ = represents the metabolic body weight (kg) of the animal

(ii) *Microbial nitrogen yield (mny) (g/d) = X * 70/(0.83 * 0.116 * 1000) = 0.727X* (7)

Where:

70 represents the N content of purines (mg N/mmol), 0.83 is the average digestibility of mixed microbial purines based on observations reported in literature (Chen and Gomes, 1992),

0.116 is the proportion of purine N in the total microbial N of mixed rumen microbes,

1/1000 is to convert the estimate from mg to g/day.

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(iii) Microbial true protein (MTP), digestible microbial true protein and efficiency of microbial nitrogen supply were calculated according to the AFRC (1993).

Microbial true protein (MTP) (g/d) = 0.80 x mny x 6.25 (8)

Digestible microbial true protein (g/d) = 0.85 x MTP (9)

Efficiency of microbial nitrogen supply (E_{mns}) (g/kg DOMR) = mny/DOMR (10)

Nutrient supply kinetics

Dietary inputs from the chemical composition of the feeds and intake values on the experiment conducted were used to calculate nutrient supply kinetics using the Small Ruminant Nutritional System (SRNS) by Sassari University (Italy), Texas A&M University, and Cornell University version 1.9. 5105. The chemical composition shown in table 3.1 in Chapter 3 was used to predict nutrients supply (rumen N balance, rumen balance % of required, peptide balance, peptide balance % of required, dietary net energy intake, crude protein intake, metabolisable protein intake, bacterial metabolisable protein, feed metabolisable protein, dietary intake protein % CP, soluble protein % CP, microbial protein concentration, urea cost, live weight change) in commercial goat feed and Silverleaf desmodium, Velvet bean, Cowpea in growing goats.The diets were calculated in a 4x4 Latin square.

6.3.5 Statistical analysis

Nutrient intake and apparent digestibility and microbial protein production were analysed using the General Linear Model (GLM) procedures for Latin cross-over designs (SAS, 2013). Nutrient supply kinetics and the rumen fermentation parameters were analysed using the General Linear Model (GLM) procedures for Latin 4 x 4 square design (SAS, 2013). Animal and period were used as the blocks. The following model was used for the analysis:

$$Y_{ijkl} = \mu + A_i + P_j + D_k + (APD)_{ijk} + e_{ijkl}$$

Where:

Y_{ijkl} is the dependent variable e.g., DM intake or nitrogen retention, N;

μ is the overall treatment mean for the Y_{ijkl} parameter;

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A_i is the ith fixed effect of the animal; P_j is the jth fixed effect of the period; D_k is the kth fixed effect of the forage and e_{ijkl} is the random residual error, assuming it is normally and independently and identically distributed with a mean of zero.

PDIFF of SAS (2013) was used for the comparison of treatment means at 0.05.

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6.5 Results

Chemical composition

Crude protein content of the four treatments were significantly different with Cowpea containing the highest followed by the commercial feed (Table 6.1). Silverleaf desmodium contained significantly the highest amount of dry matter and tannins (P <0.05).

Feed intake and apparent digestibility

Total dry matter intake was significantly highest (P <0.05) in the goats supplemented with Cowpea followed by Velvet bean, Silverleaf desmodium and lastly commercial goat feed (Table 6.2). Faecal excretion, organic matter faecal excretion, organic matter intake and apparent digestibility coefficient of dry matter were not significantly different (P >0.05) among the treatments except commercial goat feed. Digestible organic matter intake, organic matter digestibility and metabolisable energy intake were significantly highest (P <0.05) in goats on commercial goat feed followed by Silverleaf desmodium, Cowpea and then Velvet bean.

Nitrogen intake and retention

Nitrogen intake was significantly highest (P <0.05) in goats on commercial goat meal followed by Cowpea, Silverleaf desmodium and then Velvet bean. Nitrogen retention was negative for goats supplemented with Velvet bean and Silverleaf desmodium, but positive with Cowpeas supplement and commercial feed (P <0.05) as shown in Table 6.3. Nitrogen retention in goats on commercial feed was significantly different (P >0.05) from goats on Cowpea, Silverleaf desmodium and Velvet bean. Moreover, nitrogen retention for goats on Cowpea was not significantly different from goats on Silverleaf desmodium.

Microbial protein production

There were no significant differences (P <0.05) in microbial protein synthesis; the animals on the commercial feed diet were as efficient as those on Silverleaf desmodium, Cowpea and Velvet bean in microbial protein production (Table 6.4). The Cowpea and the commercial feed diets were significantly high (P <0.05) in nitrogen and the microbial protein syntheses were also higher than

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the other diets. Digestible microbial true protein, digestible organic matter in the rumen, microbial true protein, microbial nitrogen yield and allantoin were all significantly different (*P <0.05*).

Nutrition supply kinetics

Feed metabolisable protein was significantly higher when supplementing with Cowpea than with Silverleaf desmodium and Velvet bean (*P <0.05*). However, dietary net energy intake, metabolisable protein intake and microbial protein concentration were higher with Silverleaf desmodium supplementation than with Cowpea and Silverleaf desmodium. However, soluble protein %CP was higher in when supplementing with Cowpea than with Silverleaf desmodium and Velvet bean but not significant (*P >0.05*) (Table 6.5).

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Table 6.1: Chemical composition, g/kg dry matter (DM) of *Desmodium uncinatum* (Silverleaf desmodium), *Mucuna pruriens* (Velvet bean) *Vigna unguiculata* (L.), Walp (Cowpea) and commercial goat feed

Feed	DM	CP	P	Ca	ASH	CF	MADF	Tannins
	g/kg							
M. pruriens	804^{e}	148^{c}	2.0^{d}	2.4^{d}	121.3^{a}	253^{a}	390^{b}	4.4^{b}
D. uncinatum	964^{a}	163^{b}	3.0^{c}	4.8^{b}	46.1^{e}	228^{b}	270^{c}	11.2^{a}
V. unguiculata	855^{d}	180^{a}	3.2^{b}	3.8^{c}	88.1^{c}	204^{d}	225^{d}	1.2^{c}
Goat feed	865^{c}	163^{b}	5.0^{a}	10.0^{a}	56.1^{d}	111^{e}	185^{e}	ND
Hay	889^{b}	74.3^{d}	ND	ND	106^{b}	215^{c}	616^{a}	ND
SEM	1.1	0.79	0.06	0.11	0.72	0.45	3.36	0.09

DM = dry matter g.kg^{-1}, CF = crude fibre, MADF =modified acid detergent fibre, P= phosphorus, Ca= calcium, ^{a,b,c} Means in the same column with different superscripts are significantly different ($P < 0.05$), SEM = standard error of the mean, ND = not determined

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Table 6.2: Average daily dry matter intake and apparent digestibility in goats given commercial goat feed (CF) (Control), or veld hay supplemented with 300 g/kg (air dry weight basis) Velvet bean (VB), Silverleaf desmodium (SD), Cowpea (CW) and legume hays

Parameter	Treatment				
	VB	SD	CW	CF	SEM
Dry matter intake (g/d)					
Veld hay	335[a]	317[a]	330[a]	ND	20.6
Legume hay	296[bc]	294[b]	300[a]	ND	0.61
Total intake	635[a]	617[ab]	630[a]	544[bc]	21.8
DM faecal excretion (g/d)	392[a]	355[a]	365.2[a]	294[a]	27.9
OM faecal excretion	359[a]	320[a]	330[a]	218[b]	21.6
Organic matter intake (g/d)	569[a]	570[a]	570[a]	513[a]	19.6
Digestible organic matter intake (DOMI) (g/d)	210[bc]	250[ab]	240[ab]	295[a]	21.2
ME intake (MJ/d)	3.10[bc]	3.92[ab]	3.77[ab]	4.61[a]	0.33
Apparent digestibility coefficient					
Dry matter(DMD)	0.38[a]	0.42[a]	0.43[a]	0.46[a]	0.10
Organic matter (OMD)	0.37[b]	0.44[b]	0.43[b]	0.57[a]	0.04

[a, b, c] Means in the same row with different superscripts are significantly different ($P < 0.05$), DMD = dry matter digestibility, OMD = organic matter digestibility, SEM = Standard error of means, ND not determined

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Table 6.3: Nitrogen (N) intake and retention (g/d) in goats given commercial goat feed (CF) (Control), or veld hay supplemented with Velvet bean (VB), Silverleaf desmodium (SD) and Cowpea (CW) legume hays

Parameter	Treatment diet				
	VB	SD	CW	CF	SEM
N intake	11.1^{c}	11.6^{bc}	12.6^{b}	14.17^{a}	0.32
N in faeces	7.08^{a}	6.16^{a}	6.27^{a}	5.71^{a}	0.44
N in urine	5.29^{d}	5.93^{b}	5.45^{c}	6.33^{a}	0.04
Total N in faeces and urine	12.4^{a}	12.1^{a}	11.7^{b}	12.04^{a}	0.42
N retention	-1.29^{d}	-0.46^{c}	0.84^{b}	2.13^{a}	0.40

[a, b, c] Means in the same row with different superscripts are significantly different ($P < 0.05$), SEM standard error of the mean

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Table 6.4: Excretion of purine derivatives (PD) and the microbial protein production by goats given commercial goat feed (CF) (Control), or veld hay supplemented with Velvet bean (VB), Silverleaf desmodium (SD) and Cowpea (CW) legume hays

	Treatment diet				
Parameter	VB	SD	CP	CF	SEM
Allantoin (mMol/d)	3.63^{d}	4.27^{b}	3.80^{c}	4.67^{a}	0.04
Microbial nitrogen yield (^{1}MNY) (g/d)	2.64^{d}	3.10^{b}	2.76^{c}	3.40^{a}	0.03
Microbial true protein (MTP) (g/d)	13.2^{d}	15.5^{b}	13.8^{c}	17^{a}	0.14
Digestible Microbial true protein (g/d)	11.2^{d}	13.2^{b}	11.7^{c}	14.4^{a}	0.12
Digestible organic matter in the rumen (DOMR)	136^{c}	162^{ab}	156^{ab}	192^{a}	11.1
$^{1}E_{mns}$ (g/kg DOMR)	2.21	2.01	2.06	1.95	0.23

$^{a, b, c}$ Means in the same row with different superscripts are significantly different ($P < 0.05$), $^{1}E_{mns}$ = efficiency of microbial nitrogen synthesis (AFRC, 1993), SEM = standard error of the mean

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Table 6.5: Nutrients supply in Commercial goat feed, *Mucuna pruriens* (Velvet bean), *Desmodium uncinatum* (Silverleaf desmodium) and *Vigna unguiculata* (L.), Walp (Cowpea) to growing goats

Variable	Feed				
	CF	VB	SD	CW	SEM
Rumen N balance g/day	6.09^{a}	0.94^{c}	0.48^{d}	1.58^{b}	0.13
Peptide balance % of required	99.3^{a}	72.0^{c}	55.4^{d}	84.9^{b}	1.32
Dietary Net Energy Intake Mcal/kg	1.79^{a}	1.34^{d}	1.42^{b}	1.37^{c}	0
Crude Protein Intake g/day	98.5^{a}	69^{c}	73.3^{bc}	78^{b}	1.69
Metabolisable Protein Intake g/day	49.5^{b}	52.5^{ab}	56.3^{a}	55^{a}	1.32
Feed Metabolisable Protein g/day	20.3^{a}	14.3^{c}	15.3^{c}	17^{b}	0.43
Dietary Intake Protein %CP	77.5^{a}	72.5^{b}	72.1^{bc}	71.6^{c}	0.12
Soluble Protein %CP	20^{b}	26.6^{a}	26.7^{a}	26.8^{a}	0.10
Microbial Protein Concentration g/kg DMI	82.6^{c}	82.6^{c}	88.9^{a}	86.2^{b}	0.14

Means in the same row with different letters are significantly different ($P < 0.05$); SEM = standard error of the mean

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6.6 Discussion

Nitrogen retention was lower for goats supplemented with Velvet bean and Silverleaf desmodium, compared to those on Cowpeas supplement and commercial feed. Cowpeas improved N retention to a greater extent than Silverleaf desmodium and Velvet bean. Similar results were also observed by Koralagama *et al.* (2008) where N retention was positive in sheep offered Cowpeas. The results indicate that the supplementation of veld hay with forage legumes enhanced ruminant production through improvements in digestibility and intake. Foster *et al* (2009) elucidated that supplementing poor quality basal grass diets with legume forage increased feed intake and diet digestibility by ruminant livestock and legume supplementation improves N retention by the ruminant when grass diets that do not meet ruminant energy and requirements are fed.

Low N retention was observed in the experiment on goats supplemented with Velvet bean and Silverleaf desmodium this might be due to the resolution to restrict the supplementation to intake of 3% of live weight (DM basis) could also be a reason for the low N retention as growing goats fed exclusively on high-protein tree and shrub foliages have been reported to consume up to 5% of their live weight as DM (Theng Kouch *et al.*, 2003). Nevertheless, the nitrogen intake values obtained in this study were higher than those (3.04-3.19 g/day) reported by Ajayi *et al.* (2005) for goats fed some forages legumes as supplements to basal diet of *Panicum maximum*. Fadiyimu *et al.* (2010) reported higher values (9.28g/day) for sheep supplemented at various levels with forage legumes to *Panicum maximum.* However, the nitrogen intake values are lower than those (6.48-7.49 g/day) obtained by Asaolu *et al.* (2011) for West African Dwarf goats fed some browse legume combinations with *Moringa oleifera*. Although some of the animals on all the treatments were in negative nitrogen balance, supplementation with the legumes reduced ($P < 0.05$) nitrogen loss. This could be attributed to the availability of more protein and energy and a higher digestibility of organic nutrients in the legumes. The negative nitrogen retention observed in this study agrees with the findings of Minson and Milford (1967) who reported -2.7 g/d nitrogen balance in sheep supplemented with *Stylosanthes guianensis* (Fine stem stylo). Getachew *et al.* (1994) obtained an improved nitrogen balance with *D. intortum* compared to *S. guanensis* and *M. axillare*. Significant difference in faecal nitrogen losses did not agree with findings by Asaolu *et al.* (2010) who reported that the faecal nitrogen was not affected by nitrogen intake.

Apparent digestibility values for all nutrients considered were similar to those reported from previous study (Etela and Larbi, 2011). However, apparent digestibility coefficients for dry matter and organic matter in Cowpeas and Silverleaf desmodium supplementations were

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slightly lower than those observed by Baloyi *et al.* (2008). The higher dietary condensed tannin concentration in the Silverleaf desmodium is associated with the improved nitrogen retention (Gwanzura *et al.*, 2012). The tannin may inactivate rumen microbes and enzymes and protect nitrogen from degradation in the rumen by forming insoluble complexes with proteins (Waghorn *et al.*, 1994). Upon reaching the abomasum (with a low pH of 2.5-3.5), this complex solubilises (complexes between condensed tannins and protein are soluble at both low pH of less than 3.5 and at high pH, 8.5) giving the opportunity of dissociation and solubilisation in the post ruminal sites where the proteins are digested and amino acids absorbed (Jones and Mangan, 1977).

Differences in rumen microbial protein synthesis are related to dietary nitrogen concentration of the diet. There were no significant differences in microbial protein synthesis; the animals on the commercial feed were as efficient as those on Silverleaf desmodium, Cowpea and Velvet bean in microbial protein production. According to Pathak (2008), the efficiency of microbial protein synthesis greatly differs in animals fed different diets, even within similar diets. Allantoin excretion was reported to be reduced in diets with high concentrations of structural carbohydrates (Puchala and Kulasek, 1992) such as Cowpea and commercial feed diets in this study. The optimum efficiency is usually obtained when the protein: energy ratio is balanced (Puchala and Kulasek, 1992). The efficiency of microbial protein synthesis was high in the lowest protein diets, the Velvet bean and Silverleaf desmodium forages. The slow liberation of energy from the structural carbohydrates, combined with the nitrogen content in these diets could have elevated the fixation of recycled N into microbial protein (Baloyi *et al.* 2008). Therefore, the apparent conversion of dietary nitrogen to microbial nitrogen increased.

The Cowpea and the commercial feed diets were relatively high in nitrogen and the microbial protein syntheses were also higher than the other diets. It is possible that when the protein supplied in the diet exceeds the requirement of the animal tissues for nucleic acid synthesis, the animal uses a smaller proportion of the nucleic acid derivatives. Probably nucleic acid metabolism in the tissues and the proportion of endogenous to microbial nucleic acid derivatives excreted in the urine were affected by nutrient supply through changes in the *de novo* synthesis and salvage of nucleic acid (Puchala and Kulasek, 1992). Microbial protein supplies approximately two thirds of the amino acids absorbed by ruminants, differentiated by a relatively high proportion of non-protein nitrogen which has an invaluable role in the nutrition of ruminant animals (Pathak, 2008). A study by Fathia *et al.* (2008) showed that Cowpea-Millet and Cowpea-Millet-Napier grass hybrid had high feeding quality that offer good alternative to the acute shortage of good quality forages.

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Goats supplemented with forage legumes had lower peptide balance, rumen N balance supply and feed metabolisable, rumen balance and peptide balance percent of the required, crude protein intake, feed metabolisable protein, dietary intake protein than those on goat feed. This can be explained by the fact that goats have to consume more (higher level of intake) to assure their maintenance requirements, and this results in increased passage rate of feed and lower forage digestibility (Cannas, 2004). Moreover, goats have selective feeding behaviour, have their intake affected by the particle size and the fibre content of the forage and can ruminate feed particles of smaller size (Pulina *et al.*, 2013). Therefore, they have to spend more time eating and ruminating each kilogram of feed and tend to have higher digestibility of grains and high-energy diets (Cannas, 2004).

Average daily gain for goats on forage legume supplements were lower than those on commercial goat feed because various predictors of diet quality that is dietary energy concentration, digestibility, CP concentration, filling effect of forages, NDF and factors associated with dietary particle size as predictors were used in the model. Moreover, variable NE for average daily gain (ADG) depends on relative body size, nutrition level and gender. However, animals were of relatively the same age, gender and weight.

Although feed metabolisable protein was significantly higher in the goat feed compared to the legume supplements, microbial protein concentration was higher in legume supplements than in the goat feed. This is because the amount of metabolisable protein per unit of CP intake is much higher under conditions where ruminal ammonia is totally utilised for microbial protein production than under conditions where ruminal ammonia is in excess. Thus, urea cost was significantly lower in the legume supplemented diets. Silverleaf desmodium had significantly the highest microbial protein concentration because the presence of tannins and substances in the feed increases the synthesis of microbial protein and the degradation of nitrogen compounds in the rumen is reduced.

The growth of microorganisms in the rumen directly depends on the energy supply. As a result high content of starch in feed causes a positive correlative link between organic matter digested in the rumen and the level of microbial protein synthesis. Microorganisms are able to synthesize protein at a high level if animals receive high-quality forage. The level of synthesis of microbial protein is not raised by the addition of low-quality forage. According to Stern *et al.* (2006), the growth of microorganisms in the rumen of sheep fed grass and concentrated forage is higher than in the rumen of sheep fed only grass or concentrated forage.

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6.7 Conclusion

The use of forage legumes in goat production could be a solution to nutritional challenges faced by resource-constrained farmers. Higher microbial protein synthesis obtained through legume supplementation will go a long way in supplying nitrogen and amino acids for growth and reproduction. Therefore, this may improve the performance of goats thus improving both human nutrition and income generation capacity through goat sales. The forage legumes may go a long way in improving livelihood if properly adopted in the communal system for feeding livestock especially goats.

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Milk production in goats on veld hay supplemented with selected forage legumes

7.1 Abstract

This research investigated the effects of supplementing low quality veld hay with *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* forage legumes to milk production in indigenous goats. Sixteen (Mashona) indigenous lactating goats weighing 30±2 kg (20±10 days post-partum) in their third parity were used in a completely randomized design experiment (14 d adaptation period and 20 d sample collection). Daily feed intake was significantly highest in goats on the commercial goat feed followed by goats on veld hay supplemented with Cowpea, Velvet bean and Silverleaf desmodium respectively ($P < 0.05$). There was significant ($P < 0.05$) difference in the milk yield of the goats on different treatments. Milk quality parameters were not significantly different ($P > 0.05$) among goats on different treatments. Daily live weight loss was significantly higher ($P < 0.05$) in goats on diets supplemented with Cowpea compared to the commercial goat feed, Silverleaf desmodium and Velvet bean. Feed conversion ratio was highest in goats supplemented with the Silverleaf desmodium, followed by Cowpea, however, they were not significantly different ($P > 0.05$). The levels of milk production attained in this study imply that some conventional commercial goat feed can be substituted with forage legumes without significantly affecting milk yield and composition in goats.

Keywords: indigenous goats; milk production; forage legume supplements

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7.2 Introduction

The goat is believed to be the most important domestic animal to man in the tropics (Debele *et al.,* 2013) because it has constantly proved to be a useful resource to poor and landless farmers in Africa. However, inadequate availability of feed is the major problem in livestock production in sub-Saharan African countries (Agyemang, 2000) because rearing systems in the communal areas are predominantly based on pastoralism and agro-pastoralism. Consequently, several authors have reported kid mortality of 10 to 30% (Awemu *et al.,* 1999; Turkson, 2003; Snyman, 2010b; Debele, 2013) and this negatively affects goat production. Relatively low birth weight, slow growth rate and insufficient milk production by goats were identified as the major limitations directly linked to elevated kid mortality and consequently a significant decline in total productivity (Husain 1993; Snyman, 2010a; 2010b). Snyman, (2010b) ranked the combination of udder problems and too little milk yield as the second major cause of kid mortality in South Africa. Therefore, strategies aimed at increasing milk production through the use of high quality, available and affordable forages for smallholder goats production could turn out to be crucial.

Alternative affordable sources of feed such as forage legumes that provide protein, vitamins and mineral elements which are lacking in mature natural grassland pastures, especially during the dry season (Baloyi *et al.*, 2008) can be an option. The superior protein levels (11 to 22 %) in these legume forages suggest that they have a great potential for utilisation as protein supplements in goat diets. However, there is need for an in-depth study of the utilisation of different forage legumes for milk production in goats. Gusha *et al.,* (2014) reported that supplementing with legume hays could improve poor quality roughage intake in goats. Snyman, (2010b) observed lower kid mortality in supplemented goats compared to those not supplemented during lactation.Therefore, this research was designed to investigate the effects of supplementing low quality veld hay with *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* forage legumes on milk production in indigenous goats.

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7.3 Materials and methods

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7.3.1 Forage

Forage materials were grown as described in Chapter 3 and harvested from legume plantations grown in summer (2013-2014) at Henderson Research Institute Mazowe farm (altitude 1630m; average day-length is 13.2 hours in summer to 11.1 hours in winter; rainfall averages 873 mm per year, temperature mean maximum are from 19.5°C (July) to 24.6°C (January); hot summer is between September and December with October being the hottest month of the year with maximum temperature above 30°C, species (*Mucuna pruriens, Vigna unguiculata* and *Desmodium uncinatum*)) were air dried under shade and milled into coarse feed (25 mm).

7.3.2 Animals and housing

The experiment was carried out at the University of Zimbabwe's Animal Science Department. Sixteen (Mashona) indigenous lactating goats with 30±2 kg body weight (20±10 d post-partum) in their third parity were used. The animals were vaccinated against enterotoxaemia (pulpy kidney) and injected with a broad spectrum anthelmintic before kidding. The sixteen animals were randomly allocated to 4 treatments consisting of veld hay supplemented at 30% of 5% body weight (BW), with (T1) commercial goat feed (CF); (T2) Velvet bean (VB), (T3) Silverleaf desmodium (SD) and (T4) Cowpea (CW). The goats were weighed and moved into metabolism crates for individual feeding and total milk production (20±10 days after kidding).

7.3.3 Experimental procedure

There were adaptation periods of 14 d and 20 d for milk production and sample collection. Water was offered at *ad libitum*. Samples of diets and feed refusals were also obtained and composited for each period. The goats were hand-milked twice per day for the experimental period of 20 days. After emptying the udder, oxytocin (oxytocin-20®manufactured by Interchemie Holland) was injected intramuscularly and after 10 minutes, the goats were milked again. Subsequent to milking, the kids were weighed (using a digital balance) and allowed to suckle their dams and weighed again following suckling to prevent drying off of the lactating does and to determine the amount of milk consumed. The quantity of milk withdrawn from each goat (total of hand milked plus kid suckled) was weighed on a digital balance. Milk sub-samples of 10 mL were collected daily for chemical analysis.

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Metabolisable energy (ME), dietary metabolisable energy (DME), metabolisable protein intake (MPI) and metabolisable protein concentration (MPC) were calculated using the Small Ruminant Nutritional System (SRNS) by Sassari University (Italy), Texas A&M University, and Cornell University version 1.9. 5105.

7.3.4 Chemical analysis

The samples collected were analysed as described in Chapter 3. Neutral detergent fibre (NDF) and lignin were analysed according to Goering and Van Soest (1979). The milk samples were analysed for fat, lactose and protein using a Bentley (2000) infrared milk analyser.

7.3.5 Statistical analysis

Feed conversion ratio (FCR) for milk production was determined by dividing the milk yield + body weight change by dry matter intake. Data was analysed using the generalized linear model procedure of SAS (SAS 2013). The means were separated using PDIFF. The following model was used:

$$Y_{ij}=\mu+R_i+A_j+\varepsilon_{ij}$$

Y_{ijk} was response variable (milk production, feed intake)

μ was the overall mean

R_i was the effect of *ith* treatment (i = 1, 2, 3 and 4)

A_j was the animal effect

ε_{ijk} were the random residuals with the a distribution of $N \sim (0\sigma^2_E)$

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7.4 Results

The chemical compositions of the dietary ingredients are shown in Table 7.1. Crude protein content of Cowpea, Velvet bean, Silverleaf desmodium and commercial goat feed were not significantly different ($P > 0.05$). Veld hay contained the lowest amount of crude protein. Cowpea had the highest CP content and a significantly higher level NDF and fat content ($P < 0.05$). Veld hay contained the highest level of NDF in comparison to the legumes. The commercial goat feed contained significantly ($P < 0.05$) higher levels of starch, DM, ash, Ca and P compared to the other experimental diets. Velvet bean had significantly the highest level of lignin compared to Cowpea and Silverleaf desmodium.

Table 7.2 shows the total dry matter intake (DMI), metabolisable energy (ME), dietary metabolisable energy (DME), CP, metabolisable protein intake (MPI) and metabolisable protein concentration (MPC). Daily feed intake on the treatment diets and hay was significantly highest ($P < 0.05$) in goats supplemented with goat feed followed by the goats on Cowpea, Velvet bean and Silverleaf desmodium respectively. Total DMI and ME intake were not different among goats on different diets ($P > 0.05$). Dietary ME intake was significantly highest ($P < 0.05$) in goats supplemented with Velvet bean and Silverleaf desmodium in comparison to the commercial goat feed and Cowpea which also differed between themselves ($P < 0.05$). Goats supplemented with Cowpea had the highest crude protein intake followed by commercial goat feed, Velvet bean and Silverleaf desmodium correspondingly. Metabolisable protein intake and metabolisable protein were not significantly different among the treatments.

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Table 7.1: Mean chemical composition (% dry matter basis) of commercial goat feed (CF), *Vigna unguiculata* (L.), Walp (CW), veld hay, *Desmodium uncinatum* (SD) and *Mucuna pruriens* (VB)

Nutrient composition	Experimental diet					
	VB	SD	CW	CF	Hay	SEM
DM	91.5^{b}	92.2^{b}	88.4^{c}	94.5^{a}	88.6^{c}	1.29
Crude protein	15.6^{a}	16.9^{a}	17.4^{a}	15.4^{a}	8.58^{b}	1.22
NDF	56.1^{c}	47.9^{e}	64^{b}	53.3^{d}	67.5^{a}	1.5
Lignin	23.5^{a}	22.9^{ab}	21.2^{b}	9.12^{d}	7.89^{c}	1.37
Starch	8.49^{b}	9.87^{b}	8.87^{b}	17.3^{a}	7.31^{b}	3.82
Fat	2.28^{c}	3.01^{b}	4.05^{a}	1.59^{d}	1.32^{d}	0.51
Ash	7.77^{b}	8.11^{b}	9.28^{b}	23.5^{a}	6.91^{b}	2.19
Phosphorus	1.87^{b}	2.19^{b}	2.57^{b}	3.79^{a}	0.78^{c}	0.44
Calcium	2.57^{c}	4.09^{b}	3.55^{b}	7.87^{a}	1.24^{d}	0.42

$^{a, b, c}$ Means in the same row with different superscripts are significantly different ($P < 0.05$), ND= not determined, NDF= neutral detergent fibre, SEM= standard error of the mean

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Milk yield in goats supplemented with commercial goat feed and Cowpea were not significantly different ($P > 0.05$). However, they were both significantly high ($P < 0.05$) compared to goats on Silverleaf desmodium and Velvet bean respectively (Table 7.3). Fat, protein, lactose and ash in the milk were not different between the animals of the four treatments ($P > 0.05$). Daily live weight loss was significantly higher in Cowpea compared to commercial goat feed, Silverleaf desmodium and Velvet bean. Feed conversion ratio was highest in goats on the Silverleaf desmodium followed by Cowpea, however, they were not significantly different ($P > 0.05$) among treatments.

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Table 7.2: Average daily intake in goats given veld hay supplemented with Velvet bean (VB), Silverleaf desmodium (SD), Cowpea (CW) legume hays and commercial goat feed (CF) (control)

Parameter	Experimental diets				
	VB	SD	CW	CF	SEM
Hay intake DM g/day	305^{c}	306^{c}	321^{b}	339^{a}	4.78
Supplement intake g/day	394^{c}	366^{d}	413^{b}	445^{a}	7.08
Total DMI g/day	698	669	729	777	41.1
ME intake Mcal/day	1.33	1.28	1.12	1.33	0.14
Dietary ME Mcal/day	1.93^{a}	1.92^{a}	1.53^{c}	1.71^{b}	0.02
CP intake g/day	86.5^{b}	85^{b}	105.^{a}	96.5^{ab}	5.03
MP intake g/day	60.3^{b}	58.3^{b}	54^{b}	94.8^{a}	4.14
MP concentration g/kg	87.4^{c}	87.3^{c}	74^{c}	122^{a}	4.09

$^{a, b, c}$ Means in the same row with different superscripts are significantly different ($P < 0.05$), SEM= standard error of the mean

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Table 7.3: Milk production and milk composition of goats given veld hay supplemented Velvet bean (VB), Silverleaf desmodium (SD), Cowpea (CW) legume hays and commercial goat feed (CF) (control)

Parameter	Experimental diets				
	VB	SD	CW	CF	SEM
Total milk g/animal/day)	405^{c}	425^{b}	447^{a}	454^{a}	5.19
Fat content %	5.63	5.4	5.7	5.55	0.43
Protein %	5.5	5.4	5.55	5.5	0.26
Lactose %	4.66	4.67	4.7	4.7	0.05
Ash %	0.68	0.69	0.7	0.7	0'03
Live weight change g/day	-56.3^{c}	-61.3^{bc}	-81^{a}	-65^{bc}	6.95
FCR	0.50	0.55	0.51	0.50	0.07

$^{a, b, c}$ Means in the same row with different superscripts are significantly different (P <0.05), FCR= feed conversion ratio, SEM= standard error of the mean

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7.5 Discussion

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Ravhuhali *et al.,* (2011) observed that most of the cultivars of Cowpea contained CP above 15% and this is collaborated by the results in this study where the CP was 17%. The CP levels in all the legumes were within the range of 120-230 g/kg DM as reported by Ranjbar, (2007) and Ajayi *et al.,* (2009). Lignin concentration of the Velvet bean was within the range of the values reported by Agyemang *et al.,* (2000). Maasdorp and Titterton, (1997) mentioned that little is known about the maturity stage at which the nutritive value of Velvet bean is optimized. Chikagwa-Malunga *et al.,* (2009) stated that maturity affects the nutritive value and secondary compound concentration in seeds, leaves, stems and pods.

In this study total dry matter intake was not significantly different among all the treatments and this agrees with on observation by Juma *et al.,* (2006) where the total dry matter intake in lactating goats was not affected by legume supplementation. Nevertheless, Voicu *et al.,* (2008) observed higher DMI than those observed in this study. Velvet bean legume consumption was lower than that of Cowpea and the commercial goat feed. Muinga *et al.,* (2003) reported that cows fed *Gliricidia* consumed more legume than those fed Velvet bean. DMI of Silverleaf desmodium forage legume was the lowest and this can be attributed to the high levels of tannins associated with the legume as reflected by the chemical composition.

Milk production trends generally followed those of DMI and CPI and this was also noted by Nyambati *et al.,* (2003).Supplementation of basal hay with forage legumes results in milk production that was significantly different some treatment diets. Juma *et al.,* (2006) showed that Velvet bean and Clitoria can give lactation performance similar to *Gliricidia* as nitrogen supplements when Napier grass basal diet was used. Moreover, the frequency of milking the goats which was 2 times a day in this trial has an effect on total milk production, fat and protein content of the milk. Increasing milking frequency from once a day to 2 times a day increases milk yield from 7% to 38% in dairy cows (Stelwagen and Knight, 1997), 15% to 48% in ewes (Negrao *et al.,* 2001) and 6% to 35% in dairy goats (Capote *et al.* 1999).

Feed conversion ratio for milk production also increases with increasing milk production. The discrepancy in milk production reported by many authors may be due to differences in the level of nutrition, level of production, lactation stage, breed and duration of milking. It should also be noted that when less frequent milking is prolonged, the decrease in milk yield is sustained by sequential developmental adaptations, initially as a down-regulation of cellular

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differentiation (Wilde *et al.* 1987) and later as net loss in mammary cell number via apoptosis (Li *et al.* 1999).

It was evident that as the lactation progressed, most of the animals lost weight during the milking period indicating higher demands for dry matter intake at higher milking production for the indigenous breed. This result substantiates the findings of Bar-Peled *et al.*, (1995) that increased dry matter intake by high producing cows did not compensate for the increased energy demands, thus the cows lost body weight. The authors further reported that dry matter intake increased by approximately 15% when milk production was increased by increasing the frequency of milking and cows on daily milking consumed 10-11% more dry matter.

Increase in dietary fibre content reduces DMI in goats as observed by Kawas *et al.*, (1991) that when forage-to-concentrate ratio is increased DMI decrease. Additionally, DMI is regulated by the physical fill in ruminants. Luginbuhl *et al.*, (2000) reported that DMI decreases linearly with increasing dietary NDF (52.4 to 62.1 %) in Boer goats and their crosses. Although, the goats were allowed *ad libitum* dry matter intake they did not respond to the high energy demand associated with milk yield by increasing dry matter intake. The DMI were not significantly different and this was also observed by Barnes *et al.* (1990). This may be due to the explanation by Blake and Custodio (1984) that increased milk yield and milk yield to feed intake ratio occurs only as long as tissue reserves are able to support the nutrient required for milk yield. Voicu *et al.*, (2008) observed negative nitrogen balance values in lactating goats. All the goats lost weight during the trial meaning some of the milk harvested was as a result of mobilization of body reserves.

The values obtained in this study on the efficiency of utilisation of feed by the goats for milk production are within the ranges obtained by other authors. Williams *et al.* (2012) reported an efficiency of feed utilisation for milk production in the range of 0.237-0.431 in (West African Dwarf (WAD) goats. The efficient utilisation of feed by the ruminants may possibly be accredited to the physiological status, breed of the animals and the quality of the ration offered. Blake and Custodio (1984) reported that feed efficiency for milk production depends on diet, environmental factors and on genetic ability of the animal to utilise these inputs to produce milk. According to Williams *et al.* (2012) hormones such as insulin, growth hormone, and prolactin cooperate to control partitioning of dietary energy into milk and body tissue and this interaction is linked with genetic variation for milk production within and between breeds.

Voicu *et al.*, (2008) recorded higher milk yield but less milk protein (3.16% -3.72%) and milk fat (3.17%-3.83%) in lactating Carpathian goats on mixed legumes diets. Morand-Fehr *et al.*,

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(2007) suggested that the fat content of goat milk is not only determined by the feed intake of goats but also the genetic potential. Monzón-Gil *et al.*, (2010) revealed that the percentage of lactose usually is not modified by dietary manipulation and in our study the percentage of milk lactose was not significantly different among treatments. The levels of fat and lactose are within the range of those observed by Zumbo *et al.*, (2004) on Nebrodi goats.

The study of Muinga *et al.*, (2000) showed no difference in milk yield in cows fed Velvet bean compared to those fed other legumes which included *Gliricidia* and Clitoria. However, according to Tendonkeng *et al.*, (2012), supplementary feeding with Leucena and Caliandra increased the overall yield of milk per animal, milk protein content and growth of the supplemented ruminants during the dry season. On the other hand, a study on cows supplemented with fresh forage of *Gliricidia*, Clitoria, Lablab or Velvet bean showed no significant difference in the mean daily milk yield (Muinga *et al.* 2003). A similar observation was made in this study.

Fat supplementation to early lactating diets in cows can increase energy density and increase milk production (Rapetti *et al.*, 2002). Supplementation of ruminant diets with a fat source results in greater efficiency for milk production (Hosam, 2011). However, milk production also increases with increase in parity in goats (Zumbo *et al.*, 2004). The levels of milk production attained in this study imply that some commercial goat feed can be substituted with forage legumes without affecting milk yield and composition in goats.

7.6 Conclusion

The three forage legumes used in this study can provide good quality forage for use as supplement to relatively low quality basal diets of veld hay on animals in the smallholder communal areas. Milk produced by these indigenous goats has unique characteristics (higher fat and protein content) making it better than the milk of specialised dairy goats.

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CHAPTER 8

Efficiency of utilisation of protein for milk production in goats on veld hay supplemented with selected legume forages

8.1 Abstract

The experiment was carried out at the University of Zimbabwe Animal Science animal house. Sixteen (East-African) indigenous lactating does 30±2 kg (20±10 days post-partum) in their third parity were used in a completely randomised design experiment. After kidding the sixteen animals were randomly allocated to 4 treatments consisted of veld hay supplemented at 30% of 5% body weight (BW) with (1) commercial goat feed (CF); (2) Velvet bean (VB), (3) Silverleaf desmodium (SD) and (4) Cowpea (CW) with four replicates. Goats supplemented with commercial goat feed produced significantly ($P < 0.05$) the lowest amount of individual amino acids concentration in milk compared to goats on Velvet bean, Silverleaf desmodium and Cowpea. Goats supplemented with Velvet bean, Silverleaf desmodium and Cowpea showed no significant ($P > 0.05$) difference in the concentration of individual amino acids secreted in milk. Commercial goat feed had significantly ($P < 0.05$) the lowest efficiencies of utilisation of individual amino acids for milk production. Among the legume forages Silverleaf desmodium showed significantly ($P < 0.05$) the lowest efficiencies of utilisation on histidine, lysine and tyrosine. Whereas, Velvet bean had significantly the highest efficiency of utilisation of phenylalanine ($P < 0.05$) compared to Silverleaf desmodium and Cowpea. Cowpea showed the highest efficiency of utilisation of aspartic acid ($P < 0.05$) for milk production. Efficiencies of utilisation of arginine, glutamic acid, glycine, isoleucine, luecine, methionine, proline, serine, threonine and valine were not significantly ($P < 0.05$) different in Velvet bean, Silverleaf desmodium and Cowpea. Therefore, the study showed that the efficiency for converting feed amino acid to milk amino is variable in legume forages.

Keywords: legume forages, amino acids, milk production, efficiency

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8.2 Introduction

Numerous trials have been undertaken to improve utilisation of dietary nutrients by feed supplementation with various additives from different sources (Abd El-Latif *et al.*, 2004 and Ahmed *et al.*, 2008). However, some of these studies have revealed that the use of tree and shrub forages as supplements to different basal diets led to improved ruminant livestock performance in terms of milk production, growth rate and reproductive efficiency (Ondiek *et al.*, 2000). Legumes (Clitoria, Cowpea and Velvet bean) are recommended for soil fertility improvement (Saha *et al.*, 1997) due to their high nitrogen content; they can also be used as nitrogen supplements in ruminant rations. Moreover, Nyambati *et al.*, (2003) showed that milk production can be increased by supplementing with Velvet bean. Legumes can be used as supplements for ruminants fed low quality forages such as maize stover and tropical-grass hays (Muinga *et al.*, 2003).

Free amino acids are the major form in which nitrogen is absorbed to meet the tissue needs, milk production and growth of ruminants (NRC, 1985). Amino acid profiles available for absorption in the small intestine (metabolisable protein) reflect predominantly the combination of microbial protein, ruminally undegradable protein and endogenous proteins (Löest, 2006). Therefore, most of the efficiency values reported for a specific amino acid are dependent on the most limiting nutrient (energy or another amino acid). An amino acid is utilised less efficiently when a nutrient other than that amino acid limits protein synthesis compared to when that amino acid is first limiting (Löest, 2006). Studies have shown that Velvet bean, Silverleaf desmodium and Cowpea forages are good CP supplement for ruminants fed low-quality forages such as maize stover and tropical grass hays (Maasdorp and Titterton, 1997; Muinga *et al.*, 2003; Macoon and Sollenberger, 2003). Whitelaw *et al.*, (1986) Hanigan *et al.*, (2006) and Apelo (2014) reported that the efficiency of converting protein to milk protein is variable. Therefore, there is a need for an in-depth study of the efficiency of utilisation of amino acids in different legume forages for milk production to validate the results and make inferences. This research focused on determining efficiency of utilisation of individual amino acids on milk production using *Desmodium uncinatum, Mucuna pruriens* and *Vigna unguiculata* forage legume supplements in indeginous goats.

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8.3 Materials and methods

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8.3.1 Feed

Forage materials harvested from legume plantations grown in summer at Henderson Research Institute in Zimbabwe were prepared as described in Chapter 7.

8.3.2 Animals and Experimental procedure

The experiment was carried out at the University of Zimbabwe Animal Science animal house and was setup as described in Chapter 7.

Sampling procedure and data collection

Adaptation period of 14 days and 20 days for milk production and sample collection were allowed to the goats. Samples of diets were collected daily during each collection period, composited and analysed as described in Chapter 7.

8.3.3 Chemical analyses

Samples of diets were collected daily during each collection period, composited and analysed as described in Chapter 7. Amino acids were analysed at Lactolab Agricultural Research Council Pretoria using the Shimadzu LC-20 AD HPLC with CBM-20A communication bus module, SIL-20A Auto sampler and RF-20A Fluorescence detector. Amino acid standard L-Norvaline (Sigma) was used.

Efficiency

Efficiency of utilisation was calculated as:
Total amino acid in milk (g) / Total amino acid in take (g) (amino acid in hay + amino acid in supplement) *100

8.3.4 Statistical analysis

Data was analysed using the general linear model procedure of SAS (SAS 2013). The means were separated using the adjusted Tukey's method. The following model was used:

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$Y_{ijk}=\mu + R_i + A_j + \varepsilon_{ijk}$

Y_{ijk} was response variable (Amino acids intake)

μ was the overall mean

R_i was the effect of *ith* treatment (i = 1, 2, 3 and 4)

A_j was the animal effect

ε_{ijk} were the random residuals with the a distribution of $N \sim (0\sigma^2_E)$

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8.4 Results

The commercial goat feed contained a significantly ($P < 0.05$) higher concentration of individual amino acids compared to Silverleaf desmodium, Velvet bean, Cowpea and veld hay (Table 8.1). Silverleaf desmodium contained a significantly ($P < 0.05$) higher concentration of amino acids than Velvet bean and Cowpea. Methionine and cysteine levels were not significantly different in the three forage legumes ($P > 0.05$). Veld hay contained significantly ($P < 0.05$) the lowest concentration of individual amino acids (Table 8.1).

Goats supplemented with Cowpea legume forage consumed significantly ($P < 0.05$) the highest amounts of alanine, aspartic acid, glutamic acid, luecine and valine in veld hay compared to the goats on Silverleaf desmodium, Velvet bean and commercial goat feed. There was however, no significant difference ($P > 0.05$) in the rest of individual amino acids in the veld hay consumed by goats in all treatments (Table 8.2). There was no significant ($P > 0.05$) difference observed in methionine consumed in all the three legume forages (Table 8.3). Goats on the commercial goat feed consumed significantly ($P < 0.05$) the highest amount of individual amino acids compared to goats on Velvet bean, Silverleaf desmodium and Cowpea (Table 8.3). Whereas, goats supplemented with Silverleaf desmodium consumed significantly ($P < 0.05$) the highest amount of amino acids compared to goats on Velvet bean and Cowpea (Table 8.4).

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Table 8.1: Mean amino acid concentration (g/kg CP) in *Mucuna pruriens* (Velvet bean), *Desmodium uncinatum* (Silverleaf desmodium), *Vigna unguiculata* (L.), Walp (Cowpea), commercial goat feed and veld hay

Amino acid	Feeds					
	VB	SD	CW	Veld hay	CF	SEM
Glycine	41.2^{c}	47.9^{b}	39.4^{d}	12.4^{e}	52.3^{a}	0.21
Alanine	44.1^{c}	59.5^{a}	43.5^{c}	13.2^{e}	50.0^{b}	0.62
Arginine	38.0^{d}	52.4^{b}	47.6^{c}	11.4^{e}	55.8^{a}	0.48
Aspartic Acid	80.5^{d}	175.9^{b}	90.5^{c}	24.1^{e}	193.1^{a}	0.44
Cysteine	6.28^{b}	7.43^{b}	6.50^{b}	ND	10.5^{a}	0.40
Glutamic acid	83.8^{d}	108.9^{b}	104.4^{c}	25.1^{e}	115.7^{a}	0.62
Histidine	14.6^{d}	24.4^{b}	16.5^{c}	ND	28.1^{a}	0.30
Isoleucine	36.5^{b}	47.2^{a}	33.6^{c}	11.0^{e}	50.0^{a}	0.83
Leucine	59.4^{c}	81.0^{b}	59.7^{c}	17.8^{d}	85.4^{a}	0.49
Lysine	39.0^{c}	67.5^{a}	42.8^{b}	11.7^{d}	70.7^{a}	1.02
Methionine	13.4^{b}	14.6^{b}	13.5^{b}	ND	16.9^{a}	0.17
Phenylalanine	40.6^{c}	59.8^{a}	52.4^{b}	12.2^{e}	55.0^{a}	0.84
Proline	37.6^{c}	52.5^{a}	44.7^{b}	11.3^{d}	55.1^{a}	0.74
Serine	33.0^{c}	54.6^{a}	39.6^{b}	9.91^{d}	50.3^{a}	0.38
Threonine	32.4^{c}	54.9^{a}	40.8^{b}	9.72^{e}	60.1^{a}	0.63
Tyrosine	22.2^{c}	30.5^{a}	24.8^{b}	6.66^{e}	36.9^{a}	1.11
Valine	43.5^{c}	60.9^{a}	46.6^{b}	13.1^{e}	65.0^{a}	1.06

^{abc} Means in the same row with different superscript are significantly different at $P < 0.05$, SEM standard error of the mean, ND-failed to be detected

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Table 8.2 Amino acid intake (g) in veld hay consumed by goats supplemented with *Mucuna pruriens* (VB), *Desmodium uncinatum* (SD), *Vigna unguiculata* (L.), Walp (CW) and commercial goat feed (CF)

Amino acid	Treatment diets				
	VB	SD	CW	CF	SEM
Glycine	3.56	3.45	3.62	3.31	0.05
Alanine	3.79^{ab}	3.68^{ab}	3.86^{a}	3.53^{b}	0.06
Arginine	3.27	3.17	3.33	3.05	0.05
Aspartic Acid	6.92^{ab}	6.71^{bc}	7.04^{a}	6.44^{c}	0.11
Glutamic acid	7.20^{ab}	6.99^{bc}	7.34^{a}	6.71^{c}	0.11
Histidine	*	*	*	*	*
Isoleucine	3.16	3.06	3.21	2.94	0.05
Leucine	5.10^{a}	4.96^{ab}	5.20^{a}	4.76^{b}	0.08
Lysine	3.36	3.26	3.42	3.13	0.05
Methionine	*	*	*	*	*
Phenylalanine	3.21	3.12	3.27	2.99	0.05
Proline	3.24	3.15	3.30	3.02	0.05
Serine	2.84	2.76	2.90	2.65	0.04
Threonine	2.79	2.71	2.84	2.60	0.04
Tyrosine	1.91	1.85	1.95	1.78	0.03
Valine	3.76^{ab}	3.65^{ab}	3.83^{a}	3.50^{b}	0.06

^{abc} Means in the same row with different superscript are significantly different at $P < 0.05$

* trace, SEM standard error of the mean

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Table 8.3 Amino acid intake (g) in *Mucuna pruriens* (VB), *Desmodium uncinatum* (SD), *Vigna unguiculata* (L.), Walp (CW) and commercial goat feed (CF) consumed by goats

Amino acid	Treatments				
	VB	SD	CW	CF	SEM
Glycine	14.4^{c}	16.8^{b}	13.8^{c}	18.3^{a}	0.47
Alanine	15.4^{c}	20.8^{a}	15.2^{c}	17.5^{b}	0.58
Arginine	13.3^{d}	18.4^{b}	16.7^{c}	19.5^{a}	0.61
Aspartic Acid	28.2^{c}	61.6^{b}	31.7^{c}	67.6^{a}	4.52
Glutamic acid	29.3^{c}	38.1^{b}	36.5^{b}	40.5^{a}	1.08
Histidine	5.11^{b}	8.55^{a}	5.79^{b}	9.83^{a}	0.50
Isoleucine	12.8^{b}	16.5^{a}	11.7^{b}	17.5^{a}	0.63
Leucine	20.8^{b}	28.3^{a}	20.9^{b}	29.9^{a}	1.08
Lysine	13.7^{c}	23.6^{a}	15.0^{b}	24.7^{a}	1.28
Methionine	4.65	5.12	4.74	5.91	0.13
Phenylalanine	14.2^{b}	20.9^{a}	18.4^{a}	19.3^{a}	0.64
Proline	13.1^{c}	18.4^{a}	15.6^{b}	19.3^{a}	0.62
Serine	11.6^{d}	19.1^{a}	13.9^{c}	17.6^{b}	0.77
Threonine	11.3^{c}	19.2^{a}	14.3^{b}	21.0^{a}	1.0
Tyrosine	7.77^{c}	10.7^{b}	8.67^{c}	12.9^{a}	0.51
Valine	15.2^{b}	21.3^{a}	16.3^{b}	22.8^{a}	0.82

^{abc} Means in the same row with different superscript are significantly different at $P < 0.05$
SEM standard error of the mean

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Table 8.4 Total Amino acid intake of goats fed veld hay supplemented with *Mucuna pruriens* (VB), *Desmodium uncinatum* (SD), *Vigna unguiculata* (L.), Walp (CW) and commercial goat feed (CF)

Amino acid	Treatments				
	VB	SD	CW	CF	SEM
Glycine	18.0^{c}	20.2^{b}	17.4^{d}	21.6^{a}	0.44
Alanine	19.2^{c}	24.5^{a}	19.1^{c}	21.0^{b}	0.57
Arginine	16.6^{d}	21.5^{b}	20.0^{c}	22.6^{a}	0.59
Aspartic Acid	35.1^{d}	68.3^{b}	38.7^{c}	74.0^{a}	4.47
Glutamic acid	36.5^{d}	45.1^{b}	43.9^{c}	47.2^{a}	1.04
Histidine	5.11^{c}	8.55^{b}	5.79^{c}	9.83^{a}	0.50
Isoleucine	15.9^{c}	19.6^{b}	15.0^{d}	20.4^{a}	0.60
Leucine	25.9^{d}	33.3^{b}	26.1^{c}	34.6^{a}	1.04
Lysine	17.0^{d}	26.9^{b}	18.4^{c}	27.9^{a}	1.26
Methionine	4.65^{c}	5.12^{b}	4.74^{c}	5.92^{a}	0.13
Phenylalanine	17.4^{d}	24.1^{a}	21.6^{c}	22.2^{b}	0.63
Proline	16.4^{d}	21.5^{b}	18.9^{c}	22.3^{a}	0.60
Serine	14.4^{d}	21.9^{a}	16.8^{c}	20.3^{b}	0.75
Threonine	14.1^{d}	21.9^{b}	17.1^{c}	23.6^{a}	0.98
Tyrosine	9.68^{d}	12.5^{b}	10.6^{b}	14.7^{a}	0.50
Valine	19.0^{d}	25.0^{b}	20.2^{c}	26.3^{a}	0.80

^{abc} Means in the same row with different superscript are significantly different at $P < 0.05$
SEM standard error of the mean

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Goats supplemented with commercial goat feed produced significantly (P <0.05) the lower amounts of individual amino acids concentration in milk compared to goats on Velvet bean, Silverleaf desmodium and Cowpea. Goats supplemented with Velvet bean, Silverleaf desmodium and Cowpea showed no significant (P >0.05) differences in the concentrations of individual amino acids secreted in milk (Table 8.5). Goats on the commercial goat feed produced significantly (P <0.05) the lowest amount of total leucine, arginine, glutamic acid, isoleucine, lysine, proline and serine in milk (Table 8.6). The rest of the total individual amino acids were not significantly (P >0.05) different among the treatments.

Methionine showed the highest value for efficiency of utilisation. Commercial goat feed had significantly (P <0.05) the lowest efficiency of utilisation of individual amino acids for milk production. Among the legume forages Silverleaf desmodium showed significantly (P <0.05) the lowest efficiencies of utilisation on histidine, lysine and tyrosine. Whereas, Velvet bean had significantly the highest efficiency of utilisation of phenylalanine (P <0.05) compared to Silverleaf desmodium and Cowpea. Cowpea showed the highest efficiency of utilisation of aspartic acid (P <0.05) for milk production. The efficiencies of utilisation of arginine, glutamic acid, glycine, isoleucine, luecine, methionine, proline, serine, threonine and valine were not significantly (P <0.05) different in Velvet bean, Silverleaf desmodium and Cowpea (Table 8.7).

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Table 8.5: Amino acid composition (g/kg) in milk produced from goats on *Mucuna pruriens* (VB), *Desmodium uncinatum* (SD), *Vigna unguiculata* (L.), Walp (CW) and commercial goat feed (CF) and veld hay.

Amino acid	Experimental diets				
	VB	SD	CW	CF	SEM
Glycine	1.2^{a}	0.9^{a}	1.0^{a}	0.4^{b}	0.13
Alanine	1.2^{a}	1.5^{a}	1.6^{a}	0.6^{b}	0.14
Arginine	3.6^{a}	3.3^{a}	3.4^{a}	1.6^{b}	0.38
Aspartic acid	1.6^{b}	3.9^{a}	3.6^{a}	2.3^{ab}	0.38
Glutamic acid	5.8^{ab}	9.5^{a}	10.1^{a}	4.4^{b}	0.78
Histidine	2.6^{a}	1.1^{ab}	1.3^{ab}	0.4^{c}	0.29
Isoleucine	2.5^{a}	2.5^{a}	2.5^{a}	1.3^{b}	0.22
Leucine	4.2^{a}	4.5^{a}	4.7^{a}	2.3^{b}	0.39
Lysine	4.6^{a}	5.2^{a}	5.6^{a}	2.8^{b}	0.33
Methionine	2.2^{a}	2.3^{a}	2.4^{a}	1.0^{b}	0.31
Phenylalanine	3.2^{a}	2.4^{a}	2.5^{a}	1.2^{b}	0.38
Proline	7.5^{a}	5.0^{ab}	5.3^{ab}	2.4^{b}	0.96
Serine	2.7^{a}	2.5^{a}	2.7^{a}	1.2^{b}	0.29
Threonine	2.4^{a}	2.3^{a}	2.3^{a}	0.9^{b}	0.27
Tyrosine	2.4	2.6	3.4	1.8	0.23
Valine	3.4^{a}	3.3^{a}	3.4^{a}	1.6^{a}	0.32

^{abc} Means in the same row with different superscript are significantly different at $P < 0.05$
SEM standard error of the mean

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Table 8.6: Total amino acid in milk (g/Kg CP) produced from goats on *Mucuna pruriens* (VB), *Desmodium uncinatum* (SD), *Vigna unguiculata* (L.), Walp (CW) and commercial goat feed (CF) and veld hay.

Amino acid	Treatments				
	VB	SD	CW	CF	SEM
Glycine	0.46	0.38	0.42	0.18	0.05
Alanine	0.47	0.65	0.69	0.27	0.06
Arginine	1.45^{a}	1.41^{a}	1.49^{a}	0.72^{b}	0.15
Aspartic Acid	0.71	1.64	1.62	1.02	0.17
Glutamic acid	2.33^{b}	4.02^{a}	4.52^{a}	2.01^{b}	0.34
Histidine	1.06	0.45	0.59	0.18	0.12
Isoleucine	1.00^{a}	1.01^{a}	1.09^{a}	0.60^{b}	0.09
Leucine	1.67^{ab}	1.9^{a}	2.07^{a}	1.04^{b}	0.16
Lysine	1.84^{a}	2.19^{a}	2.51^{a}	1.26^{b}	0.17
Methionine	0.88	0.97	1.08	0.44	0.13
Phenylalanine	1.30	1.00	1.09	0.54	0.15
Proline	1.38^{b}	2.13^{a}	2.35^{a}	1.09^{b}	0.19
Serine	1.10^{a}	1.06^{a}	1.20^{a}	0.52^{b}	0.12
Threonine	0.95	0.95	1.01	0.41	0.11
Tyrosine	0.97	1.09	1.52	0.82	0.10
Valine	1.36^{a}	1.40^{a}	1.49^{a}	0.73^{b}	0.13

^{abc} Means in the same row with different superscript are significantly different at $P < 0.05$
SEM standard error of the mean

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Table 8.7: Efficiency (%) of utilisation of individual amino acids in goats on veld hay supplemented with *Mucuna pruriens* (VB), *Desmodium uncinatum* (SD), *Vigna unguiculata* (L.), Walp (CW) and commercial goat feed (CF) for milk production

Amino acid	Treatments				
	VB	SD	CW	CF	SEM
Glycine	2.58^{a}	1.89^{a}	2.43^{a}	0.84^{b}	0.44
Alanine	2.46^{a}	2.63^{a}	3.62^{a}	1.30^{b}	0.56
Arginine	8.75^{a}	6.53^{a}	7.47^{a}	3.17^{b}	0.59
Aspartic Acid	2.01^{b}	2.39^{b}	4.18^{a}	1.38^{c}	4.47
Glutamic acid	6.36^{ab}	8.90^{a}	10.3^{a}	4.26^{b}	1.04
Histidine	20.8^{a}	5.32^{c}	10.2^{b}	1.85^{d}	0.50
Isoleucine	6.25^{a}	5.14^{a}	7.31^{a}	2.94^{b}	0.60
Leucine	6.45^{a}	5.69^{a}	7.96^{a}	3.01^{b}	1.04
Lysine	10.8^{a}	8.12^{b}	13.7^{a}	4.52^{c}	1.26
Methionine	18.9^{a}	18.9^{a}	22.8^{a}	7.48^{b}	0.13
Phenylalanine	7.46^{a}	4.14^{bc}	5.05^{b}	2.45^{c}	0.63
Proline	8.39^{a}	9.89^{a}	12.4^{a}	4.83^{b}	0.60
Serine	7.63^{a}	4.85^{ab}	7.19^{a}	2.58^{b}	0.75
Threonine	6.70^{a}	4.35^{a}	5.93^{a}	1.73^{b}	0.98
Tyrosine	10.0^{a}	8.66^{ab}	14.3^{a}	5.56^{b}	0.50
Valine	7.16^{a}	5.60^{a}	7.42^{a}	2.81^{b}	0.80

^{abc} Means in the same row with different superscript are significantly different at $P < 0.05$
SEM standard error of the mean

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8.5 Discussion

Different treatments showed various efficiencies for milk production of individual amino acids. This might be due to the fact that the legume forages used in the experiment contained different amino acid profiles. The amino acid composition of each protein is different. However, milk protein synthesis is a genetically determined event and is narrowly influenced by diet. Consequently, the milk amino acid composition of a protein in the same breed is expected to be the same every time it is synthesised. However, the quantities are determined by the amount of milk produced which is highly affected by the diet.

Amino acids are the more required nutrients compared to crude protein because amino acids have numerous direct and essential utilities in metabolism (Schwab *et al.*, 2003). Balancing for amino acids has been a causative factor to elevated milk yields and superior milk amino acid levels. Several studies have most frequently identified methionine and lysine as the two most limiting amino acids for lactating animals fed maize-based rations (NRC 2001; St-Pierre, 2003; Noftsger and Noftsger *et al.*, 2005; Socha *et al.*, 2005; St-Pierre and Sylvester, 2005; Ordway *et al.*, 2009; Appuhamy *et al.*, 2011, Chen *et al.*, 2011; Lee *et al.*, 2012; Osorio *et al.*, 2013). Methionine and lysine are the first two limiting amino acids in most feeding situations due to their low concentrations in most feed proteins in relation to concentrations in rumen bacteria and in milk and tissue protein.

Studies done by Robinson *et al.*, (1998) and Patton, (2010) concluded that cows fed both rumen protected Methionine and Lysine may increase milk protein yield depending on dietary CP supply. However, Appuhamy *et al.*, (2011) reported no change in milk protein synthesis in high producing cows in their early lactation provided with additional jugular-infused Methionine and Lysine. However, significant increases in milk protein content and yield were observed with supplemental methionine and lysine. Moreover, NRC (2001) predicted percentage concentrations of methionine, lysine isoleucine, histadine, valine and leucine in metabolisable protein with the basal diet were 1.8, 6.1, 4.7, 2.2, 5.3 and 8.9 % respectively.

The concentration of milk amino acids often improves in response to supplementation with ruminally protected amino acids. Therefore, milk amino acid concentration when supplementing with the commercial goat feed was expected to be the highest compared to when supplementing with Velvet bean, Silverleaf desmodium or Cowpea. Instead, in this experiment, milk amino acid concentration decreased when very high-protein diet (the commercial goat feed) was fed. Leonardi *et al.* (2003) reported a decrease in milk protein concentration when dietary protein was improved while supplementation with rumen-escape

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methionine increased the concentration of protein in milk within both high-and their lower-protein diet groups. Precisely why milk protein concentration may respond to supplemental rumen escape methionine but not to supplemental protein that will increase milk yield is uncertain (Leonardi *et al.*, 2003).

The differences observed in the efficiency of utilisation of individual amino acids for milk production when supplementing veld hay with Velvet bean, Silverleaf desmodium and commercial goat feed can be attributed to the effects of each diet. Chemical composition of the diet plays a significant role in determining the amount amino acids available for utilisation which is the same as metabolisable protein. Metabolisable protein is the amount of true protein which is absorbed by the small intestines(amino acids) and it is supplied from rumen undegraded protein and microbial protein (Das *et al.*, 2014). The metabolisable protein is the total amino acids which are presented to the animal for maintenance, milk production, growth and foetal growth during gestation (NRC, 1996).

The nutritive significance of metabolisable protein in lactating animals is determined by its essential amino acids profile, mainly that of methionine and lysine (Whitehouse *et al.*, 2009). In this experiment goats supplemented with Silverleaf desmodium consumed a higher amount of amino acids compared to goats on Velvet bean and Cowpea. The different efficiencies observed are likely to be associated with differences in one or several essential amino acids, and supplementation of those deficient essential amino acids with different legume forages may result in the variation observed (Apelo *et al.*, 2014). However, Imaizumi *et al.*, (2010) observed that diets with the same crude protein content did not supply the same metabolisable protein quantity to the animals.

Deficiency in rumen degradable protein can suppress microbial protein synthesis without disturbing the ability to ferment carbohydrates. Therefore, lower than expected milk/feed ratios may be observed due to lesser than expected level synthesis of microbial protein. The rumen synthesis of high-quality microbial protein is responsible for complementing deficit in terms of the quality of rumen undegraded dietary protein. Nevertheless, in support of high milk yield, microbial protein contributes a declining fraction of the required protein. Therefore considerable quantities of dietary intake protein have to escape ruminal degradation so as to meet protein needs. Rulquin and Vérité, (1993) measured the passage of amino acid to the small intestine in ruminants using the multivariate analysis and observed that the amino acid composition of undegraded dietary protein and the relative input that undegraded dietary protein contributes to total protein flow is the determinant for a greater portion of the variation in amino acid balance of small intestines digesta. This is due to the variation in the amino acid

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composition of protein feeds. In this study all the treatments contained different concentrations of amino acids, NDF, lignin, starch and fat.

The maximum effect of diet on amino acid composition of small intestine digesta is observed when huge quantities of highly undegradable dietary protein feeds with amino acid profiles largely different from microbial protein (blood meal, feather meal and maize gluten meal) are fed (Titgemeyer *et al.*, 1989; Waltz *et al.*, 1989). Rulquin and Vérité (1993) reported that the protein source can have a significant effect on milk protein secretion. Therefore, the amino acid profile of various protein supplies would have a vital role in influencing the amount of milk protein produced.

The alterations in amino acid amounts linked with ruminal degradability are directly related to variations among diet proteins in both rumen fermentation and amino acid composition even though both factors are moderately linked by the hydropathy profile of proteins (González *et al.*, 2009). In consequence soluble and easily fermentable proteins contain high levels of hydrophilic and/or polar amino acids (serine, tyrosine, glycine, threonine and acid and basic amino acids) which are usually degraded to a greater extent than those integrated in insoluble proteins, which contain high amounts of hydrophobic and non-polar amino acids (branched-chain amino acids, methionine, phenylalanine and cysteine) (González *et al.*, 2014). Moreover, factors like protein locality in the cell and the exposure to the microbial enzymes and the outflow rate may additionally result in differences in ruminal digestibility among amino acids (Tamminga, 1983).

Although differences in protein composition among the legume forages was limited, glycine and cysteine also showed minimum efficiency values in other feeds, marked by decreased intestinal digestion in high fibre feeds such as lucerne hay (González *et al.*, 2009). González *et al.*, (2014), elucidated that low efficiency is associated mainly with the intestinal digestion of cell wall proteins. Glycine content in structural glycine-rich proteins is approximately 60–70% of all amino acid residues which are incorporated in the xylem tissue (Ringli *et al.*, 2001) that exhibits elevated resistance to the ruminal fermentation (Grenet *et al.*, 1987). Thus a considerable proportion of the glycine delivered to the post-ruminal sites may be from these proteins. Cysteine is protected by thionins which may have toxic effects on rumen microbes, obstruct microbial accessibility, and the existence of several di-sulfur bonds which are responsible for a high affinity hydrophobic nature and high rumen degradation resistance to the amino acid (Stec, 2006) These features may decrease their fermentation and hence enhance the proportion of cysteine presented to the intestines for utilisation.

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The rumen degradability of the veld hay supplemented with Velvet bean, Silverleaf desmodium and Cowpea determined the microbial protein yield. When studying the effect of three levels of microbial protein supply on milk composition and production in multiparous lactating Holstein cows, Raggio *et al.* (2004) observed that milk production increased linearly with increasing supply of microbial protein. Moreover, milk crude protein yield and yield of each milk protein fraction increased linearly with increased microbial protein supply. However, milk crude protein concentration increased linearly, but the proportion of true protein decreased. The observation is also true in this study where highly degradable commercial goat feed showed the least amount of milk amino acids.

Commercial goat feed showed the least efficiencies of utilisation for milk production of individual amino acids although it contained significantly the highest amounts of individual amino acids compared to the legume forages. Kalscheur *et al.*, (2006) reported that feeding ruminants low-protein diets may improve N efficiency; however that is usually associated with a decline in production, which is economically unfavourable to producers when using high producing animals. A significant increase in N efficiency of dairy cows (from 28.2 to 36.5 %) was observed when feed CP was decreased from 17.1 to 12.3% (Kalscheur *et al.*, 2006). Moreover, Ipharraguerre and Clark (2005) reported an increase in N efficiency from 25 to 33 % when CP was reduced from 18.7 to 14.8%.

Availability of nutrients for milk production is primarily determined by the digestibility of feed, as when milk production increased feed efficiency also improved (de Oliveira *et al.*, 2014). Digestibility of the feed decreases as the total dry matter intake is elevated (NRC, 2001). Gabel *et al.* (2003) reported a decrease in digestibility with increase in dietary DM intake when lactating cows fed the same diet, but with 1.4, 2.7 and 4.6 times their energy requirements for maintenance. Therefore, the digestibility of nutrients like amino acids, when measured as percentage of the total intake, reduces as the total intake is increased. Nevertheless, according to Casper *et al.* (2004), goats, sheep and cows require digestible nutrients to produce milk, meeting their nutritional needs. Even though the nutrient digestibility given as percentage in the feed reduces as the dry matter intake is elevated, the quantity of presented digestible nutrients increases as the intake of diet by the animals is improved. Accordingly, optimisation in total dry matter ingestion and nutrient digestibility will result in superior milk yield, and consequently higher nutrient efficiency (Linn *et al.*, 2007).

Another factor which might have caused different efficiencies to be observed was the NDF and lignin content of the diets used in the experiment. According to NRC (2001), diets with high NDF contents tend to reduce the DM intake of animals because the fibre has a filling

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effect in the rumen of animals (Mertens, 199[illegible]. This is due to the low passage rate and slow rate of fractional degradation through the rumen ecosystem, hence reducing the feed intake. Moreover, the fibre content of the feed is inversely related to the net energy level (de Oliveira *et al.*, 2014). Reduction in the nutrient intake has a major impact on the milk production, and thus on their nutrient efficiency. However, Carvalho *et al.* (2006) reported that 27% of NDF from high quality forage maximised the total nutrient intake and the 3.5% fat-corrected milk yield. Linn *et al.* (2007) reported that elevation in the dietary NDF content from 25 to 35% resulted in reduction in feed efficiency from 1.7 to 1.4. Moreover, the different efficiencies observed in this study may be the result of selective behaviour of goats compared with sheep and cattle. A research by Reis *et al.* (2013) showed that in a diet the most vital component is the roughage. When roughage is balanced in a diet of dairy goats there is limited waste of nutrients, in particular nitrogen as the dairy goat can consume up to 6% of its body weight (Reis *et al.*, 2013).

Body weight of the animal has an effect on efficiency of utilisation of nutrients, according to Linn *et al.*, (2009) with animals of higher body weight reflecting least efficiencies, on average 9.4% lower (Linn *et al.*, 2009). This observation points out that an animal with a high dry matter intake (that is animal with the most body weight) has a propensity to have inferior efficiency in comparison to animal with lighter weight. The noted difference is due to the requirement for maintenance, which has a direct effect on the partition of nutrients during lactation (NRC, 2001). According to Searle *et al.*, (1982) body weight is one of the factors directly correlated to net energy for maintenance. Moreover, Apelo *et al.*, (2014) reported that the ultimate profile of absorbed essential amino acid may be different for maintenance, pregnancy, milk production and growth, thus, the ideal profile may depend on stages of life cycle and different physiological states of the animal.

8.6 Conclusion

Among the legume forages Silverleaf desmodium showed the lowest efficiencies of utilisation on histidine, lysine and tyrosine. Whereas, Velvet bean had the highest efficiency of utilisation of phenylalanine ($P < 0.05$) compared to Silverleaf desmodium and Cowpea. Cowpea showed the highest efficiency of utilisation of aspartic acid ($P < 0.05$) for milk production. Efficiencies of utilisation of arginine, glutamic acid, glycine, isoleucine, luecine, methionine, proline, serine, threonine and valine were not significantly ($P < 0.05$) different among Velvet bean, Silverleaf desmodium and Cowpea.

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CHAPTER 9

General discussion, conclusion and recommendations

9.1 General discussion

There were significant differences among the forage legumes in terms of chemical composition, degradability, digestibility, microbial N yield, digestible microbial true protein, and digestible organic matter in the rumen and total milk production. According to (Karsli and Russel, (2001) and Karabulut *et al.,* (2007) differences in chemical composition of legume hays result in the differences in the *in vitro* gas production, nutrition kinetics and the estimated parameters such as metabolisable energy, organic matter metabolisation and microbial protein. Moreover there is significant correlation between the CP content and utilisation of legume hays. This result is in agreement with findings of Parissi *et al.* (2005) and Kamalak *et al.*, (2005).

Forage legumes commonly have higher concentrations of crude protein compared to grasses due to the symbiotic relationship with *Rhizobium* bacteria that habitat in root nodules and are responsible for fixing nitrogen. Therefore, there is no need for the supply of N fertilizer to legumes. Forage legumes usually contain higher concentrations of lignin, pectins, and calcium, but are lower in cellulose and NDF compared to grasses (Karabulut *et al.* 2007). However, grass and forage legumes contain approximately equal levels of ADF at comparable stages of growth (Duane, 1997). Forage legumes are superior compared to grasses in protein and mineral content (particularly calcium, magnesium, copper and cobalt.) even though their nutritive value falls slightly with age (Baloyi *et al.*, 1997).

Degradability and digestibility are important quantitative factors determining the nutritional value of the selected legume forages (*Mucuna pruriens* (Velvet bean), *Vigna unguiculata* Walp *(L)* (Cowpea) and *Desmodium uncinatum* (Silverleaf desmodium)) and the passage of undegradable proteins to the post-ruminal sites (Hvelplund and Weisbjerg, 2000). Legume forage of Cowpea showed the highest level of effective degradability of amino acid (at outflow rates 2%; 4%; 6%) followed by Silverleaf desmodium and then Velvet bean in both techniques. The readily degradable (a) and the slowly degradable fraction (b) of Cowpea were higher than those of Silverleaf desmodium and Velvet bean forages resulted in higher potentially degradable fractions (a + b) for Cowpea. The effective degradability of Cowpea at assumed passage rates was the highest followed by Velvet bean and Silverleaf desmodium. The

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obtained results by both *in sacco* and *in vitro* methods clearly indicate that the three forage legumes are degradable in the rumen to produce microbial protein. The rate of CP degradability in the rumen is related to maturity, DM, NDF and CP content (Tamminga *et al.*, 1991; Kamalak *et al.*, 2005)

The forage legumes can be efficiently utilised by ruminants to produce microbial protein. Feeding high-quality forages containing proteins that are rapidly degraded in the rumen, supplies sufficient N for utilisation by rumen microbes. Shortages of degradable N limit microbial protein synthesis in the rumen, especially when diets containing high concentrations of undegradable dietary protein are fed to animals (Sinclair *et al.*, 1995). When N supply is adequate, then the production of microbial protein will start to be determined by the energy supply (Hume *et al.*, 1970). Therefore, a readily fermentable energy source (carbohydrate) that releases energy at the similar rate as N liberated from dietary N source is vital for the most efficient utilisation of nutrients by rumen microbes (Klusmeyer *et al.*, 1990). However, according to Dewhurst *et al.*, (2009) higher intakes are pivotal to higher performance with forage legumes since effects on feed efficiency are inconsistent.

Chalupa (1975) indicated that normally 40-80 % per cent of dietary protein might be degraded in the rumen and transferred into microbial protein. The protein content of forages is very essential and limiting factor for rumen microbial production (Blummel *et al.*, 2003; Parissi *et al.*, 2005). Rumen microbial protein production requires energy and the amount of dietary protein changed into microbial protein must be a critical phase of nitrogen economy of the ruminant and should be an important factor in determining whether synchronisation by artificial procedures is necessary. The effective nitrogen degradability (at a passage rate of 0.02) is between the ranges of 32-80 % (Mgheni *et al.*, 1993) for tropical herbaceous legumes and the degradability values obtained in this study are within the same range.

However, Velvet bean grains contain several antinutritive compounds (Buckles *et al.*, 1999). In particular, the content of L-Dopa (3-4-dihydroxy-Lphenylalanine) is high (Ayala-Burgos *et al.*, 2003). However, l-dopa is degraded in the rumen (Chikagwa-Malunga *et al.*, 2008), those negative effects have not been observed in ruminants. (Sandoval-Castro *et al.*, 2003), nevertheless, the microbes that degrade l-dopa are yet to be identified.

In forage legumes such as Silverleaf desmodium nutritional value is high however, tannin levels can exceed 3% and this can act as 'undegradable dietary' protein, increasing the efficiency of digestion (Baloyi *et al.*, 2001). The high tannin levels reduce palatability until livestock acquire the taste for it; however, no toxicity has been recorded. Baloyi *et al.*, (2008)

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elaborated that tannins are capable of decreasing protein degradability by forming a complex with protein in the feed and by reducing the activities of rumen bacteria and their ability to interact with carbohydrates and proteins. Maturity influences the nutritive value and secondary compound concentration in seeds, leaves, stems and pods of forage legumes (Maasdorp and Titterton, 1997); little is known of the maturity stage at which the nutritive value of most of the legumes is optimised. Forage legumes are normally harvested between 110 and 270 days after planting (DAP) (Eilitta *et al.*, 2002), depending on location, but when grown for forage, plants are usually harvested between 90 and 120 days after planting (Eilitta *et al.*, 2002).

When the values for all the legume amino acids degradability were considered, there were significant correlations of *in vitro* and *in sacco* (0.99, 0.84 and 0.98) for Velvet bean, Silverleaf desmodium and Cowpeas respectively. *In vitro* procedure developed by Tilley and Terry (1963) has existed as an accurate *in vitro* technique for predicting diet digestibility (Stern *et al.*, 1997; Earing *et al.* 2010). However, a more efficient alternative to the Tilley and Terry method has been developed using the Ankom Daisy^{II} incubator (Ankom Technology Corp., Fairport, NY). Studies done by Wilman and Adesogan (2000) evaluated the *in vivo* and *in vitro* techniques and established that the Daisy^{II} system presented faintly less accurate estimation of ruminant *in vivo* digestibility. Moreover, Holden (1999) established the 2 systems to be comparable. In the gas technique high correlation was reported between digestibility measured *in vivo* and predicted from an *in vitro* rumen gas production technique in combination with chemical composition (Menke *et al.*, 1979). Low ruminal degradable materials leave a higher dietary protein flow into the post-ruminal sites for more efficient enzymatic digestion.

While amino acids are rapidly deaminated in the rumen in real conditions, the rumen microbial populations derive 25-50 % of their nitrogen from sources other than ammonia (Kamalak *et al*, 2005). Unaltered amino acids or peptides originate directly from feed protein, from recycled nitrogen to rumen or from turnover of bacterial and protozoal protein within the rumen (Oldham, 1981). Diets with higher amino acid content showed lower efficiency of utilisation of individual amino acids in milk.

Numerous trials have been undertaken in order to improve utilisation of dietary nutrients by feed supplementation with various additives from different sources (Abd El-Latif *et al.*, 2004 and Ahmed *et al.*, 2008). These studies have revealed that the use of tree and shrub forage legumes as supplements to different basal diets lead to improved ruminant livestock performance in terms of milk production, growth rate and reproductive efficiency (Ondiek *et al.*, 2000). Castillo *et al.*, (2001) postulated that supplementation of readily degraded energy

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will enhance the utilisation of available N in the rumen and further improve the productivity of goats through an increased efficiency of utilisation of ammonia-N for microbial protein synthesis. Moreover, Nyambati *et al.*, (2003) showed that milk production can be increased by supplementing with Velvet bean, highlighting the importance of integrating legumes into the low-input, mixed cropping systems in the tropics. Legumes can be supplemented for ruminants fed low quality forages such as maize stover and tropical grass hays (Muinga *et al.*, 2003).

Microbial protein supply, calculated microbial true protein and digestible microbial true protein were affected ($P > 0.05$) by legume supplementation. Animals on poor quality forages had the most efficient microbial protein synthesis and amino acid utilisation. However, supplementation of hay failed to meet the maintenance requirements of animals as evidenced by negative nitrogen balances in Velvet bean and Silverleaf desmodium. Pathak (2008) explained that the efficiency of microbial protein synthesis greatly differs in animals fed different diets, even within similar diets. Diets containing a blend of forage and concentrates boost the efficiency of microbial protein harmonising synthesis because of an enhanced rumen environment for the growth of more diverse bacteria. Literature indicates that there is a strong positive correlation between DMI and microbial growth (Clark *et al.*, 1992; Djouvinov and Todorov, 1994; Gomes *et al.*, 1994). Increasing the level of dry matter intake decrease the proportion of organic matter degraded in the rumen. Thus, supplementary nutrients were supplied for microbial production. In this research, protein which has lower rates of ruminal degradation have a tendency to improve the efficiency of microbial protein synthesis, possibly because of the improved utilisation of produced N by rumen microbes. Moreover, efficiency of microbial protein synthesis was greater in forages containing tannins which are capable of reducing ruminal N degradability.

Stern *et al.*, (1994) mentioned that ruminal microbial protein synthesis requires an adequate supply of nitrogen to achieve maximum efficiency. Towards obtaining utmost microbial growth, nitrogen and energy supply must be balanced (Weisbjerg 1994; Galgal 1994). Studies indicated that concentrations of 11 to 13% CP in diets are sufficient to obtain optimal microbial protein synthesis (Klusmeyer, 1990). However, microbial protein production response depends on the N source (Klusmeyer *et al.* 1990).

Synthesis of microbial protein is essential in ruminants as bacterial protein produced in the recticulo-rumen supply at least 50%-100% of all amino acids vital for growth, depending on the rumen undegraded crude protein concentration of the feed (NRC, 1996). However, production of microbial protein is energy-dependent and the energy is derived from

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fermentation of organic matter in the rumen and nitrogen consequential from fermentation of protein and non-protein nitrogen sources (Karsli and Russel, 2002). Some nutrients such as phosphorus, sulfur, potassium and vitamins are as well necessary for microbial protein synthesis. The principle is not merely the quantity of nutrients; the simultaneous ruminal fermentation and utilisation of dietary protein and carbohydrates are required for maximum protein synthesis and microbial growth (Sinclair *et al.*, 1995). Several researchers have reported that the increase of peptides and/or amino acids in the rumen significantly increase microbial growth (Wallace, 1991; Grishwold *et al.* 1996).

The high level of protein in forage legumes and extensive degradation means that forage legumes contain high levels of quickly degradable N. This leads to inefficient utilisation and particularly high urinary N output and low amino acid efficiency of utilisation (Dewhurst *et al.*, 2003; Cohen *et al.*, 2006; Dewhurst *et al.*, 2009).

Live weight gain is a result of mostly the amino acid supply and energy-yielding substrates conveyed to the tissues, determined by the genetic limit for protein synthesis, which will by no means reached for ruminants depending solely on pasture. According to Poppi and McLennan (1995) amino acid supply in ruminants relies on the protein content of the feed, its net transfer bypassing the rumen to the intestines as undegraded feed protein, microbial protein and absorption from the post-ruminal sites. Therefore, accretion of protein depends on the efficiency of use of absorbed protein, which is reliant on the availability of limiting essential amino acids and non-protein energy-yielding substrates.

The results showed that Cowpea is a legume forage of high quality for animal production. Thus improvements in meat and milk production could be realised if Cowpea forage is used as a supplementary feed to low quality forage (Baloyi *et al.*, 2008). Numerically high milk yields were obtained in the commercial goat feed followed by Cowpea, Silverleaf desmodium and Velvet bean forage legume supplemented treatments. Daily live weight loss was significantly higher ($P > 0.05$) in the treatment with Cowpea supplementation compared to the commercial goat feed, Silverleaf desmodium and Velvet bean. Feed conversion ratio was highest in goats supplemented with the Silverleaf desmodium followed by Cowpea, however, they were not significantly different ($P > 0.05$).

Lactating dairy goats require forage to maintain a normal milk fat content. In addition to the quantity of forage consumed, the particle size, type of forage, fermentation rate, and surface exchange properties influence rumination, milk yield, and milk fat content. Milk fat decrease can be observed in high producing dairy goats during early lactation. A high forage ration

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results in higher chewing activity which increases salivation and favours the growth of cellulolytic microbes and production of acetic acid. Increase in acetate to propionate ratio in the rumen promotes the synthesis of milk fat, as acetate is the major precursor of milk fat.

Effects of forage legumes on gross milk composition are usually small (James and Nyallu, 2000; Dewhurst *et al.*, 2009). However, the relatively high efficiency of utilisation of lysine and methionine can be due to the fact that they are essential amino acids and are limiting. Therefore, the majority of these amino acids can originate directly from the amino acids of bacterial protein, produced from endogenous urea nitrogen (Michalski *et al.*, 2014). Moreover, the branched-chain amino acids (leucine and isoleucine) contain high levels of endogenous urea nitrogen and this is as a result of these amino acids being synthesised by ruminal microbes using urea nitrogen, because ruminants cannot synthesise them on their own (Milchalski *et al.*, 2007). Efficiency of utilisation of these amino acids was low when the diet contained a high level of protein because the amount of dietary amino acid was sufficient for microbial protein synthesis and only a small part of endogenous urea nitrogen was recycled to the rumen (Zhai *et al.*, 2007).

9.2 Conclusion

Conclusion on the experiments carried out to determine degradability, digestibility, effect on supplementation to low quality hay on intake, microbial protein yield, nitrogen retention and milk production when using the forage legumes *Mucuna pruriens* (Velvet bean), *Vigna unguiculata* Walp *(L)* (Cowpea) and *Desmodium uncinatum* (Silverleaf desmodium) are:

1. On Ankom^{RF} degradability technique, the volumes of gases produced ranged from 61.4 to 70.4 ml, with an average of 64.16ml, whilst the fractional rate of gas production varied between 4.6 and 5.6%/h with a mean of 5%/h. Effective gas production, (a), (b) and (c) were significantly different among the three legumes (P<0.05). Cowpea produced significantly (P<0.05) the highest amount of gas followed by Velvet bean.
2. *In vitro* Daisy^{II} and *in sacco* Nylon-Bag techniques showed that dry matter, CP and P effective degradability of Cowpea were the highest and significantly different from Velvet bean and Silverleaf desmodium (P *<0.05*). Dry matter effective degradability of Velvet bean was higher than that of Silverleaf desmodium. However, the CP effective degradability of Silverleaf desmodium was significantly higher than Velvet bean. Effective degradability of Ca was significantly higher in Velvet bean forage followed by Cowpea.

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3. When both *in sacco* and *in vitro* techniques were compared for dry matter degradability of the three forage legumes, a significant relationship was obtained at $P<0.001$. When the values for the legume dry matter degradability were considered, there were significant correlations 0.93, 0.72 and 0.82 for Velvet bean, Silverleaf desmodium and Cowpeas respectively. The effective degradability values of both techniques were numerically within the same ranges.
4. Comparisons of the two techniques *in vitro* and *in sacco* showed some significant differences in rate of disappearance of amino acids. Legume forage of Cowpea showed the highest level of effective degradability of amino acid (at outflow rates 2%; 4%; 6%) followed by Silverleaf desmodium and then Velvet bean in both techniques. When both *in sacco* and *in vitro* techniques were compared for overall degradability of amino acids in all the 3 forage legumes a significant relationship was obtained at $P<0.001$. When the values for all the legume amino acids degradability were considered, there were significant correlations 0.99, 0.84 and 0.98 for Velvet bean, Silverleaf desmodium and Cowpeas respectively.
5. When supplemented to low quality hay *Mucuna pruriens* (VB), *Vigna unguiculata* Walp *(L)* (CW) and *Desmodium uncinatum* (SD) the total nitrogen intake was significantly high in the commercial goat feed followed by CW, SD and VB respectively ($P<0.05$). Microbial protein supply, calculated microbial true protein and digestible microbial true protein were affected ($P>0.05$) by legume supplementation. Animals on poor quality forages had the most efficient microbial protein synthesis. However, supplementation of hay failed to meet the maintenance requirements of animals as evidenced by negative nitrogen balances in VB and SD.
6. The three legumes were supplemented to lactating goats and daily feed intake on the treatment diets and hay was significantly higher in goats on commercial goat feed followed by the goats on veld hay supplemented by Cowpea, Velvet bean and Silverleaf desmodium , respectively ($P<0.05$). Total dry matter intake (DMI) and metabolisable energy (ME) intake were not significantly different between the different-diet fed animals ($P>0.05$). Higher milk yields were obtained on the commercial goat feed followed by Cowpea, Silverleaf desmodium and Velvet bean forage legume supplemented treatments.
7. Daily live weight loss was significantly higher ($P>0.05$) in treatment supplemented with Cowpea compared to the commercial goat feed, Silverleaf desmodium and Velvet bean.
8. The disappearance profiles of these forage legumes indicated that they can be used as alternative protein supplements by smallholder farmers to feed ruminants because they can be degraded in the rumen. Moreover, supplementation of poor quality veld

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hay with forage legumes could improve utilisation especially in the dry season. The levels of milk production attained in this study imply that some conventional commercial goat feed can be substituted with forage legumes without significantly affecting milk yield and composition in goats.

9. Among the legume forages Silverleaf desmodium showed the lowest efficiencies of utilisation of histidine, lysine and tyrosine, as Velvet bean had significantly the highest efficiency of utilisation of phenylalanine ($P < 0.05$) compared to Silverleaf desmodium and Cowpea. Cowpea showed the highest efficiency of utilisation of aspartic acid ($P < 0.05$) for milk production. Efficiencies of utilisation of arginine, glutamic acid, glycine, isoleucine, luecine, methionine, proline, serine, threonine and valine were not significantly ($P < 0.05$) different in Velvet bean, Silverleaf desmodium and Cowpea.

9.3 Recommendations

The levels of milk production attained in this study imply that some commercial goat feed can be substituted with forage legumes without affecting milk yield and composition in goats. Therefore, the resource-poor farmers in the rural areas can supplement ruminants with the forages without affecting milk production and composition. However, a broad study is required to be done using specialised dairy breeds to assess the effects of forage legumes compared to conventional supplements on milk production. The substitution of expensive conventional protein supplements with locally grown forage legumes may improve productivity through the reduction of expenditure on feeds. Moreover, the use of the legumes as protein supplements may improve kid weaning rate because the legumes showed the same effects on milk production and composition as compared to commercial goat feed.

The three forage legumes used in this study can provide good quality forage for use as supplement to relatively low quality basal diets of veld hay on animals in the smallholder communal areas. Milk produced by these indigenous goats has unique characteristics (higher fat and protein content) making it better than the milk of specialised dairy goats. Therefore, a study on crossbred (native and specialised goats) is necessary to evaluate the effects of the selected legumes on milk quality and quantity without significantly increasing overhead expenses.

Efficiency of utilisation of specific legumes for milk production were higher in the selected legume forages compared to the commercial goat feed. High quality protein diets tend to have

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lower efficiency of utilisation in ruminants due to rumen fermentation. Therefore, legume forages supplemented to goats can be utilised efficiently for milk production compared to commercial goat feed. However, studies need to be done to evaluate the effects of different levels of legume forage supplementations on the efficient of utilisation for milk production and milk quantity in goats.

Cowpea showed higher effective (58.6 %) rumen CP degradability value compared to Silverleaf (46.1 %) and Velvet bean (38 %) at 0.06/h outflow rate. Silverleaf produced (15.5 g/day) higher digestible microbial true protein compared to Cowpea (13.8 g/day) and Velvet bean (13.2 g/day). There was no significant difference in efficiency of microbial N synthesis 2.21, 2.01 and 2.06 for Velvet bean, Silverleaf and Cowpea respectively. The higher amount of digestible microbial true protein from the degradability of Silverleaf and lower values of degradability (higher undegradability) CP caused the legume to have higher efficiency of utilisation in comparison to Cowpea and Velvet bean. Higher DM intake values were observed in animals on Cowpea compared to animals on Silverleaf and Velvet bean. Nitrogen retention was higher on animals on Cowpea (0.84 g/day) compared to animals on Silverleaf (-0.46 g/day) and Velvet bean 9-1.29 g/day). Milk production was not significantly different amongst the treatments 405 g/day, 425 g/day and 447 g/day in Velvet bean, Silverleaf and Cowpea respectively.

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Daisyll dry matter degradability

FEED	CONSTANT	DEGRADABILITY LSMEAN	Standard Error	Pr > ltl	LSMEAN Number
X	A	12.8756000	0.4029763	<.0001	1
X	B	47.6575333	0.4029763	<.0001	2
X	C	0.1134667	0.4029763	0.7815	3
Y	A	13.3065000	0.4029763	<.0001	4
Y	B	40.1491000	0.4029763	<.0001	5
Y	C	0.1605000	0.4029763	0.6951	6
Z	A	17.6887000	0.4029763	<.0001	7
Z	B	48.0773000	0.4029763	<.0001	8
Z	C	0.1325000	0.4029763	0.7461	9

Least Squares Means for effect FEED*CONSTANT
Pr > ltl for H0: LSMean(i)=LSMean(j)
Dependent Variable: DEGRADABILITY

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	<.0001	0.4594	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	0.4709	<.0001
3	<.0001	<.0001		<.0001	<.0001	0.9351	<.0001	<.0001	0.9737
4	0.4594	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	0.9351	<.0001	<.0001		<.0001	<.0001	0.9614
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
8	<.0001	0.4709	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
9	<.0001	<.0001	0.9737	<.0001	<.0001	0.9614	<.0001	<.0001	

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Daisyll crude protein degradability

FEED	CONSTANT	DEGRADABILITY LSMEAN	Standard Error	Pr > ltl	LSMEAN Number
X	A	17.4483333	1.5758343	<.0001	1
X	B	67.1733000	1.5758343	<.0001	2
X	C	0.0266333	1.5758343	0.9867	3
Y	A	17.3346667	1.5758343	<.0001	4
Y	B	53.5752333	1.5758343	<.0001	5
Y	C	0.0745333	1.5758343	0.9628	6
Z	A	30.7049333	1.5758343	<.0001	7
Z	B	59.9774333	1.5758343	<.0001	8
Z	C	0.0524667	1.5758343	0.9738	9

Least Squares Means for effect FEED*CONSTANT
Pr > ltl for H0: LSMean(i)=LSMean(j)
Dependent Variable: DEGRADABILITY

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	<.0001	0.9599	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	0.0047	<.0001
3	<.0001	<.0001		<.0001	<.0001	0.9831	<.0001	<.0001	0.9909
4	0.9599	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	0.0101	<.0001
6	<.0001	<.0001	0.9831	<.0001	<.0001		<.0001	<.0001	0.9922
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
8	<.0001	0.0047	<.0001	<.0001	0.0101	<.0001	<.0001		<.0001
9	<.0001	<.0001	0.9909	<.0001	<.0001	0.9922	<.0001	<.0001	

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FEED	CONSTANT	DEGRADABILITY LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
X	A	12.8593667	0.7348358	<.0001	1
X	B	75.3421667	0.7348358	<.0001	2
X	C	0.1201667	0.7348358	0.8719	3
Y	A	24.0220000	0.7348358	<.0001	4
Y	B	44.6862333	0.7348358	<.0001	5
Y	C	0.1855333	0.7348358	0.8035	6
Z	A	37.6533333	0.7348358	<.0001	7
Z	B	54.9128000	0.7348358	<.0001	8
Z	C	0.0617000	0.7348358	0.9340	9

Least Squares Means for effect FEED*CONSTANT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: DEGRADABILITY

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	<.0001	<.0001		<.0001	<.0001	0.9505	<.0001	<.0001	0.9558
4	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	0.9505	<.0001	<.0001		<.0001	<.0001	0.9065
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
8	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
9	<.0001	<.0001	0.9558	<.0001	<.0001	0.9065	<.0001	<.0001	

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Daisy[II] Calcium dissappearance

FEED	CONSTANT	DEGRADABILITY LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
X	A	14.7659000	1.0785141	<.0001	1
X	B	73.2637000	1.0785141	<.0001	2
X	C	0.1153333	1.0785141	0.9160	3
Y	A	16.6057333	1.0785141	<.0001	4
Y	B	72.2368000	1.0785141	<.0001	5
Y	C	0.0633867	1.0785141	0.9538	6
Z	A	30.5631000	1.0785141	<.0001	7
Z	B	61.2712667	1.0785141	<.0001	8
Z	C	0.0512333	1.0785141	0.9626	9

Least Squares Means for effect FEED*CONSTANT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: DEGRADABILITY

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	<.0001	0.2433	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001	0.5093	<.0001	<.0001	<.0001	<.0001
3	<.0001	<.0001		<.0001	<.0001	0.9732	<.0001	<.0001	0.9669
4	0.2433	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	0.5093	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	0.9732	<.0001	<.0001		<.0001	<.0001	0.9937
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
8	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
9	<.0001	<.0001	0.9669	<.0001	<.0001	0.9937	<.0001	<.0001	

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FEED	CONSTANT	GAS LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
X	A	1.7539500	1.5412289	0.2845	1
X	B	59.1706050	1.5412289	<.0001	2
X	C	0.0458000	1.5412289	0.9769	3
Y	A	3.1304500	1.5412289	0.0728	4
Y	B	55.8102050	1.5412289	<.0001	5
Y	C	0.0557500	1.5412289	0.9719	6
Z	A	2.4500000	1.5412289	0.1464	7
Z	B	64.8371550	1.5412289	<.0001	8
Z	C	0.0498000	1.5412289	0.9749	9

Least Squares Means for effect FEED*CONSTANT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: GAS

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	0.4534	0.5434	<.0001	0.4559	0.7568	<.0001	0.4544
2	<.0001		<.0001	<.0001	0.1575	<.0001	<.0001	0.0287	<.0001
3	0.4534	<.0001		0.1907	<.0001	0.9965	0.2986	<.0001	0.9986
4	0.5434	<.0001	0.1907		<.0001	0.1920	0.7620	<.0001	0.1912
5	<.0001	0.1575	<.0001	<.0001		<.0001	<.0001	0.0025	<.0001
6	0.4559	<.0001	0.9965	0.1920	<.0001		0.3005	<.0001	0.9979
7	0.7568	<.0001	0.2986	0.7620	<.0001	0.3005		<.0001	0.2994
8	<.0001	0.0287	<.0001	<.0001	0.0025	<.0001	<.0001		<.0001
9	0.4544	<.0001	0.9986	0.1912	<.0001	0.9979	0.2994	<.0001	

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FEED	OUTFLOW	GAS LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
X	A	42.7505000	1.8662291	<.0001	1
X	B	44.2083500	1.8662291	<.0001	2
X	C	48.4601000	1.8662291	<.0001	3
Y	A	33.1708000	1.8662291	<.0001	4
Y	B	35.6296000	1.8662291	<.0001	5
Y	C	38.1487500	1.8662291	<.0001	6
Z	A	27.2351000	1.8662291	<.0001	7
Z	B	30.0150000	1.8662291	<.0001	8
Z	C	31.6258000	1.8662291	<.0001	9

Least Squares Means for effect FEED*OUTFLOW
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: GAS

i/j	1	2	3	4	5	6	7	8	9
1		0.5941	0.0587	0.0055	0.0245	0.1152	0.0002	0.0009	0.0023
2	0.5941		0.1416	0.0024	0.0100	0.0473	0.0001	0.0004	0.0010
3	0.0587	0.1416		0.0003	0.0009	0.0036	<.0001	<.0001	0.0001
4	0.0055	0.0024	0.0003		0.3758	0.0919	0.0511	0.2624	0.5727
5	0.0245	0.0100	0.0009	0.3758		0.3648	0.0112	0.0623	0.1636
6	0.1152	0.0473	0.0036	0.0919	0.3648		0.0025	0.0131	0.0355
7	0.0002	0.0001	<.0001	0.0511	0.0112	0.0025		0.3197	0.1305
8	0.0009	0.0004	<.0001	0.2624	0.0623	0.0131	0.3197		0.5567
9	0.0023	0.0010	0.0001	0.5727	0.1636	0.0355	0.1305	0.5567	

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FEED	CONSTANT	DEGRADABILITY LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
X	A	14.3536000	1.1510241	<.0001	1
X	B	46.7664000	1.1510241	<.0001	2
X	C	0.0828400	1.1510241	0.9434	3
Y	A	14.1691667	1.1510241	<.0001	4
Y	B	49.7444000	1.1510241	<.0001	5
Y	C	0.0412933	1.1510241	0.9718	6
Z	A	17.6974000	1.1510241	<.0001	7
Z	B	53.4147667	1.1510241	<.0001	8
Z	C	0.0846333	1.1510241	0.9422	9

Least Squares Means for effect FEED*CONSTANT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: DEGRADABILITY

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	<.0001	0.9110	<.0001	<.0001	0.0548	<.0001	<.0001
2	<.0001		<.0001	<.0001	0.0839	<.0001	<.0001	0.0007	<.0001
3	<.0001	<.0001		<.0001	<.0001	0.9799	<.0001	<.0001	0.9991
4	0.9110	<.0001	<.0001		<.0001	<.0001	0.0439	<.0001	<.0001
5	<.0001	0.0839	<.0001	<.0001		<.0001	<.0001	0.0368	<.0001
6	<.0001	<.0001	0.9799	<.0001	<.0001		<.0001	<.0001	0.9791
7	0.0548	<.0001	<.0001	0.0439	<.0001	<.0001		<.0001	<.0001
8	<.0001	0.0007	<.0001	<.0001	0.0368	<.0001	<.0001		<.0001
9	<.0001	<.0001	0.9991	<.0001	<.0001	0.9791	<.0001	<.0001	

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In sacco crude protein degradability

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FEED	CONSTANT	DEGRADABILITY LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
X	A	9.1248667	0.7818756	<.0001	1
X	B	84.3214000	0.7818756	<.0001	2
X	C	0.0398433	0.7818756	0.9599	3
Y	A	18.6934333	0.7818756	<.0001	4
Y	B	65.6627000	0.7818756	<.0001	5
Y	C	0.0300000	0.7818756	0.9698	6
Z	A	16.3579667	0.7818756	<.0001	7
Z	B	82.7134667	0.7818756	<.0001	8
Z	C	0.0373367	0.7818756	0.9624	9

Least Squares Means for effect FEED*CONSTANT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: DEGRADABILITY

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	0.1631	<.0001
3	<.0001	<.0001		<.0001	<.0001	0.9930	<.0001	<.0001	0.9982
4	<.0001	<.0001	<.0001		<.0001	<.0001	0.0489	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	0.9930	<.0001	<.0001		<.0001	<.0001	0.9948
7	<.0001	<.0001	<.0001	0.0489	<.0001	<.0001		<.0001	<.0001
8	<.0001	0.1631	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
9	<.0001	<.0001	0.9982	<.0001	<.0001	0.9948	<.0001	<.0001	

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In sacco Phosphorus dissappearance

FEED	CONSTANT	DEGRADABILITY LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
X	A	21.8830333	2.6550866	<.0001	1
X	B	59.0840000	2.6550866	<.0001	2
X	C	0.0977000	2.6550866	0.9711	3
Y	A	11.1743667	2.6550866	0.0005	4
Y	B	60.9110667	2.6550866	<.0001	5
Y	C	0.0911433	2.6550866	0.9730	6
Z	A	24.1425667	2.6550866	<.0001	7
Z	B	62.3926000	2.6550866	<.0001	8
Z	C	0.0454367	2.6550866	0.9865	9

Least Squares Means for effect FEED*CONSTANT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: DEGRADABILITY

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	<.0001	0.0106	<.0001	<.0001	0.5548	<.0001	<.0001
2	<.0001		<.0001	<.0001	0.6324	<.0001	<.0001	0.3899	<.0001
3	<.0001	<.0001		0.0086	<.0001	0.9986	<.0001	<.0001	0.9890
4	0.0106	<.0001	0.0086		<.0001	0.0085	0.0028	<.0001	0.0083
5	<.0001	0.6324	<.0001	<.0001		<.0001	<.0001	0.6978	<.0001
6	<.0001	<.0001	0.9986	0.0085	<.0001		<.0001	<.0001	0.9904
7	0.5548	<.0001	<.0001	0.0028	<.0001	<.0001		<.0001	<.0001
8	<.0001	0.3899	<.0001	<.0001	0.6978	<.0001	<.0001		<.0001
9	<.0001	<.0001	0.9890	0.0083	<.0001	0.9904	<.0001	<.0001	

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FEED	CONSTANT	DEGRADABILITY LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
X	A	12.3294667	2.0007960	<.0001	1
X	B	63.2538667	2.0007960	<.0001	2
X	C	0.0543767	2.0007960	0.9786	3
Y	A	25.9653000	2.0007960	<.0001	4
Y	B	61.1004000	2.0007960	<.0001	5
Y	C	0.0340033	2.0007960	0.9866	6
Z	A	20.4152667	2.0007960	<.0001	7
Z	B	69.2902000	2.0007960	<.0001	8
Z	C	0.0593400	2.0007960	0.9767	9

Least Squares Means for effect FEED*CONSTANT
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: DEGRADABILITY

i/j	1	2	3	4	5	6	7	8	9
1		<.0001	0.0004	0.0001	<.0001	0.0004	0.0105	<.0001	0.0004
2	<.0001		<.0001	<.0001	0.4565	<.0001	<.0001	0.0469	<.0001
3	0.0004	<.0001		<.0001	<.0001	0.9943	<.0001	<.0001	0.9986
4	0.0001	<.0001	<.0001		<.0001	<.0001	0.0655	<.0001	<.0001
5	<.0001	0.4565	<.0001	<.0001		<.0001	<.0001	0.0097	<.0001
6	0.0004	<.0001	0.9943	<.0001	<.0001		<.0001	<.0001	0.9930
7	0.0105	<.0001	<.0001	0.0655	<.0001	<.0001		<.0001	<.0001
8	<.0001	0.0469	<.0001	<.0001	0.0097	<.0001	<.0001		<.0001
9	0.0004	<.0001	0.9986	<.0001	<.0001	0.9930	<.0001	<.0001	

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The GLM Procedure
Least Squares Means

Trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	13.7959246	0.1388361	<.0001	1
GM	16.9815733	0.1388361	<.0001	2
SD	15.5220796	0.1388361	<.0001	3
VB	13.2030797	0.1388361	<.0001	4

N balance

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	3.79530251	0.03819426	<.0001	1
GM	4.67168454	0.03819426	<.0001	2
SD	4.27017319	0.03819426	<.0001	3
VB	3.63220899	0.03819426	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		<.0001	0.0001	0.0234
2	<.0001		0.0003	<.0001
3	0.0001	0.0003		<.0001
4	0.0234	<.0001	<.0001	



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trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	13.7959246	0.1388361	<.0001	1
GM	16.9815733	0.1388361	<.0001	2
SD	15.5220796	0.1388361	<.0001	3
VB	13.2030797	0.1388361	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		<.0001	0.0001	0.0234
2	<.0001		0.0003	<.0001
3	0.0001	0.0003		<.0001
4	0.0234	<.0001	<.0001	

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	329.744800	20.591199	<.0001	1
GM	0.000000	20.591199	1.0000	2
SD	317.301600	20.591199	<.0001	3
VB	335.522000	20.591199	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		<.0001	0.6841	0.8493
2	<.0001		<.0001	<.0001
3	0.6841	<.0001		0.5546
4	0.8493	<.0001	0.5546	

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Least Squares Means

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	365.200000	27.905921	<.0001	1
GM	293.820000	27.905921	<.0001	2
SD	355.032500	27.905921	<.0001	3
VB	392.175000	27.905921	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		0.1205	0.8053	0.5198
2	0.1205		0.1719	0.0470
3	0.8053	0.1719		0.3830
4	0.5198	0.0470	0.3830	

Least Squares Means

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	299.500000	0.608105	<.0001	1
GM	-0.000000	0.608105	1.0000	2
SD	294.000000	0.608105	<.0001	3
VB	295.750000	0.608105	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		<.0001	0.0007	0.0048
2	<.0001		<.0001	<.0001
3	0.0007	<.0001		0.0881
4	0.0048	<.0001	0.0881	

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Least Squares Means

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	629.744800	21.785250	<.0001	1
GM	543.747800	21.785250	<.0001	2
SD	617.301600	21.785250	<.0001	3
VB	635.522000	21.785250	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		0.0315	0.7003	0.8574
2	0.0315		0.0542	0.0247
3	0.7003	0.0542		0.5758
4	0.8574	0.0247	0.5758	

Least Squares Means

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	570.228055	19.613083	<.0001	1
GM	513.243548	19.613083	<.0001	2
SD	569.583789	19.613083	<.0001	3
VB	569.388250	19.613083	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		0.0857	0.9822	0.9768
2	0.0857		0.0885	0.0894
3	0.9822	0.0885		0.9946
4	0.9768	0.0894	0.9946	

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Least Squares Means

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	3.77223673	0.33272941	<.0001	1
GM	4.62817754	0.33272941	<.0001	2
SD	3.92195446	0.33272941	<.0001	3
VB	3.29636985	0.33272941	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		0.1188	0.7611	0.3509
2	0.1188		0.1841	0.0299
3	0.7611	0.1841		0.2320
4	0.3509	0.0299	0.2320	

Least Squares Means

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	329.958200	21.551210	<.0001	1
GM	218.455170	21.551210	<.0001	2
SD	319.777773	21.551210	<.0001	3
VB	359.428387	21.551210	<.0001	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		0.0106	0.7497	0.3709
2	0.0106		0.0159	0.0036
3	0.7497	0.0159		0.2410
4	0.3709	0.0036	0.2410	

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Least Squares Means

trt	y LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	0.42512049	0.09612276	0.0045	1
GM	0.45875102	0.09612276	0.0031	2
SD	0.42378895	0.09612276	0.0045	3
VB	0.37947997	0.09612276	0.0076	4

Least Squares Means for effect trt
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: y

i/j	1	2	3	4
1		0.8129	0.9925	0.7485
2	0.8129		0.8056	0.5810
3	0.9925	0.8056		0.7555
4	0.7485	0.5810	0.7555	

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Amino Acid Concentration in Hay: Least Squares Means Adjustment for Multiple Comparisons: Tukey

LEGUME	AA	AAHAY LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	ALA	3.8577	0.05466	<.0001	1
CP	ARG	3.33165	0.05466	<.0001	2
CP	ASP	7.04323	0.05466	<.0001	3
CP	GLU	7.33548	0.05466	<.0001	4
CP	GLY	3.6239	0.05466	<.0001	5
CP	HIS	0	0.05466	1	6
CP	ILEU	3.21475	0.05466	<.0001	7
CP	LEU	5.20205	0.05466	<.0001	8
CP	LYS	3.41933	0.05466	<.0001	9
CP	MET	0	0.05466	1	10
CP	PHE	3.2732	0.05466	<.0001	11
CP	PRO	3.30243	0.05466	<.0001	12
CP	SER	2.8962	0.05466	<.0001	13
CP	THR	2.84067	0.05466	<.0001	14
CP	TYR	1.94639	0.05466	<.0001	15
CP	VAL	3.82848	0.05466	<.0001	16
GM	ALA	3.5277	0.05466	<.0001	17
GM	ARG	3.04665	0.05466	<.0001	18
GM	ASP	6.44073	0.05466	<.0001	19
GM	GLU	6.70798	0.05466	<.0001	20
GM	GLY	3.3139	0.05466	<.0001	21
GM	HIS	0	0.05466	1	22
GM	ILEU	2.93975	0.05466	<.0001	23
GM	LEU	4.75705	0.05466	<.0001	24
GM	LYS	3.12683	0.05466	<.0001	25
GM	MET	0	0.05466	1	26
GM	PHE	2.9932	0.05466	<.0001	27
GM	PRO	3.01993	0.05466	<.0001	28
GM	SER	2.64845	0.05466	<.0001	29
GM	THR	2.59767	0.05466	<.0001	30
GM	TYR	1.77989	0.05466	<.0001	31
GM	VAL	3.50098	0.05466	<.0001	32
SD	ALA	3.6762	0.05466	<.0001	33
SD	ARG	3.1749	0.05466	<.0001	34
SD	ASP	6.71185	0.05466	<.0001	35
SD	GLU	6.99035	0.05466	<.0001	36
SD	GLY	3.4534	0.05466	<.0001	37
SD	HIS	0	0.05466	1	38
SD	ILEU	3.0635	0.05466	<.0001	39
SD	LEU	4.9573	0.05466	<.0001	40

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SD	LYS	3.25845	0.05466	<.0001	41
SD	MET	0	0.05466	1	42
SD	PHE	3.1192	0.05466	<.0001	43
SD	PRO	3.14705	0.05466	<.0001	44
SD	SER	2.75994	0.05466	<.0001	45
SD	THR	2.70702	0.05466	<.0001	46
SD	TYR	1.85481	0.05466	<.0001	47
SD	VAL	3.64835	0.05466	<.0001	48
VB	ALA	3.7884	0.05466	<.0001	49
VB	ARG	3.2718	0.05466	<.0001	50
VB	ASP	6.9167	0.05466	<.0001	51
VB	GLU	7.2037	0.05466	<.0001	52
VB	GLY	3.5588	0.05466	<.0001	53
VB	HIS	0	0.05466	1	54
VB	ILEU	3.157	0.05466	<.0001	55
VB	LEU	5.1086	0.05466	<.0001	56
VB	LYS	3.3579	0.05466	<.0001	57
VB	MET	0	0.05466	1	58
VB	PHE	3.2144	0.05466	<.0001	59
VB	PRO	3.2431	0.05466	<.0001	60
VB	SER	2.84417	0.05466	<.0001	61
VB	THR	2.78964	0.05466	<.0001	62
VB	TYR	1.91142	0.05466	<.0001	63
VB	VAL	3.7597	0.05466	<.0001	64

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Amino Acid Concentration in Legume: Squares Means Adjustment for Multiple Comparisons: Tukey

LEGUME	AA	AALEGUME LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	ALA	15.2355	0	.	1
CP	ARG	16.6705	0	.	2
CP	ASP	31.6785	0	.	3
CP	GLU	36.5435	0	.	4
CP	GLY	13.804	0	.	5
CP	HIS	5.7855	0	.	6
CP	ILEU	11.7495	0	.	7
CP	LEU	20.8915	0	.	8
CP	LYS	14.973	0	.	9
CP	MET	4.739	0	.	10
CP	PHE	18.354	0	.	11
CP	PRO	15.6275	0	.	12
CP	SER	13.8565	0	.	13
CP	THR	14.287	0	.	14
CP	TYR	8.666	0	.	15
CP	VAL	16.324	0	.	16
GM	ALA	17.5	0	.	17
GM	ARG	19.53	0	.	18
GM	ASP	67.585	0	.	19
GM	GLU	40.495	0	.	20
GM	GLY	18.305	0	.	21
GM	HIS	9.835	0	.	22
GM	ILEU	17.5	0	.	23
GM	LEU	29.89	0	.	24
GM	LYS	24.745	0	.	25
GM	MET	5.915	0	.	26
GM	PHE	19.25	0	.	27
GM	PRO	19.285	0	.	28
GM	SER	17.605	0	.	29
GM	THR	21.035	0	.	30
GM	TYR	12.915	0	.	31
GM	VAL	22.75	0	.	32
SD	ALA	20.818	0	.	33
SD	ARG	18.354	0	.	34
SD	ASP	61.5545	0	.	35
SD	GLU	38.1185	0	.	36
SD	GLY	16.7545	0	.	37
SD	HIS	8.554	0	.	38
SD	ILEU	16.52	0	.	39

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SD	LEU	28.3465	0	.	40
SD	LYS	23.618	0	.	41
SD	MET	5.124	0	.	42
SD	PHE	20.937	0	.	43
SD	PRO	18.3855	0	.	44
SD	SER	19.096	0	.	45
SD	THR	19.2185	0	.	46
SD	TYR	10.6715	0	.	47
SD	VAL	21.3115	0	.	48
VB	ALA	15.4245	0	.	49
VB	ARG	13.2895	0	.	50
VB	ASP	28.1575	0	.	51
VB	GLU	29.33	0	.	52
VB	GLY	14.42	0	.	53
VB	HIS	5.117	0	.	54
VB	ILEU	12.7855	0	.	55
VB	LEU	20.8005	0	.	56
VB	LYS	13.65	0	.	57
VB	MET	4.648	0	.	58
VB	PHE	14.2205	0	.	59
VB	PRO	13.1495	0	.	60
VB	SER	11.5605	0	.	61
VB	THR	11.34	0	.	62
VB	TYR	7.77	0	.	63
VB	VAL	15.225	0	.	64

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Amino Acids Consumed in the Legume: Least Squares Means Adjustment for Multiple Comparisons: Tukey

LEGUME	AA	AACONSUMED LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	ALA	19.0932	0.05466	<.0001	1
CP	ARG	20.0022	0.05466	<.0001	2
CP	ASP	38.7217	0.05466	<.0001	3
CP	GLU	43.879	0.05466	<.0001	4
CP	GLY	17.4279	0.05466	<.0001	5
CP	HIS	5.7855	0.05466	<.0001	6
CP	ILEU	14.9643	0.05466	<.0001	7
CP	LEU	26.0936	0.05466	<.0001	8
CP	LYS	18.3923	0.05466	<.0001	9
CP	MET	4.739	0.05466	<.0001	10
CP	PHE	21.6272	0.05466	<.0001	11
CP	PRO	18.9299	0.05466	<.0001	12
CP	SER	16.7527	0.05466	<.0001	13
CP	THR	17.1277	0.05466	<.0001	14
CP	TYR	10.6124	0.05466	<.0001	15
CP	VAL	20.1525	0.05466	<.0001	16
GM	ALA	21.0277	0.05466	<.0001	17
GM	ARG	22.5767	0.05466	<.0001	18
GM	ASP	74.0257	0.05466	<.0001	19
GM	GLU	47.203	0.05466	<.0001	20
GM	GLY	21.6189	0.05466	<.0001	21
GM	HIS	9.835	0.05466	<.0001	22
GM	ILEU	20.4398	0.05466	<.0001	23
GM	LEU	34.6471	0.05466	<.0001	24
GM	LYS	27.8718	0.05466	<.0001	25
GM	MET	5.915	0.05466	<.0001	26
GM	PHE	22.2432	0.05466	<.0001	27
GM	PRO	22.3049	0.05466	<.0001	28
GM	SER	20.2534	0.05466	<.0001	29
GM	THR	23.6327	0.05466	<.0001	30
GM	TYR	14.6949	0.05466	<.0001	31
GM	VAL	26.251	0.05466	<.0001	32
SD	ALA	24.4942	0.05466	<.0001	33
SD	ARG	21.5289	0.05466	<.0001	34
SD	ASP	68.2664	0.05466	<.0001	35
SD	GLU	45.1089	0.05466	<.0001	36
SD	GLY	20.2079	0.05466	<.0001	37
SD	HIS	8.554	0.05466	<.0001	38
SD	ILEU	19.5835	0.05466	<.0001	39

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SD	LEU	33.3038	0.05466	<.0001	40
SD	LYS	26.8765	0.05466	<.0001	41
SD	MET	5.124	0.05466	<.0001	42
SD	PHE	24.0562	0.05466	<.0001	43
SD	PRO	21.5326	0.05466	<.0001	44
SD	SER	21.8559	0.05466	<.0001	45
SD	THR	21.9255	0.05466	<.0001	46
SD	TYR	12.5263	0.05466	<.0001	47
SD	VAL	24.9599	0.05466	<.0001	48
VB	ALA	19.2129	0.05466	<.0001	49
VB	ARG	16.5613	0.05466	<.0001	50
VB	ASP	35.0742	0.05466	<.0001	51
VB	GLU	36.5337	0.05466	<.0001	52
VB	GLY	17.9788	0.05466	<.0001	53
VB	HIS	5.117	0.05466	<.0001	54
VB	ILEU	15.9425	0.05466	<.0001	55
VB	LEU	25.9091	0.05466	<.0001	56
VB	LYS	17.0079	0.05466	<.0001	57
VB	MET	4.648	0.05466	<.0001	58
VB	PHE	17.4349	0.05466	<.0001	59
VB	PRO	16.3926	0.05466	<.0001	60
VB	SER	14.4047	0.05466	<.0001	61
VB	THR	14.1296	0.05466	<.0001	62
VB	TYR	9.68142	0.05466	<.0001	63
VB	VAL	18.9847	0.05466	<.0001	64

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Total Amino Acids Consumed:

Tukey Comparison Lines for Least Squares Means of LEGUME*AA

			AACONSUMED LSMEAN	LEGUME	AA	LSMEAN Number
	A		74.0257	GM	ASP	19
	B		68.2664	SD	ASP	35
	C		47.203	GM	GLU	20
	D		45.1089	SD	GLU	36
	E		43.879	CP	GLU	4
	F		38.7217	CP	ASP	3
	G		36.5337	VB	GLU	52
	H		35.0742	VB	ASP	51
	I		34.6471	GM	LEU	24
	J		33.3038	SD	LEU	40
	K		27.8718	GM	LYS	25
	L		26.8765	SD	LYS	41
	M		26.251	GM	VAL	32
N	M		26.0936	CP	LEU	8
N			25.9091	VB	LEU	56
	O		24.9599	SD	VAL	48
	P		24.4942	SD	ALA	33
	Q		24.0562	SD	PHE	43
	R		23.6327	GM	THR	30
	S		22.5767	GM	ARG	18
T	S		22.3049	GM	PRO	28
T	U		22.2432	GM	PHE	27
V	U		21.9255	SD	THR	46
V	W		21.8559	SD	SER	45
V	W	X	21.6272	CP	PHE	11
V	W	X	21.6189	GM	GLY	21
	W	X	21.5326	SD	PRO	44
		X	21.5289	SD	ARG	34
	Y		21.0277	GM	ALA	17
	Z		20.4398	GM	ILEU	23
A	Z		20.2535	GM	SER	29
A	Z		20.2079	SD	GLY	37
A	Z		20.1525	CP	VAL	16
A			20.0022	CP	ARG	2
	B		19.5835	SD	ILEU	39
	C		19.2129	VB	ALA	49
	C		19.0932	CP	ALA	1
	C		18.9847	VB	VAL	64
	C		18.9299	CP	PRO	12

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	D	18.3923	CP	LYS	9
	E	17.9788	VB	GLY	53
	F	17.4349	VB	PHE	59
	F	17.4279	CP	GLY	5
G	F	17.1277	CP	THR	14
G	H	17.0079	VB	LYS	57
I	H	16.7527	CP	SER	13
I	J	16.5613	VB	ARG	50
	J	16.3926	VB	PRO	60
	K	15.9425	VB	ILEU	55
	L	14.9643	CP	ILEU	7
M	L	14.6949	GM	TYR	31
M	N	14.4047	VB	SER	61
	N	14.1296	VB	THR	62
	O	12.5263	SD	TYR	47
	P	10.6124	CP	TYR	15
	Q	9.835	GM	HIS	22
	Q	9.68142	VB	TYR	63
	R	8.554	SD	HIS	38
	S	5.915	GM	MET	26
	S	5.7855	CP	HIS	6
	T	5.124	SD	MET	42
	T	5.117	VB	HIS	54
	U	4.739	CP	MET	10
	U	4.648	VB	MET	58

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Amino acids in Milk: Least Squares Means Adjustment for Multiple Comparisons: Tukey

LEGUME	AA	AAMILK LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	ALA	0.6903	0.16448	<.0001	1
CP	ARG	1.4935	0.16448	<.0001	2
CP	ASP	1.61623	0.16448	<.0001	3
CP	GLU	4.51735	0.16448	<.0001	4
CP	GLY	0.42308	0.16448	0.0109	5
CP	HIS	0.58965	0.16448	0.0004	6
CP	ILEU	1.09278	0.16448	<.0001	7
CP	LEU	2.074	0.16448	<.0001	8
CP	LYS	2.51	0.16448	<.0001	9
CP	MET	1.08225	0.16448	<.0001	10
CP	PHE	1.09278	0.16448	<.0001	11
CP	PRO	2.3535	0.16448	<.0001	12
CP	SER	1.204	0.16448	<.0001	13
CP	THR	1.01475	0.16448	<.0001	14
CP	TYR	1.51635	0.16448	<.0001	15
CP	VAL	1.4939	0.16448	<.0001	16
GM	ALA	0.2724	0.16448	0.0994	17
GM	ARG	0.71535	0.16448	<.0001	18
GM	ASP	1.02375	0.16448	<.0001	19
GM	GLU	2.00918	0.16448	<.0001	20
GM	GLY	0.1816	0.16448	0.271	21
GM	HIS	0.18175	0.16448	0.2706	22
GM	ILEU	0.6017	0.16448	0.0003	23
GM	LEU	1.0442	0.16448	<.0001	24
GM	LYS	1.25993	0.16448	<.0001	25
GM	MET	0.44265	0.16448	0.0078	26
GM	PHE	0.5448	0.16448	0.0011	27
GM	PRO	1.07825	0.16448	<.0001	28
GM	SER	0.52255	0.16448	0.0018	29
GM	THR	0.4086	0.16448	0.0139	30
GM	TYR	0.8172	0.16448	<.0001	31
GM	VAL	0.7379	0.16448	<.0001	32
SD	ALA	0.647	0.16448	0.0001	33
SD	ARG	1.41025	0.16448	<.0001	34
SD	ASP	1.63938	0.16448	<.0001	35
SD	GLU	4.024	0.16448	<.0001	36
SD	GLY	0.38213	0.16448	0.0213	37
SD	HIS	0.45475	0.16448	0.0063	38
SD	ILEU	1.00825	0.16448	<.0001	39
SD	LEU	1.9	0.16448	<.0001	40

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SD	LYS	2.1865	0.16448	<.0001	41
SD	MET	0.9665	0.16448	<.0001	42
SD	PHE	0.9975	0.16448	<.0001	43
SD	PRO	2.13338	0.16448	<.0001	44
SD	SER	1.0615	0.16448	<.0001	45
SD	THR	0.95513	0.16448	<.0001	46
SD	TYR	1.08725	0.16448	<.0001	47
SD	VAL	1.40113	0.16448	<.0001	48
VB	ALA	0.47288	0.16448	0.0045	49
VB	ARG	1.45038	0.16448	<.0001	50
VB	ASP	0.70543	0.16448	<.0001	51
VB	GLU	2.325	0.16448	<.0001	52
VB	GLY	0.46445	0.16448	0.0053	53
VB	HIS	1.0623	0.16448	<.0001	54
VB	ILEU	0.99745	0.16448	<.0001	55
VB	LEU	1.67113	0.16448	<.0001	56
VB	LYS	1.84225	0.16448	<.0001	57
VB	MET	0.87653	0.16448	<.0001	58
VB	PHE	1.30088	0.16448	<.0001	59
VB	PRO	1.37628	0.16448	<.0001	60
VB	SER	1.0999	0.16448	<.0001	61
VB	THR	0.94785	0.16448	<.0001	62
VB	TYR	0.97105	0.16448	<.0001	63
VB	VAL	1.35963	0.16448	<.0001	64

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Efficiency of utilisation: Least Squares Means Adjustment for Multiple Comparisons: Tukey

LEGUME	AA	AAEFFICIENCY LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
CP	ALA	3.61751	1.49509	0.0165	1
CP	ARG	7.47309	1.49509	<.0001	2
CP	ASP	4.17768	1.49509	0.0058	3
CP	GLU	10.3029	1.49509	<.0001	4
CP	GLY	2.42998	1.49509	0.1058	5
CP	HIS	10.1919	1.49509	<.0001	6
CP	ILEU	7.30954	1.49509	<.0001	7
CP	LEU	7.95518	1.49509	<.0001	8
CP	LYS	13.6576	1.49509	<.0001	9
CP	MET	22.8371	1.49509	<.0001	10
CP	PHE	5.05612	1.49509	0.0009	11
CP	PRO	12.4431	1.49509	<.0001	12
CP	SER	7.19315	1.49509	<.0001	13
CP	THR	5.92903	1.49509	0.0001	14
CP	TYR	14.3041	1.49509	<.0001	15
CP	VAL	7.41899	1.49509	<.0001	16
GM	ALA	1.29545	1.49509	0.3874	17
GM	ARG	3.16799	1.49509	0.0355	18
GM	ASP	1.38225	1.49509	0.3565	19
GM	GLU	4.25604	1.49509	0.0049	20
GM	GLY	0.84001	1.49509	0.5749	21
GM	HIS	1.84799	1.49509	0.2181	22
GM	ILEU	2.94341	1.49509	0.0505	23
GM	LEU	3.01384	1.49509	0.0453	24
GM	LYS	4.51975	1.49509	0.0029	25
GM	MET	7.48352	1.49509	<.0001	26
GM	PHE	2.4493	1.49509	0.1031	27
GM	PRO	4.83371	1.49509	0.0015	28
GM	SER	2.57928	1.49509	0.0862	29
GM	THR	1.72896	1.49509	0.249	30
GM	TYR	5.56113	1.49509	0.0003	31
GM	VAL	2.81068	1.49509	0.0617	32
SD	ALA	2.63372	1.49509	0.0798	33
SD	ARG	6.5311	1.49509	<.0001	34
SD	ASP	2.3943	1.49509	0.111	35
SD	GLU	8.90177	1.49509	<.0001	36
SD	GLY	1.88603	1.49509	0.2088	37
SD	HIS	5.31623	1.49509	0.0005	38
SD	ILEU	5.13593	1.49509	0.0007	39

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SD	LEU	5.69185	1.49509	0.0002	40
SD	LYS	8.11913	1.49509	<.0001	41
SD	MET	18.8622	1.49509	<.0001	42
SD	PHE	4.13863	1.49509	0.0062	43
SD	PRO	9.88882	1.49509	<.0001	44
SD	SER	4.8482	1.49509	0.0014	45
SD	THR	4.34805	1.49509	0.0041	46
SD	TYR	8.66402	1.49509	<.0001	47
SD	VAL	5.60139	1.49509	0.0002	48
VB	ALA	2.46111	1.49509	0.1015	49
VB	ARG	8.75093	1.49509	<.0001	50
VB	ASP	2.01212	1.49509	0.1801	51
VB	GLU	6.36413	1.49509	<.0001	52
VB	GLY	2.58239	1.49509	0.0858	53
VB	HIS	20.7602	1.49509	<.0001	54
VB	ILEU	6.25335	1.49509	<.0001	55
VB	LEU	6.44725	1.49509	<.0001	56
VB	LYS	10.8291	1.49509	<.0001	57
VB	MET	18.8581	1.49509	<.0001	58
VB	PHE	7.45506	1.49509	<.0001	59
VB	PRO	8.3968	1.49509	<.0001	60
VB	SER	7.6332	1.49509	<.0001	61
VB	THR	6.70333	1.49509	<.0001	62
VB	TYR	10.029	1.49509	<.0001	63
VB	VAL	7.15851	1.49509	<.0001	64

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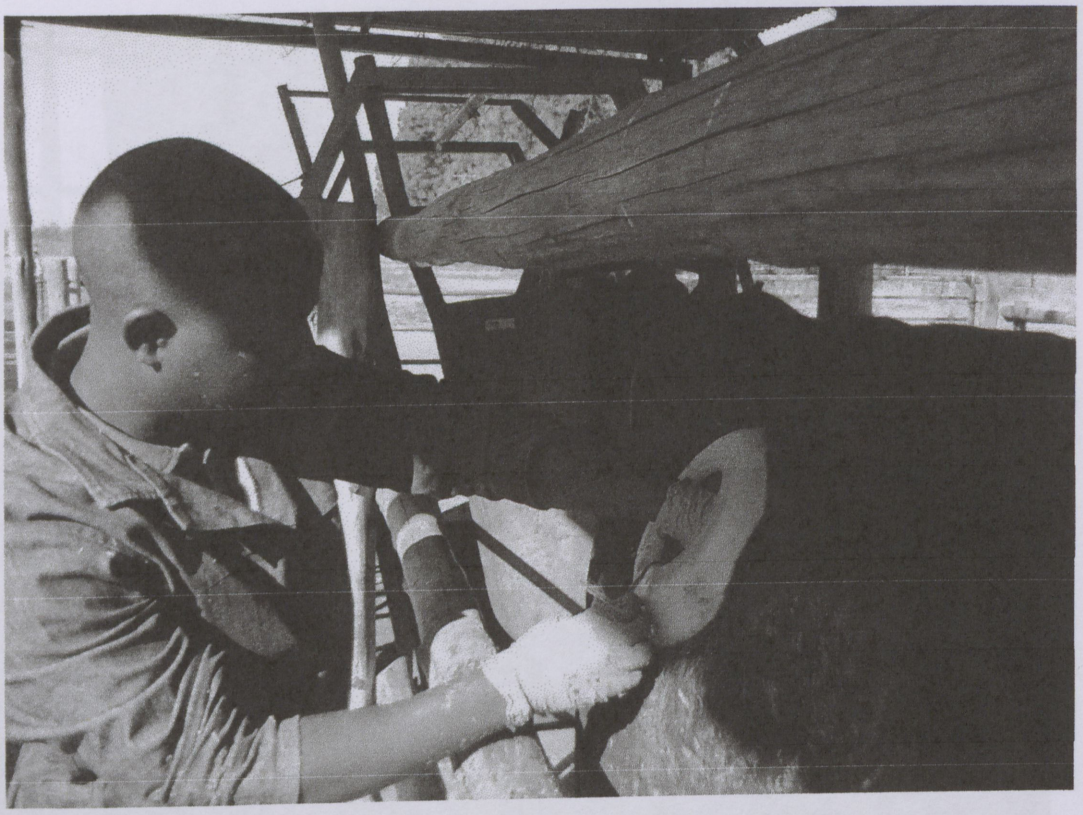

Fig 1: Insertion of Nylon-Bag into the rumen

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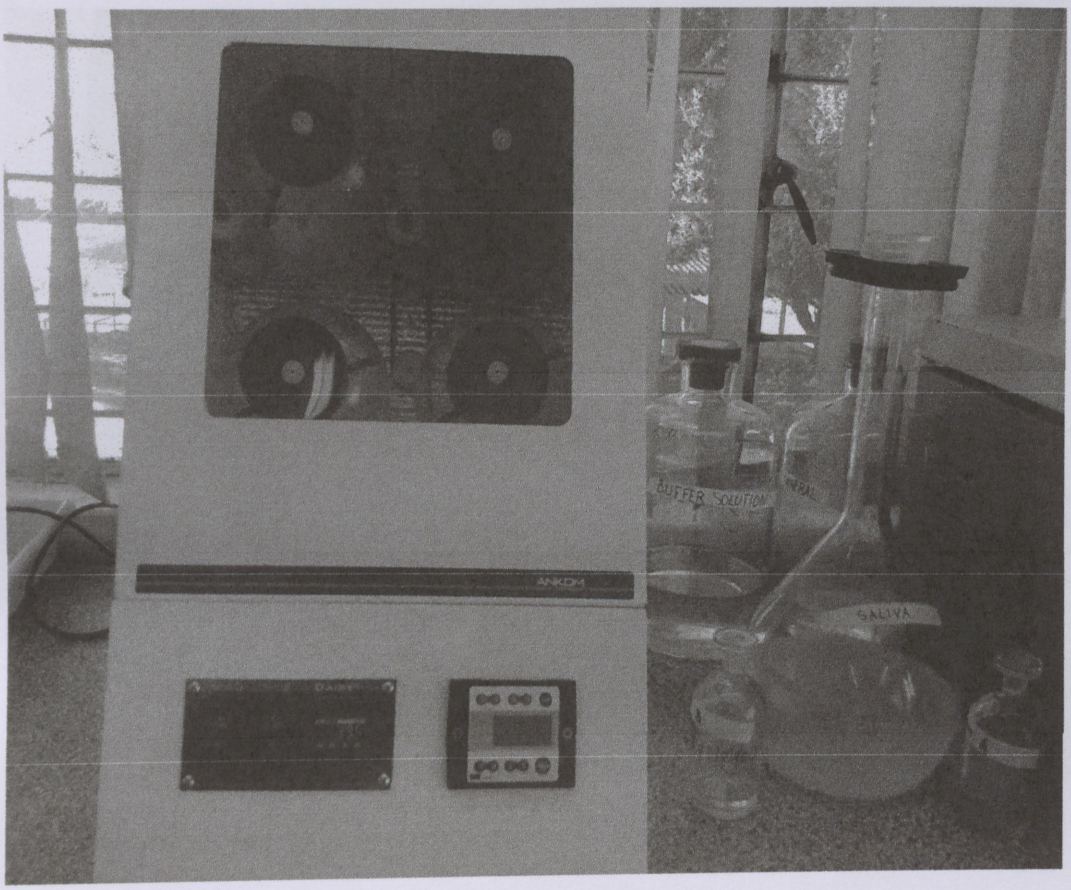

Fig 2: Daisy^{II} incubator

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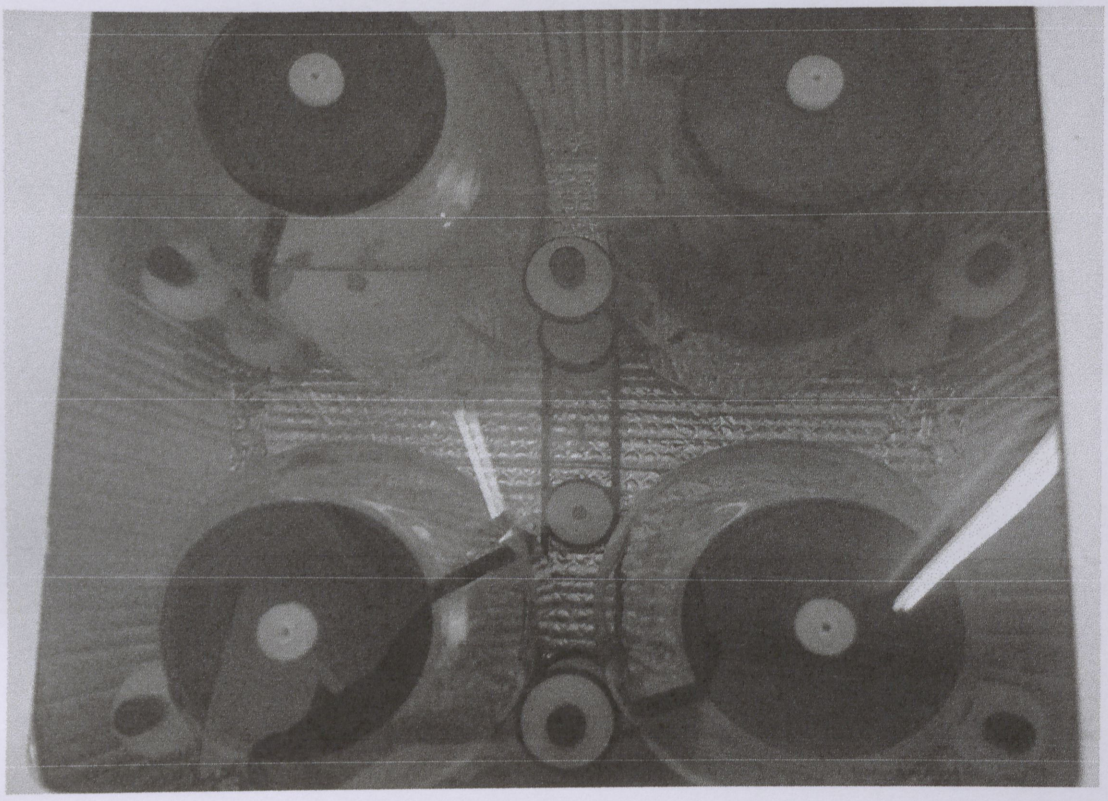

Fig 3: Daisy^{II} incubator jars

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Fig 4: Ankom^{RF} gas technique set up

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