

Nutritive value of *Cassia sturtii*, *Sutherlandia microphylla* and
Medicago sativa for sheep

By

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Declaration

I **Jacqueline Tucker** declare that the thesis/dissertation, which I hereby submit for the degree **MSc (Agric) Animal Science (Nutrition science)** at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Abbreviations

ADF	Acid detergent fibre
ADL	Acid detergent lignin
ADS	Acid detergent solution
AIDS	Acquired immune deficiency syndrome
<i>A.nummularia</i>	<i>Atriplex nummularia</i>
AOAC	Association of Official Analytical Chemists
Ca	Calcium
<i>C. sturtii</i>	<i>Cassia sturtii</i>
CF	Crude fibre
cm	Centimeters
CO ₂	Carbon dioxide
CP	Crude protein
CPD	Protein rumen degradability
Cr	Chromium
CT	Condensed tannins
Cu	Copper
d	Day
DAPA	Diaminopimelic acid
df	Dilution factor
DHP	Dihydroxypyridine
DM	Dry matter
DMD	Dry matter digestibility
DOM	Digestible organic matter
DOMI	Digestible organic matter intake
DOMR	Digestible organic matter in the rumen
EE	Ether extract
Fe	Iron

g	Gram
GABA	Gamma-aminobutyric acid
GIT	Gastro-intestinal tract
h	Hour
H	Hydrogen
ha	Hectare
HCl	Hydrochloric acid
HClO ₄	Perchloric acid
H ₂ SO ₄	Hydrogen sulphate
HNO ₃	Nitric acid
INRA	Inland Northwest Research Alliance
IVDMD	<i>In vitro</i> dry matter digestibility
IVOMD	In Vitro Organic Matter Digestibility
K	Potassium
k _d	Rate of digestion
kg	Kilogram
k _i	Rate of intake
k _p	Rate of passage
l	Litre
LW	Live weight
m	Meters
M	Molar
Mcal	Mega calorie
ME	Metabolizable energy
ml	Milliliter
mm	Millimeter
mmol	Millimol
mg	Milligram
Mg	Magnesium

MJ	Mega joule
Mn	Manganese
MRT	Mean retention time
<i>M. sativa</i>	<i>Medicago sativa</i>
N	Nitrogen
Na	Sodium
NaOH	Sodium hydroxide
NAN	Non-ammonia-nitrogen
NDF	Neutral detergent fibre
NDFI	Neutral detergent fibre intake
NDS	Neutral detergent solution
NE	Net energy
NH ₃ -N	Ammonia nitrogen
NRC	National Research Council
°C	Degree celsius
OM	Organic matter
OMD	Organic matter degradability
OMI	Organic matter intake
%	Percent
P	Phosphorus
PD	Purine derivatives
pH	H-ion concentration
RDN	Rumen degradable nitrogen
rpm	Revolutions per minute
SD	Standard deviation
Se	Selenium
<i>S. microphylla</i>	<i>Sutherlandia microphylla</i>
<i>T. sinuatum</i>	<i>Tripteris sinuatum</i>
µg	Microgram

μl	Microlitre
μm	Micrometer
μmol	Micromole
VFA	Volatile fatty acids
$W^{0.75}$	Metabolic weight
w/v	Weight/volume
Zn	Zinc

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Abstract

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The aim of this study was to assess the potential nutritive value for sheep, of two drought tolerant leguminous shrubs (*Cassia sturtii* and *Sutherlandia microphylla*) in terms of chemical composition, degradation parameters, digestibility, rumen fermentation parameters, intake, microbial nitrogen synthesis and nitrogen balance as well as the rumen kinetics when compared to that of *Medicago sativa*.

The crude ash concentration of all three forages differs, with *S. microphylla* and *C. sturtii* lower than *M. sativa*. *M. sativa* has a crude ash concentration almost twice the amount of both *S. microphylla* and *C. sturtii*. Wilcock *et al.*, (2004) reported ash values for *C. sturtii* stems and leaves of 53 and 73 g/kg and that of *S. microphylla* at 25 and 64g/kg respectively. Values for *C. sturtii* are lower while those of *S. microphylla* compare well to the average of the whole plant.

The mean CP and CF concentration differed between species with *C. sturtii* having the lowest CP and *M. sativa* the highest. *S. microphylla* had the highest CF while *M. sativa* had the lowest.

The NDF and ADF levels of the samples varied between all three species with *S. microphylla* being the highest and *M. sativa* the lowest. Values for *C. sturtii* were in between those of the two other forages.

The ADL concentration of *S. microphylla* was higher than both *C. sturtii* and *M. sativa*. The degree of lignification in *C. sturtii* was high (23.8% of NDF was ADL). The degree of lignification of *S. microphylla* was 26.8%, which is higher than that of *C. sturtii*, while *M. sativa* is the same as *C. sturtii*.

The calcium concentrations of *C. sturtii* and *M. sativa* are similar and have a higher concentration than *S. microphylla*. *M. sativa* and *C. sturtii* had a higher phosphorus concentration than *S. microphylla*. With respect to magnesium (Mg), *C. sturtii* and *M. sativa* have a similar composition while *S. microphylla* has a lower concentration.

The iron concentration of all three plants differs, with *M. sativa* having the lowest concentration and *C. sturtii* the highest. The copper concentrations in *M. sativa* and *C. sturtii* were similar, while that of *S. microphylla* was slightly lower. The zinc concentrations in *M. sativa* and *C. sturtii* were similar, while that of *S. microphylla* was slightly higher. Manganese concentration of all three species differs, with *C. sturtii* being the lowest and *S. microphylla* the highest. The plants from this trial were analysed for selenium but none or very insignificant levels were found and were not worth reporting.

The apparent DM digestibility of *S. microphylla* is significantly lower than *M. sativa* while it did not differ significantly from *C. sturtii*. *C. sturtii* did not differ significantly from both *M. sativa* and *S. microphylla*. The CP digestibility of all three species did not differ significantly, however that of *M. sativa* is numerically higher. With regards to the apparent NDF digestibility, *C. sturtii* and *S. microphylla* differ significantly to *M. sativa* with lower NDF digestibility values. The apparent OM digestibility followed the same trend as that of apparent DM digestibility.

The average intake was very different between species, with *C. sturtii* being the lowest and *M. sativa* the highest. The animals consuming either *C. sturtii* or *S. microphylla* tended to lose body weight during the experimental period, while those eating *M. sativa* gained body weight.

Voluntary intake parameters of *C. sturtii* and *S. microphylla* were lower and differed significantly between *M. sativa*. The DM intake of *M. sativa* was higher than both *C. sturtii* and *S. microphylla*.

The ME was the highest for *M. sativa* while *S. microphylla* was significantly different and had the lowest value. *C. sturtii* had an ME value similar to both *M. sativa* and *S. microphylla*. The ME intake of *S. microphylla* was 2.89 MJ/day compared to that of *M. sativa* of 8.57 MJ/day. Rumen NH₃-N concentrations of *C. sturtii* were the lowest and differed significantly from *S. microphylla* and *M. sativa*.

Sheep receiving *C. sturtii* had the lowest total rumen VFA concentration and was significantly different from *M. sativa* which had the highest value. *S. microphylla* had a similar total VFA concentration to both *C. sturtii* and *M. sativa*.

C. sturtii had the lowest proportion of acetate but did not differ significantly compared to *S. microphylla*, while both were significantly different to *M. sativa*, which had the highest value. The propionate concentration for all three forages did not differ significantly. *S. microphylla* had the highest fibre concentration, therefore leading to higher acetate concentrations than *C. sturtii* but not higher than *M. sativa*, suggesting the fibre of *S. microphylla* is less digestible. This is supported by the low apparent NDF digestibility for *S. microphylla*. Nitrogen intake was highest for *M. sativa* and was significantly different from *C. sturtii* and *S. microphylla*. The same trend followed for faecal and urinary nitrogen output as well as nitrogen retention. The nitrogen retention for all species was positive with *C. sturtii* being the lowest. These values compare well to the CP content of the three forages with *C. sturtii* the lowest and *M. sativa* the highest concentration.

The daily urinary allantoin elimination did not differ between *C. sturtii* and *S. microphylla* but was significantly different and higher for *M. sativa*.

The amount of microbial nitrogen supplied to the animal (g/day and g/kg DOMI) followed the same trend as allantoin.

M. sativa had significantly higher a-values (soluble fraction) for both DM and NDF degradation compared to the two shrub species at a rate constant of 0.02/h. *C. sturtii* had a higher b-value (potentially degradable fraction) for DM degradation compared to *S. microphylla* which shows that *S. microphylla* DM component was most readily soluble. For NDF, however, the b-values didn't differ among the species. Species had also no effect on the c-values (rate of degradation of the potentially degradable fraction b) of both DM and NDF. Therefore all species appear to have a similar potential source of energy for use by micro-organisms in the rumen. Effective DM degradability of *C. sturtii* and *S. microphylla* was similar while that of *M. sativa* was significantly higher. The effective NDF degradability for *C. sturtii* and *S. microphylla* was similar and *M. sativa* again had a significantly higher NDF degradability.

The rumen DM degradability for all three species showed a similar trend but much higher values than the apparent DM digestibility. The rumen NDF degradability values were almost identical to those reported for apparent NDF digestibility. The rate of intake and rate of digestion for *C. sturtii* and *S. microphylla* did not differ significantly, while that of *M. sativa* was the highest and significantly different. The rate of passage for all three species was similar. The percent NDF digested in the rumen differed significantly between all three species with *C. sturtii* being the lowest and *M. sativa* the highest. The percent NDF passing from the rumen also differed significantly between all three species, however this time *C. sturtii* being the highest and *M. sativa* the lowest, which corresponds well to the values for NDF digested in the rumen.

It is concluded that *C. sturtii* and *S. microphylla* are of a slightly lower nutritional value for sheep than *M. sativa*. If these two leguminous fodder species were to be used as maintenance feed, some other supporting source of energy would need to be supplied in order for these sheep to be maintained over a long period. The negative effect of all fibre related parameters (CF, NDF, ADF and ADL) in *C. sturtii* and *S. microphylla*, reduced digestibility as well as intake, leading to a forage of lower nutrient value as compared to *M. sativa*. The effect of anti-nutritional factors present in *C. sturtii* and *S. microphylla* on the digestibility of forages and nutrient contribution from forages needs to be studied to determine if these play a role in reducing the nutritional value.

Chapter 1

Introduction and Literature review

1.1. Introduction

Nutrition of domestic animals in semi-arid regions is mainly based on the utilization of rangeland resources, which are subject to many quantitative and qualitative variations throughout the year. Fodder trees and shrubs are an integral part of the diet of animals in such areas and often constitute the main source of protein, minerals and vitamins during the dry season. A number of fodder shrubs have been identified as potentially valuable species and introduced into forage production systems of the arid and semi-arid regions in the form of shrub plantations. These plantations play a key role in providing animals with forage, especially during critical periods (Bouزيد & Papanastasis, 1996).

Rangelands occupy more than 70% of South Africa's land surface. Sixty percent of this is moderately to seriously degraded. These semi-arid rangelands are under enormous pressure due to the injudicious use of natural resources and the occurrence of periodic droughts. The visible symptoms of disturbed rangelands are obvious and include degraded vegetation, bush and shrub encroachment and soil erosion (Snyman, 2003). In certain rangeland areas, natural vegetation is degraded to such an extent that the application of management practices, or even withdrawal of grazing animals, will not have an effect on the recovery. In such cases more drastic reclamation measures need to be applied to re-establish the vegetation and ensure sustainable animal production (Snyman, 2003). Rangeland deterioration begins very subtly and farmers often only realize that the land is deteriorating when drastic changes such as bare patches occur. The approaches to reclamation depend on the soil type, climate and the causes and degree of degradation. Reclamation is an expensive and time-consuming process and needs careful planning (Snyman, 2003).

The use of drought tolerant fodder shrubs and trees in semi-arid or arid areas is becoming a widely discussed topic. Not only do these plants provide a source of food for animals during the critical dry periods but they may also contribute to the reclamation of degraded rangelands. The establishment of drought tolerant crops also makes more efficient use of the available moisture, an important consideration in areas where rainfall and irrigation is limited. The establishment and maintenance of such crops is economical as they have a high recovery ability after utilization (Sparks, 2003). These browse plants may also serve as a cheap

alternative feed source which can sustain and increase ruminant production on land not suitable for crop production. Forage from such rangelands is deficient in nitrogen, energy and some minerals and cannot support production (Ngwa *et al.*, 2002). As supplements these plants can benefit low-resource farmers who cannot afford the purchase of conventional protein sources. The nutritive value of these plants has also not been extensively researched, but more interest is now being shown in them, especially indigenous species. Published information shows that these fodder species, particularly the leguminous ones are able to provide forage that could maintain a productive state in livestock. If a proper management strategy is developed and followed, a lot of progress can be made and these plants can be properly utilized.

1.1.1 Desertification in South Africa

Semi-arid districts are defined as those in which the average annual rainfall was 300-600mm and where rain-fed crops succeed in four out of five years without irrigation. Arid districts are those in which the average annual rainfall was less than 300mm and conditions are generally too dry to grow the most drought-resistant crops without irrigation (Dean & MacDonald, 1994).

In Southern Africa, a region which is to a very large extent covered by arid land, the topic of desertification inspires a great deal of discussion and debate. According to the 1978 United Nations conference on the subject, desertification can be defined as the '...diminution or destruction of the biological potential of the land', in other words a conversion from a more productive state to a less productive one. Desertification according to Dean and MacDonald (1994) may be defined as a functionally irreversible decline in useable secondary production from untransformed semi-arid and arid rangelands due to local human influence and is indicated by long-term changes in total system diversity. The prediction that the area covered by desert in South Africa is increasing in size involves changes in the ratios between grass cover and shrub cover (Dean & MacDonald, 1994). The Karoo used to be perennial, palatable grassland (sweetveld) prior to habitation by European farmers. Today the occurrence of overgrazing and trampling has been so heavy that the grasses and shrubs have thinned out and have been replaced by unpalatable shrubs characteristic of the Karoo today (Dean & MacDonald, 1994). Selective and persistent grazing has played an important role in occurrence of unpalatable shrubs. The percentage of grass cover increases from the west to east following the rainfall gradient. The summer rainfall in the eastern regions provides a hot, wet season favouring the growth of C4 grasses (these grasses produce a 4-carbon molecule during photosynthesis which

make them more adapted to warm and hot seasonal conditions, therefore growth periods occur during the warm season). The fact that the grass cover is decreasing in this region and the shrubs taking over provides an impression that the Karoo is spreading eastwards (Dean & MacDonald, 1994).

The consequence of desertification is that it is accompanied by severe soil erosion. Therefore, the topsoil is no longer protected by grass cover and is lost by erosion and grasses can no longer re-establish themselves. Invasion by unpalatable Karoo bushes does not prevent erosion because their growth habit does not protect the topsoil (Van Breda & Barnard, 1991). There are large areas of the Karoo where the grazing quality has deteriorated and continuing to do so, especially since most of the topsoil has been eroded. Where palatable shrubs have been replaced by unpalatable ones, recovery may be hampered by the fact that many succulents and shrubs do not have soil-stored seeds. Palatable shrubs that have been heavily browsed are unlikely to be replaced if they die, even if browsing pressures are temporarily lifted (Van Breda & Barnard, 1991). There is also no natural restoration process whereby unpalatable shrubs are replaced by palatable ones. Field trials that have attempted to reintroduce certain palatable species have been met with limited success. Many farmers and arid-zone ecologists are aware of degraded farms and patches in these areas (Van Breda & Barnard, 1991). Hoffmann & Cowling (1990) suggest that karroid dwarf shrub lands are resilient to sustained grazing pressure, but nevertheless note that grazing in the Karoo subtly alters species composition and population structure. A large proportion of the highly palatable perennial shrub species of the winter rainfall region are currently rated as both scarce and fast disappearing or vulnerable (Van Breda & Barnard, 1991).

The Karoo is a complex ecosystem, which we do not yet fully understand. The soil, rainfall, temperature and seasonality gradients are pronounced. The rainfall varies from 50 – 500mm per year, with a marked increase in unpredictability in the drier regions to the west. The vegetation is also varied and includes succulents, shrubs and grasses. In the past it was believed that in order to minimise veld deterioration and maximise yields farmers should adopt a specific grazing management system. Today the systems are more farm specific (Lovegrove, 1993).

Over the years the demand for wool and other animal products provided the economic incentive for overstocking and overgrazing. Today, although the Karoo represents about one third of South Africa's farmland, the gross annual income from their agricultural products is only 6.2 percent (%) of the countries gross annual income. It has been estimated that for a farming unit in the Karoo to be economically viable, it must support at least 1200 small stock such as

sheep (Lovegrove, 1993). There is a possibility that large tracts of unproductive and denuded Karoo land could be granted to subsistence farmers but the result will be uncontrolled overgrazing and an accelerated rate of desertification and erosion (Lovegrove, 1993). There are alternatives to overstocking so that the area can be made economically viable. Veld conservation, through understanding of plant establishment and the conditions needed for this, is the most important requirement. Lower stocking rates, which result in the preservation of vegetation, generate far greater long-term gross income for the farmer than high stocking rates (Lovegrove, 1993).

Grazing by domestic livestock is thought to be a major force leading to dry land degradation in the semi-arid and arid rangelands of Southern Africa (Dean & MacDonald, 1994). It has been shown that decreases in stocking rates reflect decreases in the carrying capacity/secondary productivity of rangelands and this may be one of the symptoms of desertification. The present stocking rates in the semi-arid and arid regions reflect the maximum number of livestock units that rangelands can carry. Since stocking rates have been reduced, it is logical to suspect that the productivity of forage plants of these rangelands has declined over the past few decades. Changes in the absolute and relative abundance of fodder plants, together with irreversible losses of topsoil and changes in infiltration rates in semi-arid and arid rangelands has reduced the biomass of domestic livestock that can be carried on these rangelands (Dean & MacDonald, 1994).

1.2. Leguminous trees and shrubs

Legumes are known for their ability to grow in a symbiotic relationship with nitrogen-fixing bacteria. Many legumes are also very drought resistant. Legumes are superior to grasses in protein and mineral concentration and their nutritive value declines less with age. There are, however, certain nutritional disorders associated with certain legumes (McDonald *et al.*, 2002). Legumes can improve the quality of the diet of ruminants in three ways: by increasing the protein concentration of the diet; by increasing the intake of energy and protein by animals and by increasing the length of time that green forages is available to animals (Minson, 1994).

Animals grazing mature grass pastures often supplement their diet by consuming the foliage of trees and shrubs, many of which are legumes. The collective term applied to food obtained this way is 'browse'. In addition to the plants being harvested by animals, browse can be cut and fed to animals. The foliage of leguminous trees is high in protein 200-300g/kg dry matter (DM)) and minerals, but is also high in fibre (500-600 neutral detergent fibre (NDF)/kg

DM) (McDonald *et al.*, 2002). Foliage of some species also has a high concentration of condensed tannins, which can be problematical. Low to moderate concentrations of tannin precipitate soluble plant proteins and thus protect them against digestion in the rumen, but if the proteins are too firmly bound to the tannins they are not digested in the small intestine (McDonald *et al.*, 2002). Tannins can thus act as toxins and/or digestion inhibitors, with resultant suppression of feed intake and passage rate (McSweeney *et al.*, 2001). Tannins and other constituents may reduce the palatability of browse, so that its nutritional value may be rather as a food reserve, to be utilized when grass herbage is no longer available (McDonald *et al.*, 2002). Some of the commonly known browse species include leucaena (*Leucaena leucocephala*), sesbania (*Sesbania sesban*) and acacia (*Acacia angustissima*) (McDonald *et al.*, 2002).

Animal production from tropical legumes will be limited if they contain insufficient minerals or protein and if these nutrients cannot be obtained from other sources. The potential of legumes to provide these nutrients varies with the element and between legume species, stage of growth, and other variables (Minson, 1994). Tropical legumes contain, on average, 0.29% phosphorus, which is slightly higher than tropical grasses (0.22%). The calcium level (1.21% vs. 0.40%) is also higher and is usually well above that required by grazing ruminants, the magnesium trend being similar. The sodium concentration of most tropical legumes is, however, low (0.05%), which is the minimum dietary level recommended for ruminant production. However, certain non-leguminous fodder shrubs, such as *Atriplex*, may contain higher levels of sodium. Tropical legumes generally have a high crude protein concentration which declines slowly over time and even when mature they generally contain more than nine percent as such can be used as protein supplements to mature tropical grasses, leading to an increase in intake of grass (Minson, 1994). The chemical composition of legume forage hays may differ due to a variation in the leaf content of the hay and stage of growth. Some losses of leaf material can occur during harvesting and drying. The leaves contain more protein while the stems more fibre (neutral and acid detergent fibre) (Mupangwa *et al.*, 2000). The protein concentration in leaves and stems is 23% and 17% respectively with the solubility of the crude protein (CP) in leaves being lower, which may be due to a high tannin content. The apparent digestibility of the CP varies considerably. Most of this variation is positively associated with the level of CP. The capacity of legumes to produce an ammonia-rich rumen environment and by-pass protein that provides amino acids to the lower gastro-intestinal tract (GIT) gives an additional advantage of this type of feed (Ngwa *et al.*, 2002). The gross energy concentration in tropical legumes appears to be similar to those in grasses and temperate legumes. Not many values for metabolisable and nett energy have been published. The quantity of dry matter eaten by

ruminants is the single most important factor controlling production. Unless large quantities of legume are eaten, production will be low due to a low intake of digestible energy (Minson, 1994).

A disorder that is frequently encountered in cattle and sheep grazing on legume-dominated pastures is bloat. Lucerne is one of those posing a serious problem (McDonald *et al.*, 2002). The primary cause of bloat is the retention of the fermentation gasses in a stable foam, preventing their elimination by eructation. Soluble leaf proteins are thought to play a major role in the formation of the foam. Legumes that contain significant concentrations of condensed tannins (>20g/kg) are unlikely to cause bloat, probably because of the ability of tannins to precipitate soluble proteins. Another disorder associated with grazing sheep on pure lucerne is a sudden death syndrome termed 'redgut' (McDonald *et al.*, 2002). This is thought to be caused by the rapid passage of highly digestible forage through the rumen that causes increased fermentation in the large intestine. A large number of species are known to contain compounds, which have oestrogenic activity, the activity of these compounds can be increased as a result of metabolism in the rumen (McDonald *et al.*, 2002). The consumption of oestrogenic pasture plants by sheep leads to severe infertility and post-natal death in lambs. The infertility can persist for long periods after the ewes have been removed from the pastures. Cattle grazing these pastures do not appear to suffer infertility problems (McDonald *et al.*, 2002).

Leucaena contains a toxic amino acid, mimosine. In the rumen it is converted to dihydroxypyridine (DHP), a compound with goitrogenic properties. In countries with a natural population of leucaena, grazing animals' possess a rumen microorganism capable of destroying DHP (McDonald *et al.*, 2002).

All legumes appear to have the C3 photosynthetic pathway, but they can still be separated into cool and warm season types based on their adaptation to temperature. The photosynthesis response of Lucerne (*Medicago sativa*) to light is intermediate between cool and warm season grasses even though the leaf anatomy and enzymes involved with CO₂ fixation are clearly C3 (Nelson & Moser, 1994).

Leguminous multipurpose trees that are rich in nitrogen and widely used in the tropics offer an opportunity for use as nitrogen supplements to livestock fed on crop residues. Supplementation of *L. leucocephala* and *S. sesban* to sheep provided higher concentrations of rumen metabolites, which naturally improved rumen function and feed digestibility (Bonsi *et al.*, 1995).

1.3. Plant species

1.3.1 *Cassia sturtii*

Forage shrubs are useful forages for arid areas because of their adaptation and productivity in dry climates and poor soils (Ventura *et al.*, 2004). These plants can increase the carrying capacity of the land for grazing animals. They also have the potential to restore degraded rangelands. One of the most promising, in the Israeli experience, is *Cassia sturtii*, an exotic shrub from southern Australia (Faucon, 2001). It is a legume belonging to the family *Fabaceae*. The plant is an evergreen shrub, up to 1.8m tall and 1.8m wide with grey-green leaves and yellow flowers. It flowers during April. Once established it can survive with little water and is, therefore, able to tolerate drought and full sun (Faucon, 2001). It remains palatable year round, has good grazing resistance and the leaves have a reasonably high protein concentration (12%). Annual dry matter yields of about 1 ton per ha in a 200 mm rainfall area have been recorded (Pasternak *et al.*, 1986). Figure 1.1 gives an idea of the physical appearance of *C. sturtii*.



Figure 1.1 The flowers and leaves of *C. sturtii* (University of Pretoria Experimental Farm)

Relative to *Atriplex* species, *C. sturtii* recovered better after browsing; it recovered its biomass in the two highest planting densities and in the three lower densities; it had approximately 70% of its initial biomass at the beginning of a fifth browsing season. *C. sturtii* out-yielded *A. canescens* in edible biomass production but not *A. nummularia* (Benjamin *et al.*, 1995).

Wilcock *et al* (2004) reported a DM yield for *C. sturtii* (University of Pretoria Experimental Farm) over a 6 month period of approximately 500 g DM per plot (plot size; 60m in length and 17.5m wide). The growth of these plants may have been affected by frost during the winter; however the plants did recover quickly. As the plants increased in size, there was a decrease in percentage leaf material (Wilcock *et al*, 2004). This leaf and stem material were analysed for *in vitro* digestible organic matter (IVDOM), ash and CP. The results are shown in table 1.1.

Table 1.1 The *in vitro* digestible organic matter, ash and crude protein concentrations of the leaves and stems of *C. sturtii* (Wilcock *et al.*, 2004)

Nutrient	Stems (<3mm)	Leaves
IVDOM %	41.8	55.4
Ash g/kg DM	53	73
CP g/kg DM	76	147

The concentration of nutrients and IVDOM observed in leaf material was higher than in the stems. Similarly Van Niekerk *et al.* (2004,a) reported CP values for leaf material of *C. sturtii* from 114 to 147 g/kg DM and IVDOM varied from 529 to 574 g/kg DM. Both authors stated that *C. sturtii* proved to have fair potential and met requirements as a fodder crop.

In a trial conducted by Ventura *et al.* (2004), the nutritive value of three forage shrubs endemic from Canary Islands (*Bituminaria bituminosa*, *Rumex lunaria*, *Adenocarpus foliosus*) and two introduced shrubs (*Acacia salicina*, *Cassia sturtii*) were studied throughout a year. Although the above species have been widely used in ruminant feed, little research has been carried out to determine their nutritive value. Ruminal degradability and *in vitro* digestibility trials were conducted on the above legume shrubs.

The chemical composition of *Cassia sturtii* used in the trial conducted by Ventura *et al.*, (2004) is shown in Table 1.2, 1.3 and 1.4.

Table 1.2 Chemical composition (g/kg DM) of the edible portion of *C. sturtii* (Ventura *et al.*, 2004)

	DM	OM	CP	NDF	ADF	ADL
Mean	446	937	102	390	254	88

Table 1.3 Mineral (g/kg DM) concentration of the edible portion of *C. sturtii* (Ventura *et al.*, 2004)

	Ca	P	Mg	Na
Mean	13.4	1.7	1.2	3.6

Table 1.4 Organic matter and protein ruminal degradability (OMD, CPD, %), *in vitro* dry matter and organic matter digestibility (IVDMD, IVOMD, %) and digestible organic matter (DOM, g/kg DM) of the edible portion of *C. sturtii* (Ventura *et al.*, 2004)

	OMD	CPD	IVDMD	IVOMD	DOM
Mean	53.5	56.4	42.7	38.8	364

C. sturtii had the lowest CP concentration (mean 10%), compared to the endemic forage shrubs (Canary Islands) which varied between 15 and 18.5%. The fibre concentration was moderate (390g NDF/kg DM), but with a relatively high lignification (25% of NDF was ADL) and similar to the endemic species. The *C. sturtii* in this trial was shown to contain a significant amount of tannins. Organic matter (OM) and protein degradability was moderate (53.5 and 56.4% respectively). IVOMD and DOM values were low, probably because of the negative effects of tannins on digestibility. The mean NE concentration was low (3.2-3.3Mj/kg DM). The results of this research suggest that more than 1.5kg DM of *C. sturtii* is needed to provide the energy and protein requirements for the maintenance of a 50kg goat (INRA, 1988; as referred to by Ventura *et al.*, 2004). However the tannin content could affect the intake of these shrubs (Ventura *et al.*, 2004).

Some of the *Cassia* spp. have been shown to contain toxic compounds which make ruminants and monogastrics susceptible to poisoning by *Cassia* spp. A few articles have been published on the toxicological effects of *Cassia obtusifolia* and *Cassia occidentalis* which is also part of the *Fabaceae* (legume) family. Several compounds that bind strongly to cell membranes occur in *Cassia* spp., but the specific toxin responsible for muscle degeneration has not been identified (Herbert *et al.*, 1983). The toxin induces acute muscle and liver degeneration that can be rapidly fatal in most animals. The greatest concentration of the toxin appears to be in the seeds with *C. obtusifolia* and *C. occidentalis* considered to be more toxic than other species, but all *Cassia* spp. should be considered toxic unless proven otherwise (Mercer *et al.*, 1967). Pods

from *C. occidentalis* fed to cattle at a rate of 0.5 percent of their body weight induced severe muscle degeneration and poisoning occurred when the green plant was eaten at 0.4 to 12 percent of body weight (O'Hara *et al.*, 1969). Suliman and Shommein (1986) showed that at lower doses, *Cassia* spp. can cause diarrhea and decreased weight gain. The plant is not very palatable and tends to reduce feed intake. Some of the clinical signs are abdominal pain, straining and diarrhea are thought to be due to the irritant effects of anthraquinones in *Cassia* spp. Depending on the amount of plant or seed pods consumed, muscle degeneration begins several days after, causing weakness and recumbancy. The urine may be coffee coloured due to myoglobinuria. The levels of serum enzymes creatine kinase and aspartame transaminase are usually elevated. Renal failure may develop and in severe cases hepatic failure may be the predominant organ failure leading to death of the animal. Gross lesions at postmortem examination consist primarily of pale skeletal muscles similar to those seen in white muscle disease (O'Hara *et al.*, 1969).

Although these species are known to be troublesome weeds of the South eastern United States, Hawaii, Mexico and most of the tropical world, the studies conducted, have shown them to be deleterious. Whether the species *C. sturtii* also contains some of these toxic compounds is questionable but one worth investigating.

1.3.2 *Sutherlandia microphylla*

Sutherlandia microphylla (family-Leguminosae) is widely distributed in the Karoo, but prefers disturbed and gravelly soils. It is a soft shrub about 0.3-1.5 m tall and has a typical pea flower, scarlet to orange on colour. The fruit is an inflated pod, red when young and pale brown and papery when ripe. Although it is very palatable with a high nutritional value and good production, it is not, however, long lived. It is often grazed down to bare stems (Le Roux *et al.*, 1994). *Sutherlandia frutescens* is another species of the same family which grows in the same areas and only grows to 0.2-0.5m tall. The flowers and fruit are very similar. It has the same uses as *S. microphylla* but this species is often used in medicine (Le Roux *et al.*, 1994). The physical appearance of the flower of *S. microphylla* can be seen in Figure 1.2.



Figure 1.2 The flower of *S. microphylla* (University of Pretoria Experimental Farm)

Wilcock *et al.* (2004) reported that *S. microphylla* had the highest DM yield over a 6 month period, of approximately 1600 g DM per plot (plot size; 60m in length and 17.5m wide) when compared to *C. sturtii* of 500 g DM per plot and *Tripteris sinuatum* of 500g DM per plot (plot size; 60m in length and 17.5m wide). However, *S. microphylla* had the largest percentage decrease in leaf material with increase in plant size. The harvested material was analyzed for IVDOM, ash and CP concentrations. The results are shown in Table 1.5.

Table 1.5 The *in vitro* digestible organic matter, ash and crude protein concentrations of the leaves and stems of *S. microphylla* (Wilcock *et al.*, 2004)

Nutrient	Stems (<3mm)	Leaves
IVDOM %	38.9	66.0
Ash g/kg DM	25	64
CP g/kg DM	88	225

Sutherlandia is regarded as an indigenous multi-purpose medicinal plant in Southern Africa. Its efficacy as a safe tonic for diverse health conditions comes from a long history of use in numerous cultures. *Sutherlandia* assists the body to mobilize its own resources to cope with diverse physical and mental stresses and is, therefore, more correctly known as an adaptogenic tonic. Some of its uses include immune support, stress, depression, cancer, diabetes, gastritis and rheumatoid arthritis. Prof. Ben-Erik van Wyk (University of Johannesburg) and Dr. Carl Albrecht (Cancer association of South Africa) studied the chemistry of *Sutherlandia*. Four known key compounds contribute to the efficacy of this medicinal plant and are described below in more detail. The potent non-protein amino acid L-canavanine is an L-arginine antagonist with

antiviral, antibacterial, antifungal and anticancer activities and is found in significant levels in the leaves of *Sutherlandia*. Canavanine is distinctively immunosuppressive of critical cellular immune response against cancer cells and infectious organisms. *Sutherlandia* is, therefore, potentially an extremely dangerous plant and ought not to be sold as a safe and efficacious plant (Thomson, 2002).

Several legumes, often the whole plant, but in particular the seeds, contain this known toxic chemical canavanine, that, after being eaten, is imported into protein in place of arginine. Some herbivores, which are mixed feeders, have developed several survival defenses of their own. A number of canavanine-degrading bacteria may break down sufficient amounts of the dietary canavanine so that the toxic effects of this compound are reduced when ruminants eat these foods (Thomson, 2002). The earliest toxicity reports were of observed effects in rats fed canavanine-containing meal. Later experiments quantified the mammalian toxicity of canavanine, with 20mg/kg body weight having no effect, 200mg/kg showing clear damage and 2g/kg leading to death, all within 24 hours. When 1g/kg of arginine was fed with 200mg/kg canavanine, no toxicity was observed (Thomson, 2002). An explanation of the toxic effects was a disturbance of protein metabolism related to the disturbance of arginine functions. Relatively moderate canavanine feeding was observed to lead a reduction in normal weight gain. Milk production reduction was markedly reduced after feeding canavanine to dairy cows. When feed was given with protein-rich fodder, few ill effects were noted. Canavanine is rapidly metabolised in the liver, yet damage in this and other organs is reported. Post mortems of animals allowed to graze freely on canavanine rich plants have revealed lesions and haemorrhages of the lymph glands. Canavanine has, furthermore, been determined to be a vitamin B₆ antagonist (Thomson, 2002).

The compound Pinitol, which is an antidiabetic agent and Gamma-aminobutyric acid (GABA) an inhibitory neurotransmitter, have both been isolated from the leaves of *S. microphylla* as well. Asparagines have also been found (Thomson, 2002).

The published biological activities of these compounds appear to validate some of the traditional uses of *S. microphylla*, and further support the use of *S. microphylla* as a quality of life tonic in cancer and HIV-AIDS patients (Van Wyk, 2004 personal communication).

Very little research has been done on the nutritive value of this indigenous shrub for domestic animals. The fact that it is indigenous may lead to great interest in its use as a fodder shrub in the semi-arid rangelands of South Africa, but careful research needs to be conducted because the shrub may be potentially toxic.

1.3.3 *Medicago sativa* (Lucerne)

Lucerne (*Medicago sativa*) is an important forage legume widely planted in temperate areas and in many tropical and sub-tropical countries. It is commonly grown on its own and used as a hay crop (McDonald *et al.*, 2002). In Table 1.5 an example of the dry matter composition of lucerne is presented.

Table 1.6 Composition of the dry matter of lucerne (McDonald *et al.*, 2002)

	Pre-bud	In-bud	Early flower
Crude fibre (g/kg)	220	282	300
Ash (g/kg)	120	82	100
Crude protein (g/kg)	253	205	171
Digestible organic matter (g/kg)	670	620	540
Metabolisable energy (MJ/kg)	10.2	9.4	8.2

The value of lucerne hay lies in its relatively high crude protein concentration, which may be as high as 200g/kg if it is made from a crop cut in the early bloom stage (McDonald *et al.*, 2002). The traditional method of conserving green crops is that of haymaking. The aim of haymaking is to reduce the moisture content of the green crop to a low level as rapidly as possible to inhibit the action of plant and microbial enzymes. The custom of cutting the crop in a mature state, when moisture content is at its lowest, is clearly a sensible procedure for rapid drying and maximum yield, but unfortunately the more mature the herbage, the poorer the nutritive value (McDonald *et al.*, 2002). If optimal drying conditions are present hays made from early cut crops will be of higher nutritive value than those made from mature crops. Chemical changes such as oxidation, leaching and mechanical damage arise during the drying process and result in a loss of valuable nutrients (McDonald *et al.*, 2002).

Protein in lucerne forage is degraded both rapidly and extensively in the rumen. Forage protein is often wasted due to excessive ammonia formation in the rumen. The excess soluble protein, and/or protein in relation to available energy, often leads to an inefficient use of lucerne forage protein by ruminants (Bekker, 1995). The synchronous supply of nitrogen (N) and energy to rumen micro-organisms will maximise the capture of rumen degradable nitrogen (RDN) and

optimise microbial growth rate and efficiency resulting in an increased duodenal amino-N flow (Bekker, 1995).

The nutritive value and rumen kinetics are of particular interest and an experiment conducted by Bekker (1995) supplies some useful information on these particular parameters. The mean chemical composition over the three periods is shown in Table 1.7.

Table 1.7 Fibre parameters (% of DM) of lucerne in autumn, spring and summer. (Bekker, 1995)

Parameter	Period		
	Autumn	Spring	Summer
NDF	35.7	38.9	39.6
ADF	27.7	31.0	31.2
ADL	6.81	7.36	7.11
Cellulose	20.9	23.7	24.1
Hemicellulose	7.95	7.87	8.34

Significant seasonal effects were observed for NDF, ADF, ADL and cellulose (Bekker, 1995).

The effect of season was significant for organic matter intake (OMI) and digestible OMI (DOMI) where intakes in the third period were lower. No cultivar effects were identified. The influence of season on the digestibility and organic matter intake is presented in Table 1.8.

Table 1.8 The influence of season on organic matter intake and digestibility by sheep. (Bekker, 1995)

Parameter	Period		
	Autumn	Spring	Summer
OMI (g/d)	964	877	634
OMI (g/kg W0.75/d)	45.4	40.9	29.9
DOMI (g/d)	601	575	417
DOMI(g/kg W0.75/d)	28.4	26.8	19.7
Total tract apparent OM digestibility (%)	61.7	65.2	65.8

Table 1.9 shows the results of total volatile fatty acid (VFA) concentration and molar proportions of VFA in the rumen. No significant seasonal effects were observed between autumn and spring (Bekker, 1995).

Table 1.9 The influence of season on VFA production and molar proportions of VFA's in the rumen of sheep. (Bekker, 1995)

Parameter	Period		
	Autumn	Spring	Summer
Total VFA (mmol/100ml)	33.5	32.3	29.0
Molar proportions			
Acetic acid	0.638	0.642	0.662
Propionic acid	0.223	0.219	0.195
Butyric acid	0.102	0.100	0.106
Valeric acid	0.036	0.039	0.037
Acetic/Propionic	2.87	2.95	3.43

The results for nitrogen intake and rumen ammonia are presented in Table 1.10.

Table 1.10 The influence of season on nitrogen intake and rumen ammonia. (Live weight basis) (Bekker, 1995)

Parameter	Period		
	Autumn	Spring	Summer
N intake (g/d)	35.2	34.1	29.2
Rumen NH ₃ -N (mmol/l)	33.7	44.4	51.9

Significant seasonal effects were observed for mean N intake and rumen ammonia concentrations. Differences between autumn and spring are attributed to seasonal effects since both were conducted at a mature growth stage. Differences between spring and summer are attributed to growth stage effects since both were conducted in the same growing season but at

different maturity stages. It follows that the effects for growth stage were more pronounced than seasonal effects. The results on a live weight basis show the trends (Bekker, 1995).

A lower total VFA production at the younger growth stage explains the lower OMI and DOMI. At the younger growth stage a greater proportion of the forage protein is of leaf origin and is highly degradable (Bekker, 1995). At a younger growth stage, total VFA production in the rumen was significantly lower than the mature stage. This is attributed to the lower intake of digestible OM. Even though N intake was lower, rumen ammonia concentration was significantly higher in the younger stage.

1.4. Nutritive value

Poor nutrition is one of the major constraints to livestock productivity in sub-Saharan Africa. This is because animals thrive predominantly on high fibre feeds which are deficient in certain nutrients essential for microbial fermentation (Dicko & Sangare, 1984). Consequently, the digestibility and intake of digestible nutrients are unavoidably low. By supplementing the diet with feeds containing the deficient nutrients the deficiencies can be mitigated. Roughage diets and supplements differ vastly in quality and, therefore, quantity eaten by the animal (Dicko & Sangare, 1984). Previously, digestibility and chemical composition were used to describe the nutritive value of fibrous feeds; however an understanding of the factors which affect rumen degradability of low-quality basal feeds and microbial protein production, as well as determining the response (performance) from feeds, will provide more efficient designing of diets that can be utilised more efficiently (Osuji *et al.*, 1993b).

Forage quality can be defined as a function of both forage intake and digestibility. Ruminant productivity is the ultimate measure of forage quality (Paterson, 1994). Compared to grasses, fodder trees and shrubs have relatively higher concentrations of CP, minerals and NDF plus ADL, while their average concentration of ADF, as well as their average dry matter digestibility (DMD) are both lower. Chemical constituents such as N, NDF, ADF and lignin are essential to predict the nutritive value of shrubs (Kaitho *et al.*, 1998). These nutrient concentrations are subject to less variation than with grasses and this particularly enhances its value as dry season feeds for livestock (Dicko & Sangare, 1984).

Supplementation of tropical grasses with legumes has been reported to result in increased dry matter intake and to improve digestibility (Ndlovu & Buchanan-Smith, 1985).

A study was conducted by Mupangwa *et al.* (2000) on the herbaceous tropical forage legumes *Cassia rotundifolia* (Cassia), *Lablab purpureus* (Lablab), *Macroptilium atropurpureum*

(Siratro) and *Stylosanthes guianensis* (Stylo) to investigate the effect of feeding four legume hays as sole diets to mature sheep on DM intake, apparent digestibility, rumen ammonia levels, urinary excretion of purine derivatives and rumen microbial protein production. Although these forage legumes have great potential as protein supplements to supplement low quality roughages, studies on them as sole diets are scarce. Very little is known about these legumes regarding its intake by ruminants and the rumen microbial protein production and levels of animal response they can elicit when given to ruminants as sole diets (Mupangwa *et al.*, 2000). The nutritional parameters of these legume hays are presented in Table 1.11.

Table 1.11 Some nutritional parameters of legume hays given to sheep as sole diets (Mupangwa *et al.*, 2000)

	Cassia	Lablab	Siratro	Stylo	SED
DM intake (g/kgW ^{0.75})	12.1	48.2	52.6	50.9	2.94
Apparent digestibility-DM	0.550	0.638	0.581	0.577	0.034
N intake (g/day)	5.75	16.5	25.1	27.7	0.83
Faecal N (g/day)	1.08	4.77	4.81	4.20	0.55
Urinary N(g/day)	10.2	23.1	29.1	23.9	3.54
N retention (g/day)	-5.53	-11.4	-8.68	-0.53	4.04
Rumen NH ₃ -N (mg N/l)	150	225	159	161	15.9
Allantoin (mmol/day)	2.89	3.29	3.58	4.73	1.16
Microbial N supply (g/day)	2.70	3.15	3.43	4.53	0.98
Microbial N supply (g/kg DOMR)	50.1	12.9	16.0	18.1	8.69

DOMR (Digestible organic matter in the rumen) = 0.65 x DOMI (g/day)

The DM intake of the other hays was higher than that of *Cassia*. This difference could be due to higher apparent DM digestibility which may result in lower rumen retention time and greater turnover rate from the rumen of sheep. *Cassia* has been reported to have a relatively low acceptability which could be due to anti-nutritive factors such as condensed tannins. The tannins are reported to reduce voluntary intake through an unpleasant sensation in the mouth and an inhibition of microbial enzyme activity (Kaitho *et al.*, 1998).

The metabolisable energy intake of sheep in the four hays in Table 1.11 was 1.30, 5.92, 5.17 and 6.03 MJ/day respectively. The maintenance requirements for ME are 6.19 MJ/day (NRC, 2007). Animals consuming any of the hays are in a negative energy and nitrogen balance

and needed to mobilise body reserves to meet requirements. The type of protein and its degradability and an incorrect protein to energy ratio are the concern. This results in a reduced efficiency of rumen ammonia utilisation leading to excess ammonia in the bloodstream and loss in the urine as urea (Mupangwa *et al.*, 2000).

This study has demonstrated that an extensive loss of legume protein as ammonia in the rumen occurs in the absence of a readily fermentable energy source, which can result in a reduction of undegraded dietary protein flowing to post-ruminal sites. The efficiency of microbial production from rations based on forage legumes can be limited by a lack of a readily available energy source and the addition of such a source may assist in giving increased ammonia utilisation and microbial protein production. The legumes can be fed as protein supplements to ruminants consuming low quality tropical grasses and crop residues (Mupangwa *et al.*, 2000).

1.5. Digestibility and voluntary feed intake

Supplementation of tropical grasses with legumes has been reported to result in increased DM intake and improve DM digestibility. They also have great potential as protein supplements to these low quality roughages (Mupangwa *et al.*, 2000).

Prediction of voluntary intake of roughages by ruminants has long been a research priority in animal feeding. The level of feed intake is dependant on feed characteristics, animal and environmental factors and their interactions (Fonesca *et al.*, 1998). Feed characteristics are recognised as the most important factor when low quality roughages are offered. In the past simple and multiple regression equations were used to predict intake and digestibility, however a simpler method of feed evaluation was proposed.

The nylon bag technique, in which samples of feed are directly incubated in the rumen, has become widely used. This technique allows rumen digestion to be studied at different periods of time, and thereby the kinetics of digestion (Fonesca *et al.*, 1998).

1.5.1 *In situ* digestibility

The history of the development of methods for the assessment of the value of feedstuffs for animal production is a long one. In the early attempts in Europe feeding trials were used, and the workers also tried to predict the nutritive value of feedstuffs by the extraction of the “solubles” with water, alkali, ether and alcohol. As knowledge increased, the early methods were modified and developed, in order to improve the reliability with which laboratory techniques

could be used to predict nutritive value to the animal. Although highly developed laboratory procedures are now available, such as acid detergent fibre, the modifications which have been introduced have often simply attempted to mimic the *in vivo* process. For the evaluation of feedstuffs, *in vivo* techniques are nearly always preferred. The use of the nylon bag has the advantage of giving a very rapid estimate of the degradation of feedstuffs in the functioning rumen (Orskov *et al.*, 1980).

The nylon bag technique provides a means of ranking feeds according to the rate and extent of degradation of dry matter, organic matter, nitrogen or other nutritional parameters. It involves incubating samples of feeds in the rumen of fistulated animals for periods of from six to 120 hours and subsequent determination of the disappearance of the different feed components (Orskov *et al.*, 1980).

1.5.2 Intake

Domestic ruminants utilize trees and shrubs as both browse and “cut and carry” branches in the stall. On rangelands, animals have the advantage of selecting from a wide choice of browse and obtaining high quality feed. Consumption of various types of forage reduces chances of poisoning. The “cut and carry” system obviates the limitation of inaccessibility of certain browse. The system also facilitates rational usage of fodder. Its disadvantages include damage to the trees due to injudicious lopping and the imposition of a limited variety of feed which may increase the risk of poisoning (Dicko & Sangare, 1984).

Regulation of intake in ruminants is primarily a function of physical fill for diets that are energetically bulky and less digestible, such as high forage diets; but intake becomes primarily a function of metabolic control for dense and highly digestible high concentrate diets. Cell wall concentration of forage diets is the best single chemical predictor of intake. Depression of dry matter digestibility associated with increased intake is less for legumes than for grasses (Milford & Minson, 1965a).

Voluntary intake is determined by offering animals a known quantity of feed and determining the amount at the end of the feeding period. Digestion and retention coefficients are determined by collecting all the excreta (urine and faeces) and analysing feed and excreta samples. The amount of some of the nutrients absorbed and retained in the body or stored can also be determined by analysing excretions such as urine (Osuji *et al.*, 1993a).

Mgheni (2000) conducted a trial to describe tropical forages in terms of its rumen degradability characteristics, digestion and passage rates of fibre and the resultant rumen pool

sizes in order to estimate the physical fill and potential intake of tropical forages. The results demonstrated that intake of these forages can adequately be described from physical fill based on degradability characteristics, rumen pool size and passage rate of NDF measured by the rumen evacuation technique. The use of NDF parameters as predictors of voluntary feed intake is recommended as a realistic estimate of forage dry mater intake because NDF was found to be distinct from microbial and endogenous materials (Mgheni, 2000).

1.5.3 Rumen evacuation

Rumen digestibility of the dietary component is a function of the rate at which it is digested (k_d) and the rate at which it passes (k_p) from the rumen. Each rate expresses the fraction of the rumen component pool which is digested (k_d) or passes (k_p) from the rumen, per unit time. The use of markers has indicated that a decreased intake is associated with a decreased passage rate, increased rumen pH and an increased rate of digestion of NDF or its components (Robinson *et al.*, 1987). The components of rumen fibre kinetics are graphically explained in Figure 1.1 below.

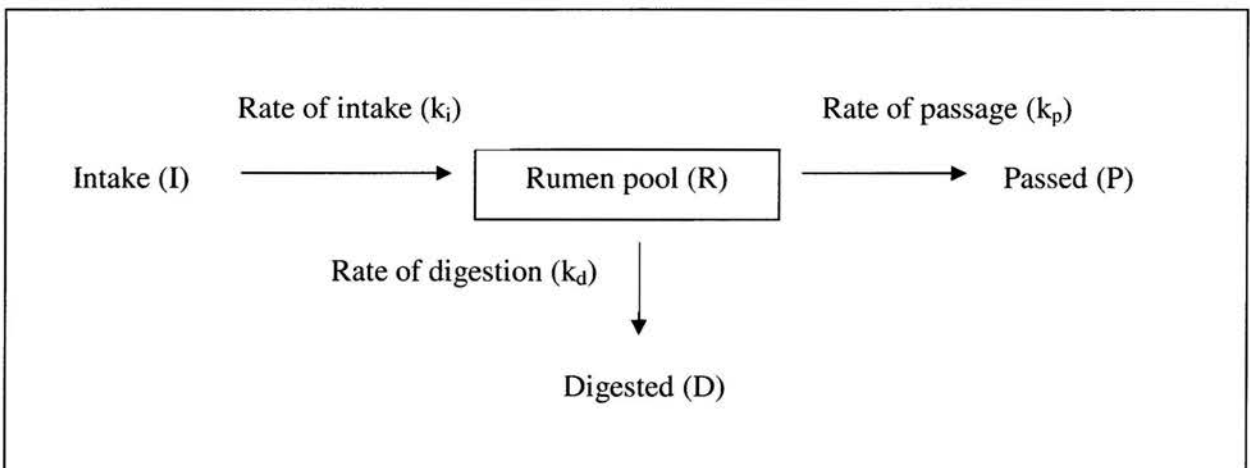


Fig. 1.3 Rumen fibre kinetics (Robinson *et al.*, 1987)

The complete removal and mixing of reticulorumen contents appears to be a valid technique in digestion studies. Rumen evacuation derived rates of digestion of fibre (k_d) are highly correlated with *in vivo* digestibility of fibre; therefore it is a useful technique in estimation of the rate of fibre digestion (Khalili, 1993).

Estimating the rate of fibre digestion in the rumen using the nylon bag technique gave results lower than the results based on the rumen evacuation data (Tamminga *et al.*, 1989). The porosity and closed surface area of the bags and the lack of rumination may provide some explanation for the different results. According to Tamminga *et al.* (1989), including rumen evacuation data in a rumen digestion model resulted in a level of rumen digestion which was much close to data observed *in vivo* than those based only on nylon bag incubations.

1.5.4 Flow rates

The extent of digestion of a feed depends on its rate of digestion and on the time the feed spends in the digestion pool. The animal's requirements are met from the digested component of intake. Flow rate is the rate (mass/time) at which digesta (feed or a marker) leaves a compartment per unit time. Flow rate or fractional outflow rate are estimated to determine the mean duration feed remains in the GIT, usually called the mean retention time (MRT). The time available for digestion in each pool ($t_{1/2}$) is also estimated since it is reported to have a strong positive correlation with OM digestibility (Grovmum & Williams, 1977). Markers or rumen evacuation can be used to estimate both rumen volume and passage rates (Osuji *et al.*, 1993a). The determination of passage rate of NDF using the rumen evacuation technique, was much lower (Huhtanen & Khalili, 1991) than that based on chromium (Cr) mordanted straw particles (Huhtanen, 1988) in cattle fed similar diets. Aitchison *et al.* (1986), Robinson *et al.* (1987) and Tamminga *et al.* (1989) observed higher rates of passage (k_p) for the Cr-mordant than the value based on rumen evacuation. The reason for these different results is that mordant particles are not digested. Recent data (Tamminga *et al.*, 1989) showed that Cr-mordanted particles gave fairly accurate estimates of the passage of indigestible cell wall materials.

There are certain impediments to particle passage from the rumen, such as the fibre mat and reticulo-omasal orifice which affect the mechanism of particle passage. There are also factors which control the passage, these include; specific gravity of a feed particle which must increase so that it can drop from the mat into the liquid layer to pass through the rumen; size of particle must be reduced by rumination and microbial digestion, which allows for passage through the fibre mat and reticulo-omasal orifice; particle shape will also affect passage rate as well as rumen volume and motility (Firkins *et al.*, 1998). Passage rates of animals with a higher rumen pool size will have a lower rate of passage than those with smaller rumens. Typical values for rate of passage of roughages are 1-6% per hour (Firkins *et al.*, 1998).

As the level of intake increases, the passage of liquid and solid digesta increase and where there is an increase in fibre concentration, the passage rate of liquid and smaller particles will increase. This effect of fibre varies with the type of roughage. When lucerne is fed as the forage the digesta separates into a liquid and fibre mat and the grain particles will fall into the liquid fraction. By increasing the amount of forage in the diet, there is an increase in rumination and therefore secretion of salivary buffers. These buffers increase the osmotic pressure of the rumen contents and therefore increase the passage of liquid digesta and grain which reduces grain digestibility in the rumen. An opposite example is one where cotton seed hulls are fed as the forage. These do not ferment as quickly as lucerne does and it also does not form a mat, but rather a homogenous mixture with liquid digesta and grain. Higher amounts of cotton seed hulls therefore reduce the passage rate of grain and increased rumination of the grain, increases digestibility in the rumen (Firkins *et al.*, 1998).

The time of day feeding can also affect the rate of passage but only really applies to protein supplements (Firkins *et al.*, 1998).

The rate of passage affects both rate and site of digestion. At a constant rate of digestion, increasing the passage rate will decrease the digestibility of a feed in the total tract and increase the proportion of digestion that occurs in the lower tract. The depression in digestibility associated with increased rate of passage is greater for starch than cellulose and may also be acceptable, if the increase in intake increases the total amount of digestible dry matter consumed. For example, in typical dairy rations a 0.9% increase in rate of passage will lead to a 1% increase in digestible dry matter intake. Grinding the feed will also be more effective in increasing digestible dry matter intake of low quality forages than high quality ones (Firkins *et al.*, 1998).

The rate of passage may also have an effect on the VFA production, where an increased rate of passage will decrease the total VFA production. This effect is associated with a reduced dry matter digestion. A higher turnover rate of the liquid fraction will increase the production and concentration of acetic acid, butyric acid and methane and decrease propionate (Firkins *et al.*, 1998).

1.5.5 Rumen fermentation

Rumen fermentation can normally provide about 70 – 100% of the amino acids supplied to ruminants while volatile fatty acids (VFA), the main end products of microbial fermentation,

supply about 70-85% of the energy absorbed. Poor quality roughages are often unable to support rumen conditions that are conducive to optimal microbial activity because of their deficiency in total N, true protein, readily fermentable carbohydrates and minerals. Leguminous forages have a potential as high quality, low cost and readily available supplements and can contribute to better utilization of poor quality roughages (Tolera & Sundstol, 2000).

The ammonia-N in the rumen fluid is the key intermediate in the microbial degradation and synthesis of protein. However, rumen ammonia-N concentration only reflects the balance between production, absorption and incorporation into microbial cells (Tolera & Sundstol, 2000). An imbalance in energy and protein fermentation has been reported to cause an accumulation of rumen ammonia nitrogen and the rate of *in situ* DM and NDF degradation are slower, which could be a result of limited available energy for incorporation of rumen ammonia N into microbial protein (Melaku *et al.*, 2004). There is a lot of disagreement in literature concerning the optimum rumen ammonia-N level for microbial activity in the process of digestion. McDonald *et al.* (1996) put the estimates in a range of 85 -300mg/l NH₃-N rumen fluid, while Abdulrazak *et al.* (1997) indicated that rumen ammonia concentration of 50-80 mg/l NH₃-N rumen fluid could be sufficient for fibre digestion. In general, the minimum value of ammonia concentration for optimal rumen function could be variable depending on the type of feed (Tolera & Sundstol, 2000).

The quantities and relative proportions of the VFA present in the rumen depend on the type of fermentation, which in turn is determined by the microbial population which depends on the type of feed and feeding regime. The proportion of acetic acid usually increases with increasing levels of cellulose in roughage diets (Orskov, 1994).

In the study done by Melaku *et al.* (2004) the rumen fermentation pattern in sheep supplemented with the various leguminous trees was a typical fermentation pattern for roughage feeds, which is characterised with a high rumen pH. High rumen pH encourages growth of cellulolytic microorganisms and the synthesis of acetate as a major product of fermentation with low proportions of propionate and butyrate. This could be attributed to the high fibre concentration in the supplement feeds. Differences in molar proportions of rumen fermentation end products can influence energy metabolism pathways, thereby efficiency of feed energy utilization. Higher molar proportions of propionate in the trees and their mixtures may have decreased energy losses in inter-species hydrogen transfer and thereby, methane production in the rumen. Therefore, supplementation with the trees and their mixtures appears to be efficient due to the higher propionate production which has a positive relationship with efficiency of energy utilization (Van Houtert, 1993).

In an experiment done by De Visser *et al.* (1992) it was discovered that rumen fluid contents increased sharply after feeding, resulting in dilution of the VFA and buffering the pH to decline. In addition, rate of absorption from the rumen increases with higher VFA concentrations. The ruminant animal has a number of mechanisms which will prevent the average VFA concentration from rising above the maximum of 150 mmol/l (Tamminga & Van Vuuren, 1988), thereby preventing a drop in pH values that inhibit the rate of degradation. It is also been shown that the production of total VFA decreases linearly as intake declined (Robinson *et al.*, 1987).

1.6. Protein

A significant variation in CP concentration (6 to 23% DM) occurs between (tree and shrub) species and even between edible parts of the same plant. In general, leaves are higher in CP than stems, almost twice as much in the case of southern African browse according to Walker (1980). Leaves also contain more CP on average than the pods although the latter were characterized by a higher organic matter and digestibility. Leguminous species were found to contain 25-30% more CP than non-leguminous plants (Dicko & Sangare, 1984). Nitrogen retention is considered as the most common index of the protein nutrition status of ruminants (Melaku *et al.*, 2004).

1.6.1 Microbial protein production

Studies on the urinary excretion of purine derivatives by ruminants have been stimulated by the possible use of their excretion as an estimator of the rumen microbial protein supplied to the animal. This is because in ruminants nucleic acids flowing to the small intestines are essentially of rumen microbial origin. Absorbed purines are degraded to hypoxanthine, xanthine, uric acid and allantoin, which are excreted in the urine and should relate quantitatively to the amount of purines and hence microbial protein absorbed. However, some of these purine derivatives originate from the animals tissues, and the endogenous contribution has, therefore, to be quantified (Chen *et al.*, 1990).

Measurement of microbial protein supply to ruminants has been an important area of study in ruminant protein nutrition. An estimate of microbial protein contribution in the intestinal protein flow is incorporated into the new protein evaluation systems already being used in different countries. The supply of microbial protein to the animal per unit feed ingested, usually

expressed as g microbial N/kg digestible organic matter fermented in the rumen (DOMR), varies by almost 4 folds (14-60gN/kg DOMR) (Agricultural Research Council, 1984). This variation is due to the influence of various factors relating to the diet or rumen environment. The effects of many of these factors have not yet been conclusively demonstrated or quantitatively defined (Chen & Gomes, 1992).

Microbial protein contributes a significant part (0.42-0.93) of the total protein flux into the small intestine in ruminants. Its proportion is usually determined with microbial markers, including nucleic acids, diaminopimelic acid (DAPA), ³²P and ¹⁵N. These methods are tedious and require cannulated animals, infusion of isotopes and separation of rumen microbes. Cannulation can also cause some changes in digestion, may reduce intake and limits the amount of experimental animals. Many attempts have been made to find an indirect measurement of microbial protein synthesis in intact animals (Djouvinov & Todorov, 1994).

The microbial protein entering the duodenum can be estimated by quantification of urinary allantoin. The nucleic acids synthesized by the rumen micro-organisms are enzymatically degraded to purine and pyrimidine bases which are absorbed; their final products are excreted in the urine with allantoin being in the greatest proportion (Tebot *et al.*, 2002). Several authors have revealed a close relationship between microbial nucleic acids reaching the small intestine and the urinary excretion of purine derivatives, especially allantoin (McAllan & Smith, 1973 and Antoniewicz *et al.*, 1980). The relative contribution of allantoin to the total excretion within each animal is relatively constant, irrespective of purine output, therefore the measurement of allantoin alone could be used an estimator of the supply of purines. In ruminants, allantoin appears to originate predominantly from nucleic acids synthesised by rumen microbes (Antoniewicz *et al.*, 1980).

1.7. Fibre

The fibre components of feeds inhibits the intake of forages due to slow digestion compared to cell solubles (Van Soest, 1982), and a limitation on feed intake is apparent when fibre concentration of feeds contains more than 500g per kg of forage DM (Van Soest, 1965). However, Milford & Minson (1965) reported that forage intake dropped sharply when sheep were fed forages with CP levels below 70g/kg DM. The digestibility of feed is closely related to its chemical composition, and feed which varies in composition is more likely to vary in digestibility. The fibre fraction of feed has the greatest influence on digestibility, with both the amount and chemical composition of the fibre being important. When forages are heated with a

neutral detergent solution, the cell contents are dissolved and the residue remaining is the NDF which contains the cell walls. The cell wall fraction is further divided into hemicelluloses and cellulose plus lignin (ADF) (McDonald *et al.*, 2002). The digestibility of the cell wall (cellulose) depends on the degree of lignification (lignin concentration of the ADF). Tropical forages generally have a lower digestibility because their leaves contain more vascular bundles, therefore more lignin, and their dense mass of cells resist digestion by micro-organisms (McDonald *et al.*, 2002).

1.8. Minerals

Mineral elements have various functions in the animal body and the concentration must be maintained to ensure functional and structural integrity of the tissues and to ensure that the growth, health and productivity of the animal are unimpaired (Underwood & Suttle, 1999). Large numbers of livestock in many parts of the world consume diets that do not meet the requirements which may lead to nutritional disorders, ranging from acute mineral deficiency or toxicity diseases to mild conditions where unsatisfactory growth, production and fertility are a result (Underwood & Suttle, 1999). There are many mineral interactions with other nutrients or minerals as well as animal and environmental factors which can affect their availability to the animal.

The concentration of minerals in forages depends on the species of plant, the type of soil on which the plant grows, the climatic conditions and the stage of maturity (Underwood & Suttle, 1999).

In foraging situations, the diet sample collected may not be a representation of the material actually eaten by the animal, because of selective grazing and soil contamination in the field. Animals show preferences for different plant parts, which can vary in mineral concentration. Estimates of mineral intake and plant composition do not take into account the absorption and utilization by the animal for example; zinc and manganese concentrations can be adequate when there are normal levels of calcium or phosphorus but deficient when high in those elements (Underwood & Suttle, 1999).

Leguminous species are generally richer in macro elements. The trace elements, notably iron, copper and zinc are also higher in legumes. Phosphorus concentrations decline with advancing maturity, as does the concentration of magnesium, zinc, copper, manganese and iron but not to the extent that phosphorus does. A decrease in mineral concentration with maturity is usually a

reflection of increases in the proportion of stem to leaf, stems and older leaves have lower mineral concentrations than young plant parts (Minson, 1990).

The concentration of calcium and potassium is usually higher than that of other minerals; the average being around 1 to 1.5% for southern African browse (Walker, 1980). Some abnormally high concentrations of some minerals such as sodium chloride and selenium may be found in browse (Dicko & Sangare, 1984).

Trace minerals are essential components in the animal body for growth and the prevention of a wide range of clinical and pathological disorders. Some naturally occurring diseases were associated with a deficiency in trace minerals and were found to respond to trace mineral supplementation. There are also many documentations of interactions between trace minerals and other minerals, for example that of copper and molybdenum (Underwood and Suttle, 1999).

Very little is known about *C. sturtii* and *S. microphylla* regarding their intakes by ruminants and other nutritional effects they may have when given to ruminants as sole diets.

The value of these forages to provide animals with sufficient nutrients for maintenance during dry periods is a neglected topic.

The research in this thesis will investigate the effect of feeding these legume hays (*C. sturtii* and *S. microphylla*) compared to *M. sativa*, as sole diets to sheep on intake, digestibility, rumen parameters and microbial protein production.

Their role in alternative sources of feed for semi-arid regions and the reclamation of degraded rangelands warrants more research.

Chapter 2

Materials and Methods

2.1 Introduction

The study consisted of three sections. The first was a digestibility trial involving feed intake, faeces and urine excretion and certain rumen parameters being measured. The second was an *in sacco* trial to measure the DM and NDF degradability of the feeds, and the third was a rumen evacuation trial measuring the passage rate of the feeds.

All three sections to the trial were conducted at the same location and involved the same animals. Measurements and analytical methods will be discussed under each section concerned.

The aim of this study was to determine the nutritive value of the two leguminous forage shrubs, namely *C. sturtii* and *S. microphylla*, compared to *M. sativa* (SA Standard cultivar), which is a well-known leguminous forage plant. By comparing the two shrubs one can determine the value to the animals and whether or not it will be sustainable to utilize these shrubs as fodder for sheep in dry areas.

2.2. Location

The trial was conducted on the Hatfield Experimental Farm of the University of Pretoria, South Africa (co-ordinates 025°15'28.9"E, 25°45'03.6"S). Pretoria is situated in the Gauteng Province at an altitude of 1360m. It is a summer rainfall area with an average annual rainfall of 650mm, of which half occurs during November to January. The temperatures are moderate in winter and occasional frost does occur. The average daily minimum and maximum temperatures are 10.3 and 24.5°C respectively.

The soil type is a Hutton form (MacVicar *et al.*, 1977), is well-drained and slightly acidic and has a good nutrient status. The Hutton type is a deep-clay-loam soil with approximately 25% clay and an effective depth of 600mm+.

2.3. Material

The *C. sturtii* (Origin: Australia), *S. microphylla* (Origin: South Africa) and *M. sativa* (SA Standard cultivar) were planted and harvested on the Hatfield Experimental farm. The *Cassia* plants were established from seedlings in February 2002 and were approximately 3 years old when harvested, while the *Sutherlandia* was seeded *in situ* in the spring/summer of the 2003/2004 season. Both plants were established under dry land conditions. The *M. sativa* was cultivated during late summer 2003 under dry land conditions. The whole plants were harvested from May to June 2004 at a height of 30cm from ground level and then sun-dried. To minimize selection of the material all harvested material was milled through a 1cm sieve size. The physical appearance of *C. sturtii* and *S. microphylla* before harvesting, are presented in Figure 2.1 and 2.2.



Figure 2.1 Physical appearance of *C. sturtii* before harvesting on the Hatfield Experimental Farm



Figure 2.2 Physical appearance of *S. microphylla* before harvesting on the Hatfield Experimental Farm

2.4. Animals

Six adult cannulated Döhne Merino wethers were used. Three of the sheep were fitted with small rumen cannulae (5cm diameter) and the other three with large rumen cannulae (10cm diameter). The same animals were used during each section of the trial.

A randomised block design was used to allocate animals to treatments. Each sheep received all three treatments, starting with one and then moving to the next treatment in the following period. The three treatments included feeding with *Cassia sturtii*, *Sutherlandia microphylla* or *Medicago sativa*. The animals were kept in individual cages in the sheep metabolism house of the Small Stock Section on the Hatfield Experimental Farm for the full duration of the experiment. For the digestibility trial they were housed on concrete floors in larger cages during the adaptation period and were put into the metabolic crates three days before the experimental period and remained in the crates for the duration of the experimental period. Thereafter they were once again placed in larger cages on concrete floors until the next experimental period. During the other two trial sections, the sheep remained on the concrete floors and sampled as necessary.

All animals received the necessary vaccinations (pulpy kidney and blue tongue) and were dosed for internal parasites before the start of the experiment. Animals were fed twice a day and

received fresh water on an *ad-lib* basis. The metabolic house was cleaned on a daily basis. The ethics committee (project nr. AUCC 041215-031) of the University of Pretoria, Animal and Wildlife Sciences Department evaluated the trial before any research was undertaken and approved the procedures.

2.5. Digestibility trial

Voluntary intake and digestibility was determined by offering animals a known quantity of feed *ad lib* and determining the amount remaining at the end of the feeding period. Digestion and retention coefficients were determined by collecting all the excreta (mainly urine and faeces) and analysing feed and excreta samples.

The digestibility trial was conducted using all six cannulated animals. The trial consisted of three periods with each animal allocated to a treatment during the period (randomized block design – see Table 2.1). This allowed for a more accurate statistical analysis to minimize the error.

In Table 2.1 the experimental outline of the digestibility trial is presented.

Table 2.1 Experimental outline of the digestibility trial conducted on six cannulated animals using the three forages *S. microphylla*, *C. sturtii* and *M. sativa*

Period 1 (16 days)	<i>S. microphylla</i> (2 sheep)	<i>C. sturtii</i> (2 sheep)	<i>M. sativa</i> (2 sheep)
Period 2 (16 days)	<i>C. sturtii</i> (2 sheep)	<i>M. sativa</i> (2 sheep)	<i>S. microphylla</i> (2 sheep)
Period 3 (16 days)	<i>M. sativa</i> (2 sheep)	<i>S. microphylla</i> (2 sheep)	<i>C. sturtii</i> (2 sheep)

The experimental procedure lasted for approximately 20 days. The first 14 days consisted of an adaptation period for the experimental diets, while the following 6 days were used for data collection, including collection of feed, orts, faeces and urine. The animals were fed *ad lib* dry feed per day during the adaptation and experimental period.

2.6 Measurements and preparation for chemical analyses

Animals were weighed at the start and end of each period to determine the change in body weight during the trial. Weighing was after a period of overnight starvation (food and water). Individual intake was recorded by weighing the feed offered and the orts daily. A daily sample of each feed (as fed) and feed refusals was obtained and stored for later analysis. At the end of each period the samples from each feed were thoroughly mixed together, oven dried at 55 °C for 48 hours and then stored until analyzed. Each animal was equipped with a faecal bag to record daily faecal excretion. Faeces were collected twice a day, weighed and a 10% representative sample was taken. The samples were pooled and then dried for 48 hours at 100°C in a force draught oven, milled and stored for later analysis. The metabolic crates used were such that the total urine output could be collected and measured daily. This was collected in a container that contained 25ml of a 10% H₂SO₄ solution to prevent loss of urinary ammonia. The urine plus acid was then stored in plastic vials and frozen until analyzed for allantoin and uric acid.

Three random samples of each feed were taken at the start of each period, then pooled into one sample to determine the chemical composition of the feeds. DM, ash, NDF and ADF, ADL, CF, CP and minerals were determined.

Feed samples (5 – 10%) were collected at each feeding and all faecal and urine excretions collected were stored at –10°C until the end of the collection period. At the end of the collection period, total faeces and urine collected was mixed and sub-sampled. The urine was analysed for protein/nitrogen concentration. Feed and faecal samples were ground to pass through a 1mm sieve and dried to determine DM concentration according to the AOAC 934.01 (2000). Total nitrogen was determined using the Dumas/Leco technique described in the AOAC 968.06 (2000). NDF and ADF was determined as described by Goering and Van Soest (1970).

On days 3 and 4 during the experimental period samples of rumen fluid were taken over a period, giving 12 samples; 08:00, 12:00, 16:00, 20:00, 24:00, 04:00, 10:00, 14:00, 18:00, 22:00, 02:00 and 06:00 respectively.

Rumen contents were collected with the aid of a 60ml syringe connected to a plastic tube. Contents were drawn up from a few locations in the rumen into the tube by aid of suction caused by the syringe. Approximately 150ml of rumen contents was collected per animal during each sampling period.

The rumen contents were drained through cheesecloth, with the solids being discarded. A 30ml sample of rumen fluid was put into a container with 5ml 0.5 M H₂SO₄ to be used for the determination of rumen ammonia nitrogen (NH₃-N) concentration with an auto analyzer (Broderick & Kang, 1980). A further 10ml sample was placed in a container with 1.0ml of a 10% sodium hydroxide (NaOH) solution for VFA analysis, using the gas chromatographic method (Webb, 1994). The samples were then pooled for each animal and then frozen for later analysis.

2.7 Chemical analysis

2.7.1 Dry matter and Ash

Dry matter was determined on the feed, orts, faeces, *in sacco* and rumen content samples. (AOAC: 934.01, 2000).

$$\%DM = (\text{Mass of oven dry sample} / \text{Mass of air dry sample}) \times 100$$

$$\%Ash = (\text{Mass of Ash} / \text{Mass of air dry sample}) \times 100$$

$$\%OM = 100 - \%Ash \text{ (DM-basis)}$$

2.7.2 Nitrogen and crude protein

CP was determined on the feed, orts and faeces samples. It was determined using the Dumas method described in the AOAC 968.06 (2000).

2.7.3 Crude fibre

CF was determined for the three feed samples using the ceramic fibre filter method described by the AOAC 962.09 (2000).

$$\text{Crude fibre \%} = [(W2 - W3) - (B2 - B3)] / W1 \times 100$$

Where W1 = original sample weight

W2 = sample weight after drying at 110°C

W3 = sample weight after ashing at 550°C

Where B2 and B3 are the average weights of all the blanks after drying and ashing respectively.

2.7.4 Neutral detergent fibre (NDF)

NDF was determined for the feed, Orts, faeces, rumen content and *in sacco* samples. The analysis was done using the Dosi fibre system (Robertson & Van Soest, 1981).

NDF was calculated as follows:

$$\% \text{NDF} = [(\text{dry mass of NDS extracted sample (g)} - \text{mass of ash (g)}) / \text{sample mass (g)}] \times 100$$

2.7.5 Acid detergent fibre (ADF)

ADF was determined according to the method by Goering & Van Soest (1988).

ADF was calculated as follows:

$$\% \text{ADF} = [(\text{dry mass of sample after ADS extraction (g)} - \text{mass of ash (g)}) / \text{sample mass (g)}] \times 100$$

2.7.6 Acid detergent lignin (ADL)

ADL was determined according to the method by Goering & Van Soest (1988).

ADL was calculated as follows:

$$\%ADL = [(W1 - W2)/W0 \text{ (sample mass)}] \times 100$$

Where $W0$ = original sample mass

$W1$ = weight after acid extraction and first overnight drying

$W2$ = weight after ashing at 550°C for three hours

2.7.7 Minerals

Samples for mineral analysis (Ca, Mg, Zn, Cu, Mn, Fe) were prepared by the method of the AOAC 935.13 (2000). Sample preparation for phosphorus was done using the AOAC 968.08 (2000) method.

Atomic absorption spectrophotometry was used to determine calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) concentrations according to the method by Giron (1973). P concentration was determined using the method by AOAC 965.17 (2000).

The mineral concentration was estimated as follows:

$$\% \text{ Element} = (\mu\text{g/g of element in sample solution} \times \text{df} \times 0.0001) / \text{sample weight (g)}$$

For Ca, Mg and P which is expressed as g/kg.

$$\text{mg/kg Element} = (\mu\text{g/g of element in sample solution} \times \text{df}) / \text{sample weight (g)}$$

For Fe, Zn, Cu and Mn which is expressed a parts per million (mg/kg)

Where df = dilution factor

2.7.8 Rumen NH₃-N

The rumen fluid samples were analyzed for rumen ammonia nitrogen concentration, using a Technicon Auto-Analyzer (Broderick & Kang, 1980).

2.7.9 Rumen Volatile fatty acids (VFA)

The determination of VFA's in the rumen is done by using the Gas chromatographic method. The apparatus used was a Varian 3300 FID Detector Gas Chromatograph, of which the gas is hydrogen and air (Webb, 1994).

The results were calculated as follows:

$$\text{mg/100ml VFA in sample} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{standard concentration} \times \text{dilution factors}$$

The above result can then be divided by the molecular mass of the VFA to obtain concentration in mmol/100ml sample (Webb, 1994).

2.8 Estimation of microbial protein supply using urinary allantoin

The urine was analyzed for allantoin to determine the microbial protein concentration in the method described by Pentz (1969).

2.8.1 Sample preparation

- 1) Total urine excretion was collected in a plastic container on a 24 hour basis from the animal for 5 – 7 days.
- 2) 100ml of a 10% H₂SO₄ was added to the urine to ensure the pH remained less than 3.
- 3) Each morning the quantity of urine voided was measured, and 3 – 5l of water was added to prevent precipitation of uric acid.
- 4) A subsample (20 – 50ml) of diluted urine was taken, and frozen for allantoin analysis.

- 5) Urine was then diluted in the laboratory as follows; for allantoin determination dilution rate was 1 in 30 times (1ml of urine + 29 ml distilled water)

2.8.2 Allantoin

A sample of urine (1 – 2ml) was transferred to a 50ml volumetric flask and diluted to volume with distilled water. 5ml of the diluted urine was pipetted into a Pyrex tube graduated at 25ml and 1ml of a 0.5 M NaOH was added and vigorously boiled in a water bath for 7 minutes and then, on removal, immersed in a water bath at 20°C. 1ml of 0.5 M NaOH was added with 5 drops additional to adjust acidity to approximately 0.02M. Five ml of the standard solution of allantoate are pipetted into a separate tube and 5 drops of 0.5 M HCl added. Then 1ml of the phenylhydrazine solution was added to each tube. The tubes were shaken and placed in a boiling water bath for 2 minutes then immediately plunged into an ice-salt bath at -10°C and chilled for 3 minutes. On removal, 3ml of a chilled hydrochloric acid was added to each tube and 1ml of potassium ferricyanide solution. The contents were well mixed. After 30 minutes the tubes were filled to the mark with distilled water and compared in a Duboscq colorimeter (Pentz, 1969).

The calculation is as follows:

$$S/U \times 0.1 \times 0.738 \times 50/5 \times 100/X = S/U \times 73.8/X = \text{mg allantoin in 100ml urine (Pentz, 1969)}$$

Where: S = reading of the standard

U = reading of the unknown

X = number of ml of urine used for initial dilution to 50ml

0.738 = dilution factor for conversion of allantoate to allantoin

2.8.3 Microbial Nitrogen supply and uptake

Microbial protein supply was estimated according to the equation proposed by Puchala and Kulasek (1992)

$$Y = e^{(0.830 + 2.089x)}$$

Where: Y = microbial nitrogen supplied (g/day)

X = urinary excretion of allantoin (mg/day)

The efficiency of the microbial protein supply in the rumen is expressed as microbial nitrogen supply (g/day) per kilogram digestible organic matter intake (DOMI) (g DM/day/kg W^{0.75}).

2.9. Rumen degradability

Ruminal dry matter and neutral detergent fibre digestibility was determined using nylon bags.

Three animals with large rumen fistula were used to measure *In situ* rumen DM and NDF degradability of the three fodder species using the technique described by Osuji *et al.* (1993a).

The animals were adapted to the feed for three days before incubation started.

The sheep were fed twice daily during the 15 day experimental period with 3 days to assess each plant.

The dried feed samples (determined dry matter concentration) were milled through a 2mm screen.

Bags (140 x 90mm) made of polyester cloth, with an average pore size of 53µm were used. The bags were dried at 60 °C for 30 minutes and then weighed.

Approximately 5g of dry sample was weighed into the oven dried bags in duplicate, which were then tied off with 100% polyester string. The bags were attached to a stainless steel disc (140g, 45mm diameter, 11mm thick and with ten holes around the edge) using the polyester string. A 50cm nylon string was attached to the metal disc to anchor the bags deep inside the rumen.

A 3 x 3 factorial experimental layout was used as illustrated in Table 2.2.

Table 2.2 Experimental layout of the rumen degradability trial

Treatment	Period 1	Period 2	Period 3
Lucerne	Animal 1	Animal 3	Animal 2
<i>Sutherlandia</i>	Animal 2	Animal 1	Animal 3
<i>Cassia</i>	Animal 3	Animal 2	Animal 1

The bags were all incubated in the rumen at once on day 1 and removed one by one using the sequential withdrawal method (Osuji *et al.*, 1993a). Bags were withdrawn at 0, 4, 8, 16, 24, 48, 72 hours respectively. After each incubation time, one bag was removed per animal, immediately dipped in ice water to prevent further microbial activity. Each bag was then rinsed under running cold water to remove microbes and degraded material smaller than the bag pores. The bags were then frozen until all bags had been removed and washed.

All the bags were then dried in a force draught oven at 60°C for 48 hours. The bags, with their contents, were then weighed. The procedure was repeated twice to give six replications for each treatment per animal treatment (Osuji *et al.*, 1993a).

The dry matter and NDF of the residue samples were then determined using the same analysis as with feed samples.

The disappearance was calculated using the following formula (Osuji *et al.*, 1993a):

$$\text{Disappearance} = (\text{SWa} - \text{BW}) \times \text{DMA} - (\text{SWb} - \text{BW}) \times \text{DMb} / (\text{SWa} - \text{BW}) \times \text{DMA}$$

Where: SWa = weight of original sample + nylon bag

BW = weight of empty nylon bag

SWb = weight of the sample + nylon bag after incubation

DMA = Dry matter/NDF of feed sample

DMb = Dry matter/NDF of residue sample

These data were then processed electronically and plotted against time using the model of DM disappearance proposed by Orskov & McDonald (1979) which summarizes the data and derives degradation parameters.

$$Y = a + b (1 - e^{-ct})$$

Where: Y = degradability at time (t)

a = intercept

b = potentially degradable fraction

c = rate of degradation of b

2.10. Rumen kinetics

The total weight of rumen contents and the passage rate were estimated by manually emptying the rumen of each animal at different times. There should be a minimum of 24 hours between consecutive emptyings (Robinson *et al.*, 1987; Tamminga *et al.*, 1989; Osuji *et al.*, 1993a). Figure 2.3 shows how the emptying was undertaken during the trial

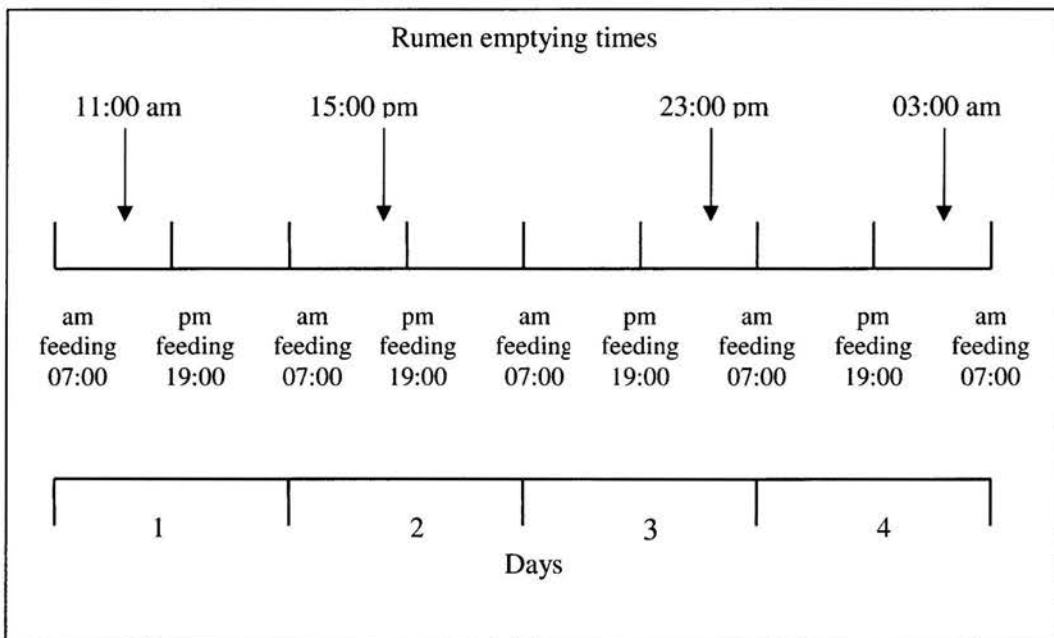


Figure 2.3 Rumen evacuation time schedule (4 & 8 hours after feeding)

Procedure used for estimating rumen volume by evacuation (Osuji *et al.*, 1993a)

1. Remove the cover of the rumen cannula and empty all rumen contents by hand into a barrel, keeping the barrel in warm water.
2. Weigh all the material, mix thoroughly and take a 2.0 – 2.5 kg sample
3. Return the remaining material to the rumen as soon as possible (The procedure should not exceed 10 minutes and the rumen should only be empty for 2-3 minutes.)
4. Dry rumen samples at 100°C for 24 hours to determine dry matter content of rumen digesta
5. Dry samples of rumen content for analysis at 60°C for 48 hours.

The kinetics of rumen NDF intake, passage rate and digestion can be calculated using the model described by Robinson *et al.* (1987), assuming steady state conditions in the rumen.

$$\text{Rate of intake (ki per hour)} = 1/24 \times (\text{intake, kg/day}) / (\text{rumen pool size, kg})$$

$$\text{Rate of passage (kp per hour)} = 1/24 \times (\text{faecal flow, kg/day}) / (\text{rumen pool size, kg})$$

$$\text{Rate of digestion (kd per hour)} = ki - kp$$

Where: Intake = kg NDF (DM basis) per day
Pool size = kg NDF (DM basis) in the rumen
Faecal flow = kg NDF (DM basis) excreted per day (Robinson *et al.*, 1987)

The extent of digestion of a feed is controlled by the relationship between rate of digestion and rate of passage (Mertens, 1993), therefore:

$$\% \text{ NDF digested in rumen} = kd / (kp + kd)$$

$$\% \text{ NDF passing from rumen} = kp / (kp + kd)$$

2.11. Statistical analysis of data

An analysis of variance with the ANOVA model (Statistical Analysis Systems, 2005) was used to determine the significant difference between different fodder species/treatments, period and animal effects for the balanced data. Means and standard errors (se) were calculated.

Significance of difference (5%) between means was determined by using Fischer's test (Samuels, 1989).

Chapter 3

Results and discussion

3.1. Chemical composition of the forages

3.1.1 Crude ash, protein and fibre components

The crude ash, protein and fibre components of the forage samples are presented in Table 3.1.

Table 3.1 Dry matter composition [Ash, CP, CF, NDF, ADF and ADL (g/kg DM basis)] of *C. sturtii*, *S. microphylla* and *M. sativa*

Parameter	Species		
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>
Ash	32.0	38.0	73.0
CP	122	136.0	165.0
CF	440.0	498.0	386.0
NDF	572.0	645.0	546.0
ADF	478.0	509.0	437.0
ADL	136.0	173.0	130.0

The crude ash concentration of all three forages differs, with *S. microphylla* and *C. sturtii* lower than *M. sativa*. *M. sativa* has a crude ash concentration almost twice the amount of both *S. microphylla* and *C. sturtii*. Wilcock *et al.* (2004) reported ash values for *C. sturtii* stems and leaves of 53 and 73 g/kg and that of *S. microphylla* at 25 and 64g/kg respectively. Values for *C. sturtii* are lower while those of *S. microphylla* compare well to the average of the whole plant. Scholtz *et al.* (2009) evaluated the nutritive value of 168 lucerne hay samples in South Africa and reported a minimum ash value of 72.5g/kg and a mean ash value of 129.7g/kg. The minimum value reported for *M. sativa* in this trial compares well to that of Scholtz *et al.* (2009). The higher ash content of *M. sativa* compared to *S. microphylla* and *C. sturtii* shows that *M. sativa* has a higher inorganic matter content.

The mean CP and CF concentration differed between species with *C. sturtii* having the lowest CP and *M. sativa* the highest. *S. microphylla* had the highest CF while *M. sativa* had the lowest. Ventura *et al.* (2004) reported average CP values for *C. sturtii* of 102.0 g/kg DM, Aganga *et al.* (2003) reported CP concentration in *C. sturtii* of 130 g/kg DM and Ahn *et al.* (1988) reported a CP value for *Cassia rotundifolia* hay of 137.5g/kg DM and lucerne hay of 250g/kg DM. The value reported in this trial for *C. sturtii* was similar to these values, while that of lucerne hay (half bud stage) was much higher than reported in this trial for *M. sativa*, showing the differences in maturity. Wilcock *et al.* (2004) reported values for the CP concentration of stems and leaves in *C. sturtii* and *S. microphylla* of 76 and 147g/kg DM and 88 and 225 g/kg DM respectively. These values compare well with the average CP concentration of the whole plant reported in this study.

Kanani *et al.* (2006) reported values for chemical composition of some other tropical forage legumes (*Dolchis lablab*, *Leucaena leucocephala* and *Desmanthus bicontortus*) as well as *M. sativa*. All forages had a CP concentration above 20%.

Van Niekerk *et al.* (2004a) reported CP values of leaves of *C. sturtii* at two sites (namely Hatfield and Lovedale in the Northern Cape) of 147g/kg and 114g/kg DM respectively. Mupangwa *et al.* (2000) reported CP values for *Cassia rotundifolia* at 182 g/kg which is higher than the value reported in this study and that reported by Ahn *et al.* (1988). Values may vary due to variation in leaf content and stage of growth. Ramirez (1998) reported values for lucerne hay CP of 16.88 which is similar to the value reported here.

Snyman (2006) reported values for three different palatability selections of *A. nummularia* of between 215.3g/kg CP for the least palatable and 243.8g/kg CP for most palatable plants. *A. nummularia* is also regarded as a drought resistant leguminous fodder shrub so comparisons may be made with the shrubs in this trial. CP values for the plants in this trial are much lower than those reported for *A. nummularia* although the values reported were considered to be high, possibly as a result of a high soil N concentration and certain rainfall and climatic conditions.

The results from Van Niekerk *et al.* (2004a) for *C. sturtii* and *A. nummularia* were 147g/kg and 208 g/kg CP respectively and 250g/kg and 407 g/kg NDF respectively. The high CP and fairly low NDF values are proof of these forages having fair potential as fodder crops for livestock.

In the trial the whole plant was fed, it therefore, included stems and leaves. There is usually a difference in the CP and CF concentration of leaves and stems (Wilcock *et al.*, 2004) and

feeding leaves only would probably provide higher CP and lower CF levels, which would probably also have an effect on digestibility. Wilcock *et al.* (2004) showed that the percentage leaf material in *C. sturtii* was between 48 – 62% and that of *S. microphylla* was between 27 – 55%. *C. sturtii* and *S. microphylla* had a lot of woody material (stems) and this material was included in the feed. Some leaf material was lost in the drying process as well. This may explain the high CF values compared to that of the *M. sativa*.

Wilcock *et al.* (2004) showed that as the plants increase in size, the percentage of leaf material decreases. Of the three species studied by Wilcock *et al.* (2004) (*C. sturtii*, *S. microphylla* and *T. sinuatum*) the *S. microphylla* exhibited the largest decrease in leaf material with age, while *C. sturtii* had the highest percentage of leaf.

A study conducted by Ventura *et al.* (2004) reported that *C. sturtii* contained tannins. Tannins in forages, at high levels, have been reported to reduce protein and DM digestibility of the forages by ruminants. At low to moderate levels, condensed tannins increase the quantity of dietary protein, especially essential amino acids, flowing to the small intestine (McMahon *et al.*, 2000). This could have an effect on the protein concentration and digestibility of the plant. Further research is necessary to determine the effect, as the tannin concentration in this study was not determined.

The NRC for small ruminants (2007) reported values for crude fibre and protein concentration in full bloom lucerne hay, of 340 g/kg CF and 160 g/kg CP which compare well to the values in this trial.

The daily requirement of CP for maintenance of a 50kg mature ewe is 66gCP/day (assuming 20% rumen undegradable intake protein, UIP) (NRC, 2007). The daily dry matter intake is 1.83% of body weight (0.92kg DM), therefore the CP requirement would be satisfied by a feed consisting of 7.2 % CP; all three feeds would therefore supply sufficient CP for maintenance, if intake was adequate. The requirement for early gestation (single lamb) is 91gCP/day; all three feeds still meet these requirements. However, only *S. microphylla* and *M. sativa* meet the requirement of early lactation (169gCP/day), with *C. sturtii* close at 154gCP/day, again provided intake is sufficient (2.51% of BW).

Relative to other conventional fodder plants, the species analyzed had nutrient levels indicative of forages of good quality. The CP concentration for *C. sturtii* and *S. microphylla* of 122 and 136 g/kg DM is more than found in several tropical grasses (Minson, 1990).

The NDF and ADF levels of the samples varied between all three species with *S. microphylla* being the highest and *M. sativa* the lowest. Values for *C. sturtii* were in between those of the two other forages. Ventura *et al.*, (2004) reported a value for *C. sturtii* for NDF, ADF and ADL of 390.0, 254 and 88 g/kg DM respectively. Mupangwa *et al.* (2000) reported NDF, ADF and ADL values for *C. sturtii* of 419g/kg, 275g/kg and 75.7g/kg respectively which were lower than those reported here, while Ahn *et al.* (1988) reported values for *C. rotundifolia* of ADF 417g/kg and ADL at 109g/kg which are more similar. The values reported for lucerne hay ADF and ADL were lower than in this trial, 359 g/kg and 84g/kg respectively (Ahn *et al.*, 1988).

Ramirez (1998) reported values for lucerne hay NDF, ADF and ADL of 45.9, 32.5 and 2.9% which are also much lower than reported here. The stage of maturity may be the differentiating factor.

Van Niekerk *et al.* (2004a) reported NDF and ADL values for *C. sturtii* leaf material at two different sites. The NDF was 250 and 223 g/kg respectively and the ADL was 75 and 71 g/kg. *C. sturtii* also had significantly lower NDF levels than the *Atriplex* spp. in that trial. These values are much lower than those obtained in this trial but cannot be compared to those in this trial because only the leaves were analysed and in this trial whole plants were evaluated. Snyman (2006) reported NDF values for *A. nummularia* of between 416.2g/kg and 455.1g/kg. These values are lower than all plants in this trial.

Kanani *et al.* (2006) reported that *M. sativa* had a numerically higher NDF, ADF and ADL value than the other forages he studied, 34.2, 26.5 and 5.49 % respectively. The forages evaluated in this trial had much higher values for NDF, ADF and ADL, which shows a lower nutritional value.

The difference between the plants used in this study and those reported in other studies could be due to the variation in leaf content of the plants and stage of growth at which the plants were harvested. In the study conducted by Mupangwa *et al.* (2000), the *C. sturtii* plants were harvested at 20 weeks and field cured, whereas those in this study were 3 years of age. An increase in the NDF concentration over time is due to an increase in the proportion of stem material (Watson *et al.*, 1987). It could also be due to loss of leaf material during the drying and milling process which consequently make it appear that the forages have a poor nutrient value.

The ADL concentration of *S. microphylla* was higher than both *C. sturtii* and *M. sativa*. The degree of lignification in *C. sturtii* was high (23.8% of NDF was ADL). According to Ventura *et al.* (2004), *C. sturtii* had a relatively high lignification (25% of NDF was ADL) which is similar to the

lignification of *C. sturtii* in this trial. The degree of lignification of *S. microphylla* was 26.8%, which is higher than that of *C. sturtii*, while *M. sativa* is the same as *C. sturtii*. The extent of cellulose digested in the rumen depends on the degree of lignification, where in older herbage with 100g lignin/kg the proportion of cellulose digested may be less than 60%. In younger herbage where there is approximately 50g lignin/kg, the proportion of cellulose digested may be as much as 80% (McDonald *et al.*, 2002). All three species have greater than 100g lignin/kg therefore cellulose digestion may be negatively affected. Lignin is a major concern for digestibility of plants as it has a high resistance to chemical digestion, as the physical properties render it inaccessible to enzymes (McDonald *et al.*, 2002). Lignin therefore has a negative effect on total organic matter digestibility (Moya-Rodriguez *et al.*, 2002).

Fibre, specifically NDF components of feeds inhibit intake of forages due to slow digestion compared to cell solubles (Van Soest, 1982) and a limitation on feed intake is apparent when fibre concentration is more than 500g/kg DM (Van Soest, 1965). Milford and Minson (1965) also reported that forage intake dropped sharply when sheep were fed forages with CP levels below 70g/kg DM. Based on these above measures one of the limitations on intake could be the fibre components as all species have an NDF value above 500g/kg. NDF also has an indirect relationship with the rate of digestion, as does ADF (McDonald *et al.*, 2002).

There has not been much research done on the composition of *C. sturtii* and *S. microphylla* over a period of time under normal grazing conditions and there are very few articles containing information on the chemical composition of these shrubs, to determine whether these values are normal and sufficient to supply enough nutrients for maintenance and even production over prolonged periods. Further research is required.

3.1.2 Minerals

3.1.2.1 Macro-minerals

The macro-mineral concentration and the calcium to phosphorus ratios are presented in Table 3.2.

Table 3.2 Macro-mineral concentration (g/kg DM basis) of *C. sturtii*, *S. microphylla* and *M. sativa* and Ca: P ratio

Parameter	Species		
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>
Ca	10.8	4.6	11.5
P	2.8	1.8	2.8
Mg	1.9	0.6	1.9
Ca: P	3.9:1	2.5:1	4.1:1

The calcium concentrations of *C. sturtii* and *M. sativa* are similar and have a higher concentration than *S. microphylla*. Van Niekerk *et al.*, (2004b) reported Ca values for *C. sturtii* leaf material of two different sites of 15.5 and 14.5 g/kg respectively, which were slightly higher than this trial, possibly due to the difference in leaf material versus the whole plant. Leguminous forages are usually satisfactory sources of calcium (Ca) for grazing livestock. Minson (1990) reported mean calcium concentrations of 10.1 g/kg DM for tropical legumes. The *C. sturtii* falls into this range for tropical legumes, however the *S. microphylla* Ca concentration is much lower. Ruminants can absorb Ca with a relatively high efficiency when necessary (Underwood & Suttle, 1999), therefore what is utilized by the animal from the forage may be adequate.

M. sativa and *C. sturtii* had a higher phosphorus concentration than *S. microphylla*. Van Niekerk *et al.*, (2004b) reported P values for *C. sturtii* leaf material at two sites in South Africa of 1.5 and 0.8 g/kg respectively. This was leaf material that was analysed, and these values are lower than those reported in this trial for the whole plant. The P status of forages varies widely depending on P status of soil, stage of maturity of the plant and climate, but distribution between leaf and stem is uniform. An exception is the tropical legume, *Stylosanthes*, which grows well on low P soils and its P status remains low. Therefore the difference in the values reported for *C. sturtii* in this trial compared to Van Niekerk *et al.*, (2004b), could be due to different soil P levels and climate and not necessarily the fact that only leaf material was used. Tropical forages were noted to contain P levels of about 2.3 g/kg DM with legumes having a higher P concentration than grasses (3.2g/kg vs. 2.7g/kg DM) (Minson, 1990).

Mupangwa *et al.* (2000) reported Ca values for *C. rotundifolia* of 16.8 g/kg and P of 1.40 g/kg which are slightly different to that in this study leading again to a possible species, maturity and soil differences.

The Ca concentration of lucerne hay as reported by NRC (2007) is 120g/kg, which is similar to the value in this trial. *M. sativa* is known to have a high Ca concentration. The P concentration was reported by NRC (2007) as 2.3g/kg, which is similar but lower than that found here, again showing differences due to soil content, stage of maturity or climate.

Ruminants differ from non-ruminants, in that a large quantity of P is secreted in the saliva during rumination, therefore the major source of P to the animal is not via the diet but rather saliva.

The NRC (2007) recommends a maintenance Ca requirement for a mature ewe (50kg) of 2.0 g/kg DM and for P, 1.5 g/kg DM, which gives a Ca: P ratio of 1.3.

The values for *S. microphylla* Ca and P are 4.6 and 1.8 g/kg DM respectively, giving a ratio of Ca: P of 2.5:1. *S. microphylla* therefore supplies sufficient Ca and P to the animals.

Ca and P values of *C. sturtii* were 10.8 and 2.8 g/kg DM respectively, with a ratio of 3.9:1, and *M. Sativa* 11.5 g/kg Ca and 2.8 P g/kg DM respectively, with a ratio of 4.1:1. The Ca: P ratio for all forages are above normal and ideal level, but can be tolerated by sheep.

S. microphylla cannot meet the P requirements after maintenance, i.e. gestation and lactation (NRC, 2007) while *C. sturtii* and *M. sativa* can meet both Ca and P requirements until late gestation. P supplementation from late gestation onwards will have to be considered for all three forages.

The maximum dietary Ca level is 1.5% and P, 0.6% of diet dry matter (NRC, 2007). Calcium toxicity can lead to hypocalcaemia and soft tissue calcification and excessive P in relation to calcium may lead to urinary calculi formation and skeletal softening. The greatest concern with P is related to the Ca: P ratio which can affect absorption of Ca or P when either is greater than 7:1 or less than 1:1. If P is deficient then a Ca: P ratio of 3.6 was detrimental for sheep, but if P is adequate then the performance is not affected at a Ca: P ratio of 5.4. In a trial with sheep, a high Ca: P ratio of 10:1 had no adverse effect with a diet containing 2.6 g P/kg DM, but severe bone disorders were noted when the diet only contained 0.8 g P/kg DM (Underwood and Suttle, 1999). The Ca: P ratio is important for optimal utilization of both these minerals, however most livestock can readily change an extreme ratio of dietary Ca: P homeostatically, to an acceptable ratio. Therefore the Ca: P ratio should only be used to define the requirements of Ca and P when the supply of either element is limiting or excessive (Underwood and Suttle, 1999). All these forages contain adequate amounts of both minerals, and therefore supply sufficient Ca and P for maintenance to the animals without needing to further supplement.

With respect to magnesium (Mg), *C. sturtii* and *M. sativa* have a similar composition while *S. microphylla* has a lower concentration. Van Niekerk *et al.*, (2004b) reported Mg values for *C. sturtii* leaf material at two sites of 2.0 and 1.2 g/kg respectively. Minson (1990) reported values for Mg in tropical legumes of 2.8 g/kg DM which is slightly higher than reported in this trial. The magnesium concentration of plants varies with species, soil and climatic conditions (Underwood and Suttle, 1999). Ventura *et al.* (2004) reported similar values to those reported in this trial, for Ca in *C. sturtii* of 13.4 g/kg DM, P of 1.7g/kg DM and Mg of 1.2 g/kg DM.

The requirement for magnesium for maintenance is 1.1g/day, early gestation 1.3g/day and early lactation 1.9g/day (NRC, 2007). Both *C. sturtii* and *M. sativa* supply sufficient Mg to the animals through all production stages, but *S. microphylla* is lower than required from the start. Underwood and Suttle (1999) reported that a marginal deficient diet (0.5 g Mg/kg DM) can eventually lead to a marginal loss of appetite. The Mg concentration in *S. microphylla* is similar to the marginal level reported. Whether or not the low Mg concentration could have affected the intake of the plant would have to be assessed in a separate trial.

3.1.2.2 Trace minerals

Trace element concentrations of the plants are represented in Table 3.3.

Table 3.3 Trace mineral concentration (mg/kg DM) of *C. sturtii*, *S. microphylla* and *M. sativa*

Parameter	Species		
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>
Fe	554.8	469.6	238.4
Cu	9.6	8.5	9.8
Zn	15.2	20.9	15.7
Mn	35.6	57.8	40.5

The iron concentration of all three plants differs, with *M. sativa* having the lowest concentration and *C. sturtii* the highest. The mean concentration of iron in legumes is 306 and grasses 264 mg Fe/kg DM. High values are attributable to soil contamination (Underwood and Suttle, 1999).

Iron levels for all three plant species were well above the maintenance, early gestation and lactation requirement for sheep of 8, 32 and 11 mg/day according to the NRC (2007). The maximum tolerable level of 500mg/kg DM, with high levels of Fe in forages (549 – 990 mg/kg DM) causing copper deficiencies in lambs (NRC, 2007). Iron is a well known antagonist of many of the other trace minerals such as copper, zinc and manganese, so levels above those required may have an antagonistic effect in the gut, reducing absorption and therefore increasing the daily requirement of these other essential trace minerals, which may have a major effect on production (reproduction and immunity) of animals (Underwood and Suttle, 1999). High Ca levels may also affect the availability of some trace minerals, such as that of Zn and Cu. The inverse relationship between Ca and Zn is well known (Underwood and Suttle, 1999).

The copper concentrations in *M. sativa* and *C. sturtii* were similar, while that of *S. microphylla* was slightly lower.

Copper, iron, sulphur and molybdenum concentrations of forages vary with species, strain and maturity of the plant and the soil conditions. Temperate grasses tend to be lower in copper than legumes (4.7 vs. 7.8 mg/kg) but under tropical conditions the position is reversed (grasses 7.8 vs. legumes 3.9 mg/kg) (Minson, 1990). The concentration of copper in the trial plants was higher than reported by Minson (1990) for tropical legumes, possibly due to species differences. There is little published information on the molybdenum in tropical legumes, but modest increases may be all that are needed to induce hypocuprosis; on average 3.9mg/kg molybdenum would give a Cu: Mo ration of 1.0. Dietary Cu: Mo ratios of <1.0 (or Fe: Cu ratios >100) indicate a high risk of disorder and ratios of 1.0 - 3.0 (or Fe: Cu between 50 – 100) indicate a marginal risk of future problems (Underwood and Suttle, 1999). The Fe: Cu ratio of *C. sturtii* and *S. microphylla* is between the 50-100 at 58:1 and 55:1 respectively indicating a marginal risk of future trace mineral imbalance problems. *M. sativa* is below the risk ratio at 24:1.

There is a three way interaction between copper, molybdenum and sulphur and it is well documented in ruminant animals. The ability of copper to meet the animals' requirements depends on this interaction mentioned above as well as the absorbability of the copper from forages. Although molybdenum and sulphur were not analysed for in this trial, the levels in the plant could affect the copper concentrations which were lower than the requirement. It may be worth studying this interaction in future trials and the effect it has on production in sheep fed these forages. As mentioned above, copper absorption is also affected by iron levels.

Contamination of forages with soil iron is common and should be taken into account when assessing the value of forages as copper sources (Underwood and Suttle, 1999).

There is also a risk of copper toxicity, especially in housed lambs and sheep receiving large amounts of concentrates with high copper levels (>15 mg/kg). Copper poisoning can be prevented by using the known antagonists of copper (molybdate containing salt licks, iron, sulphur or zinc supplements) (Underwood and Suttle, 1999).

The copper levels for *C. sturtii* and *M. sativa* are well above the maintenance, early gestation and lactation requirement for sheep of 4, 8 and 8.8 mg/day according to the NRC (2007), however *S. microphylla* just meets the requirements for maintenance.

The zinc concentrations in *M. sativa* and *C. sturtii* were similar, while that of *S. microphylla* was slightly higher. Underwood and Suttle (1999) reported values for lucerne hays of 13 – 25 mg Zn/kg DM. Ranges reported by several authors varied drastically for pasture legumes; One study of North American legume forages had a range of 20 – 60 mg Zn/kg DM while another had a range of 11 – 18 mg Zn/kg DM. The NRC (2007) reported values for lucerne hay for Zn of 23mg/kg. Differences between species contribute little to the variation in forage zinc, rather stage of maturity is more important with concentrations falling almost 50% for successive cuts in one study (Underwood and Suttle, 1999). The *S. microphylla* plants were harvested at an earlier stage, therefore less mature plants than *C. sturtii*, thus having a higher concentration of zinc. *M. sativa* compared well to the value for lucerne hay reported by Underwood and Suttle (1999), but was slightly lower than that reported by the NRC (2007).

The absorption of zinc can be affected by elements such as copper, which increase the mucosal binding of zinc.

The zinc levels for all three species are below the maintenance, early gestation and lactation requirement for sheep of 30, 42 and 59 mg/day according to the NRC (2007), therefore extra supplementation could be required. Zinc plays a major role in growth and health and is involved in more than 300 enzymes and linked to metabolism of many important nutrients. Zinc also has a major function in appetite control, with the appetite regulating hormone cholecystokinin in the intestine (NRC, 2007).

Manganese concentration of all three species differs, with *C. sturtii* being the lowest and *S. microphylla* the highest. Van Niekerk *et al.* (2004b) reported Mn and Zn values for *C. sturtii* leaf material at two sites of 37 and 40mg Mn/kg DM and 22 and 13mg Zn/kg DM. Once again these values cannot be directly compared to those in Table 3.4 as it was only leaf material that was

analysed, but it does, however, compare well. Underwood and Suttle (1999) reported values for lucerne hays of manganese concentrations that vary between 43.7 and 22.7 mg Mn/kg DM. These values vary widely with differences due to species and maturity being small. Most high values probably arise from soil contamination and can also occur during sample processing if mills with steel blades are used (Underwood and Suttle, 1999). The absorption of manganese can be negatively affected by excess or addition of inorganic phosphorus and phytate. Phytate is broken down in the rumen so will have little effect in ruminant animals. Phosphorus levels in the trial plants were not excessive therefore the possibility of manganese deficiency problems is very low.

The manganese levels for all three species are above the maintenance, early gestation and lactation requirement for sheep of 16, 29 and 21 mg/day according to the NRC (2007).

The plants from this trial were analyzed for selenium but none or very insignificant levels were found and were not worth reporting. However Van Niekerk *et al.* (2004b) reported Se for *C. sturtii* leaf material at two sites of 19 and 314µg Se/kg DM. The Se requirement of sheep is >100µg/kg, so only the plants at the second site (Lovedale) would meet the requirements. As discussed in the research by Van Niekerk *et al.* (2004b) there was a very large difference in the Se concentration of *C. sturtii* from the different sites, and it was also leaf material that was analysed. So whole plant material will dilute the Se concentration further and it may even be too low to detect. Minson (1990) reported that legumes tend to contain less selenium than grasses but the difference diminishes as the soil selenium status declines. Species differences occur in certain plants which are usually named by their manner of selenium metabolism, as 'accumulator', 'convertor' or 'indicator'. It may be necessary to supplement Se for animals being fed these plants as selenium is an essential trace mineral necessary for growth, fertility and prevention of a variety of diseases. Selenium supplementation together with vitamin E also shows a good response as both act as anti-oxidants in the animal body. Selenium can be supplemented using direct subcutaneous injection (sodium selenite, 0.1mg/kg LW at 3 monthly intervals) as well as fed orally through selenium rich plants or mineral salt (included at 0.3mg/kg DM) (Underwood and Suttle, 1999).

As mentioned above, mineral composition of forages can vary with soil fertility, plant species and stage of maturity. In this study soil fertility and the interaction between composition and maturity was not examined.

Other forms of trace mineral supplementation such as in feed organic mineral complexes and/or inorganic mineral salts as well as injectable products can be considered to ensure optimal fertility, growth and health in the animals.

3.2. Digestibility trial

3.2.1. Apparent digestibility and voluntary intake

The apparent digestibility of DM, CP, NDF and OM of the three feeds is represented in Table 3.4.

Table 3.4 Apparent digestibility (%) for DM, CP, NDF and OM of *C. sturtii*, *S. microphylla* and *M. sativa*

	Species			
Parameter	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	s.e.
DM (%)	44.57 ^{ab}	37.36 ^b	52.69 ^a	3.312
CP (%)	53.52 ^a	53.94 ^a	63.01 ^a	3.221
NDF (%)	24.57 ^a	29.75 ^a	42.73 ^b	3.208
OM (%)	45.30 ^{ab}	38.66 ^b	53.06 ^a	3.317

^{ab} Column means with the same superscript do not differ significantly ($P>0.05$) (Duncan's multiple test range)
 s.e. Standard error

The apparent DM digestibility of *S. microphylla* is significantly lower than *M. sativa* while it did not differ significantly from *C. sturtii*. *C. sturtii* did not differ significantly from both *M. sativa* and *S. microphylla*. The CP digestibility of all three species did not differ significantly, however that of *M. sativa* is numerically higher. The higher CP digestibility of *M. sativa* compared to the other plants may be due to its lower fibre concentration (see Table 3.1). With regards to the apparent NDF digestibility, *C. sturtii* and *S. microphylla* differ significantly to *M. sativa* with lower NDF digestibility values. The apparent OM digestibility followed the same trend as that of apparent DM digestibility.

The DM digestibility of *C. sturtii* is lower than reported by Mupangwa *et al.* (2000) for *C. rotundifolia* which had a DM digestibility of 55% and OM digestibility of 57.9%. Organic matter,

53.5% and protein degradability, 56.4%, of *C. sturtii* was moderate as reported by Ventura *et al* (2004), but higher than reported in this trial.

Ahn *et al* (1988) reported an *in vivo* dry matter digestibility for *C. rotundifolia* and lucerne hay of 55.5% and 65% respectively. The lucerne and *Cassia* DM digestibility in this trial was much lower. Ramirez (1998) reported similar DM and CP digestibility for lucerne hay, 58.9 and 60.9%, however that of NDF was higher than reported here, 56.96%.

Benjamin *et al.* (1995) reported apparent *in vitro* digestibility of *C. sturtii* and *A.nummularia*, DM, 50.9% and 73.5% and OM, 47.9 and 58.7 % respectively. The *A.nummularia* had a higher fodder value in term of apparent digestibility as well as a higher proportion of leaf material in the biomass eaten from the shrubs. The values reported for *C. sturtii* are similar to those reported here, but slightly higher as these were freshly browsed shrubs.

Abou El Nasr *et al.* (1996) reported values for *Atriplex nummularia* (saltbush) and *Acacia salinga* (acacia) for apparent digestibility of DM (59.6 & 60.8%), CP (47.7 & 40.6%) and NDF (64.1 & 46.3%). Although *A. nummularia* is not a legume, but *A. salinga* is, these shrubs can be compared to those in this trial, as they were also studied and can be planted under dry arid conditions. They were fed as dried fodder with similar NDF (63.8 & 69.5%), ADF (39.2 & 44.7%) and ADL (10.9 & 16.8%) values to those reported for *C. sturtii*, *S. microphylla* and *M. sativa* in this trial. Apparent dry matter digestibility of saltbush and acacia was similar to *M. sativa* but higher than *C. sturtii* and *S. microphylla*.

The apparent CP digestibility of saltbush and acacia was lower than reported for the forages in this trial, probably due to lower CP concentration, 9.1 and 10.1% respectively, and possible interaction with the condensed tannins. The apparent NDF digestibility of saltbush was much higher than all three species in this trial, and that of acacia was similar to *M. sativa*, however Abou El Nasr *et al.* (1996) reported that the level of fibre digestibility was low and could be due to high concentrations of lignin and hemicelluloses. The fibre concentration of all species is similar therefore the fibre of acacia and saltbush is more digestible than *C. sturtii* and *S. microphylla*.

The apparent DM digestibility for *C. sturtii* and *S. microphylla* are below 45% which is considered adequate for high animal performance on pastures (Kallah *et al.*, 2000). If the plants were fed as fresh forage, there might be a different situation, as drying might cause loss of some potential nutrient value. The low DM digestibility of *S. microphylla* may be explained in part by its higher fibre concentration (645 g/kg DM). Chriyaa *et al.* (1997) reported a high fibre

concentration for wheat straw (696 g/kg DM) with a consequent DM digestibility of 42.2%. The higher ADL concentration of both *C. sturtii* and *S. microphylla* explain the lower DM digestibility compared to *M. sativa*.

Generally, lignin has a negative effect on the total organic matter digestibility (Moya-Rodriguez *et al.*, 2002). *S. microphylla* had the highest lignin (ADL) concentration and lowest OM digestibility, supporting this relationship.

Condensed tannins on the other hand negatively affect the nutritional status of ruminants by reducing ruminal digestion of protein and cell wall (Moya-Rodriguez *et al.*, 2002). *C. sturtii* had the lowest CP and NDF apparent digestibility, therefore leading to a possibility of the presence of condensed tannins.

However, the lack of significant difference between all forages in OM digestibility may indicate that condensed tannin content did not depress microbial activity in the rumen and therefore the observed lower intakes of *C. sturtii* and *S. microphylla* may be due to an astringency effect in the mouth (Mupangwa *et al.*, 2000).

Ramirez (1998) concluded that low digestion of cell wall in diets containing tropical forages may be related to the chemical or physical nature of the cell wall which may affect the extent of digestion, but cannot be detected by chemical means; therefore a negative effect on digestibility may never be proved.

The simplest measure of nutritional value of CP is its apparent digestibility and a close correlation between CP concentration and apparent digestibility has been reported (Milford and Minson, 1965,b). It can be expressed by the general equation $Y = 70 \log X - 15$, where $Y =$ CP digestibility and $X =$ CP concentration (Milford and Minson, 1965(b)). The predicted CP digestibilities of *C. sturtii*, *S. microphylla* and *M. sativa* were 61.05, 64.35 and 70.22% respectively. The apparent CP digestibilities reported in this trial were lower than the predicted values.

The data on voluntary intake of the three species during the feeding trial is presented in Table 3.5.

Table 3.5 Average intake (g/day) and change in body weight and voluntary intake (g/day/kg W^{0.75}) of DM (DMI), CP (CPI), NDF (NDFI), OM (OMI) and the digestible organic matter intake (DOMI) for *C. sturtii*, *S. microphylla* and *M. sativa*

Parameter	Species			s.e.
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	
Average intake (g/day)	301.4	468.1	1158.8	
Change in body weight (kg)	-6.9	-8.9	+5.1	
DMI (g/day/kg W ^{0.75})	21.20 ^a	26.42 ^a	52.54 ^b	5.386
CPI (g/day/kg W ^{0.75})	2.74 ^a	2.96 ^a	7.41 ^b	0.696
NDFI (g/day/kg W ^{0.75})	11.57 ^a	18.44 ^a	30.65 ^b	3.387
OMI (g/day/kg W ^{0.75})	20.42 ^a	25.53 ^a	48.55 ^b	5.095
DOMI (g DM/day/kg W ^{0.75})	9.14 ^a	9.69 ^a	26.15 ^b	2.697

^{ab} Column means with the same superscript do not differ significantly (P>0.05) (Duncan's multiple test range)
 s.e. Standard error

The average intake was very different between species, with *C. sturtii* being the lowest and *M. sativa* the highest. The animals consuming either *C. sturtii* or *S. microphylla* tended to lose body weight during the experimental period, while those eating *M. sativa* gained body weight.

Voluntary intake parameters of *C. sturtii* and *S. microphylla* were lower and differed significantly between *M. sativa*.

The DM intake of *M. sativa* was higher than both *C. sturtii* and *S. microphylla*. This difference could be attributed to higher apparent DM digestibility which may have resulted in lower rumen retention time and greater turnover of particulate matter from the rumen of sheep (Mupangwa *et al.*, 2000) offered *M. sativa* compared to the other two forages.

Minson (1994) reported an estimate for the general relationship between intake and DM digestibility of a range tropical legume to be as:

$$\text{DM intake (g/kg W}^{0.75}\text{)} = 1.76 \text{ DM digestibility} - 44.5 \text{ (r=0.86)}$$

Using this relationship, the potential DM intake for the three species, *C. sturtii*, *S. microphylla* and *M. sativa* would be 33.94, 21.25 and 48.23 g/kg $W^{0.75}$ respectively. The value obtained in this study for *C. sturtii* was lower than the estimated intakes, while those of *S. microphylla* and *M. sativa* were higher. The intake of *C. sturtii* and *S. microphylla* was also below the 80 g/kg $W^{0.75}$ suggested by Crampton *et al.*, (1960) for forages (early bloom, chopped, dehydrated legume forage) possibly due to the effect of anti-nutritional factors present in the *C. sturtii* and *S. microphylla*.

Ramirez (1998) reported a CP intake for lucerne hay of 8 g/day/kg $W^{0.75}$; however that of DM and NDF was higher than reported here, 86.1 and 54.3 g/day/kg $W^{0.75}$ respectively, which corresponds well to the chemical composition of the lucerne fibre, indicating a cut that had a higher nutritive value.

Mupangwa *et al* (2000) reported values for *C. rotundifolia*; DMI and OMI of 12.1 and 10.9 g/kg $W^{0.75}$ respectively which are much lower than the respective values in this trial.

NDF concentration of plants is negatively correlated with intake in ruminants. The NDF concentration of *S. microphylla* was the highest with a high degree of lignification, thus decreasing the intake significantly compared to *M. sativa*. However the NDF and ADL concentration as well as degree of lignification of *C. sturtii* was lower than *S. microphylla* but the voluntary intake was lower. This could suggest that the lignin in *S. microphylla* was more localized than that of *C. sturtii*.

Animals prefer a diet with a lower fibre concentration, as there is a tendency for the palatability to decrease with increase in NDF concentration. The intake of the various feeds may show this effect.

Abou El Nasr *et al.* (1996) reported values for *A. nummularia* (saltbush) and *A. salinga* (acacia) for voluntary intake of DM (53.7 & 49.7 g/day/kg $W^{0.75}$), CP (4.89 & 5.02 g/day/kg $W^{0.75}$) and NDF (34.3 & 34.5 g/day/kg $W^{0.75}$) similar to *M. sativa* in this experiment. Differences in intake between the acacia and saltbush compared to *C. sturtii* and *S. microphylla* might therefore not be due to fibre constraints but rather palatability or anti-nutritional factors which may have a greater effect.

Abou El Nasr *et al.* (1996) reported that ensiling or feeding fresh plant of the acacia and saltbush hays appeared to promote an increased nutritive value and could have a significant role in feeding small ruminants in arid and semi-arid areas. Whether there is an effect of feeding ensiled or fresh plants of the forages in this trial would have to be examined.

The above mentioned effect of reduced intake that may be due to some anti-nutritional factors to a greater degree than NDF was not examined in this trial. It was noted in Chapter 1 that *C. sturtii* and *S. microphylla* contain certain compounds, but whether or not these affect the composition, intake and digestibility is unknown.

Thomson (2002) suggested that the lower intake of *S. microphylla* may be due to the physical attributes of the plant, as well as palatability and other possible anti-nutritional factors that could lead to reduced intake and digestibility. This may lead to a reduced ruminal turnover and rate of digestion which can be caused by an inhibition in microbial activity and inhibition of microbial enzymes (Salawu. 1997).

Snyder *et al.*, (2007) reported that the leguminous tree *Robinia pseudoacacia* – Black Locust) contained high levels of tannins and that these high levels negatively affected voluntary feed intake and NDF digestibility. This could be due to substrate being protected by tannins from hydrolysis and by the direct inhibition of digestive enzymes (Snyder *et al.*, 2007).

Other studies have also shown that *C. sturtii* does contain anti-nutritional factors such as condensed tannins (Ventura *et al*, 2004) which may reduce the acceptability; as can be seen in Table 3.5 the average intake of *C. sturtii* was the lowest. Mupangwa *et al.* (2000) reported values for condensed tannin concentration in *C. rotundifolia* which were the highest out of all four tropical legume hays tested (29.5 g/kg). This value was lower than the values of 50-100g/kg DM (Barry & Duncan, 1984) and 65-90 g/kg DM (Barry & Manley, 1986) reported to cause a reduction in voluntary intake in sheep.

According to Bailey (1985), many species of *Cassia* are toxic. Some species such as the *C. rotundifolia* used in the trial conducted by Ahn *et al*, (1988) did not appear to contain any deleterious compounds. The sheep ate well throughout the study with voluntary intakes of 1.09 kg DM/day which is close to that predicted for material with high fibre concentration and low digestibility. However the intake reported was in contrast to field observations where stock avoided eating the *cassia*, the difference may be due to loss of volatile anti nutritional or unpalatable compounds during hay making.

It may be necessary to do further research into this topic of anti-nutritional and toxic factors in this plant species and to determine which parts of the plants contain these substances as well as their effect on intake and digestibility. Other physical factors such as the fact that *C. sturtii* had very hard and woody stems, may have a negative effect on intake and digestibility and the

very pungent smell of *S. microphylla* which could affect palatability and therefore intake, should also be studied.

The daily dry matter intake of *S. microphylla* and *C. sturtii*, 480.1g/day and 366.4g/day respectively is below that required for long term maintenance of a 50kg sheep (1.83% of body weight, 0.91kg/day) (NRC, 2007). It was noted that the animals that received the *S. microphylla* and *C. sturtii* treatments did lose weight during each period. *M. sativa* had a daily dry matter intake of 1064.3 g/day, therefore could meet the requirements with consequent gain in body mass. None of the forages on their own could support early gestation (1.16kg/day) or early lactation (1.26kg/day) intake requirements (NRC, 2007).

These forages should be supplemented with energy supplements such as maize to improve their utilization.

3.2.2 Metabolisable energy

The metabolisable energy of a food is the digestible energy less the energy lost in the urine and combustible gases, which consist almost entirely of methane, Methane production is closely related to intake, and at the maintenance level of nutrition about 7-9 percent of the gross energy is lost as methane. When methane production cannot be measured directly it is estimated as 8 percent of gross energy intake. (McDonald *et al.*, 2002)

In this trial the gross energy and digestible energy was not determined and was therefore estimated. An alternative to chemical analysis for predicting the energy value of food is the assessment of digestibility by fermentation *in vitro* and the prediction of metabolisable energy value from the digestible organic matter content of the food. For roughages given to ruminants the following formula can be used:

$$\text{ME (MJ/kg DM)} = 0.016 (\text{DOMD})$$

Where DOMD = g digestible organic matter per kg dry matter (McDonald *et al.*, 2002)

The metabolisable energy (ME) values and ME intake of the three species in presented in Table 3.6.

Table 3.6 Metabolisable energy (MJ/kg DM) and ME intake (MJ/day) for *C. sturtii*, *S. microphylla* and *M. sativa*

Parameter	Species			s.e.
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	
ME (MJ/kg DM)	6.975 ^{ab}	5.978 ^b	7.842 ^a	0.508
ME intake (MJ/day)	2.512 ^b	2.890 ^b	8.570 ^a	0.916

^{ab} Column means with the same superscript do not differ significantly ($P > 0.05$) (Duncan's multiple test range)
 s.e. Standard error

The ME was the highest for *M. sativa* while *S. microphylla* was significantly different and had the lowest value. *C. sturtii* had an ME value similar to both *M. sativa* and *S. microphylla*.

The value for *M. sativa* (lucerne hay) compares well to that reported by McDonald *et al.*, 2002, of 7.8 MJ/kg DM.

The requirements for ME intake per day for maintenance, early gestation and early lactation of a 50kg ewe are 7.32, 9.25 and 12.55 MJ/day (NRC, 2007).

Animals consuming *M. sativa* had sufficiently high intakes to meet ME requirements for maintenance and some gain, while animals consuming *C. sturtii* and *S. microphylla* were not able to meet the energy requirements and therefore, were in a negative energy balance and would need to meet the deficit by mobilising body reserves, which was clear in the loss of body weight during the experimental period. Mupangwa *et al* (2000) reported an ME intake for *C. rotundifolia* of 1.30 MJ/day which is even lower than that reported in this trial. Some other forms of energy would be needed to support maintenance for longer periods.

3.3. Rumen parameters and fermentation

The rumen parameters were compared in this section of the trial, with specific attention given to rumen ammonia concentration ($\text{NH}_3\text{-N}$) and the three important volatile fatty acids (VFA), acetate, propionate and butyrate.

3.3.1 Rumen Ammonia ($\text{NH}_3\text{-N}$)

The rumen $\text{NH}_3\text{-N}$ for the three forages is compared in Table 3.7.

Table 3.7 Rumen ammonia concentration in sheep for the species, *C. sturtii*, *S. microphylla* and *M. sativa*

Parameter	Species			s.e.
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	
Rumen ammonia (NH ₃ -N) (mg/100ml)	8.15 ^a	16.86 ^b	14.47 ^b	1.521

^{ab} Column means with the same superscript do not differ significantly ($P>0.05$) (Duncan's multiple test range)
 s.e. Standard error

Rumen NH₃-N concentrations of *C. sturtii* were the lowest and differed significantly from *S. microphylla* and *M. sativa* which were similar, with *S. microphylla* being slightly higher.

The rumen ammonia N concentrations were above the recommended optimal level of 5mg/100ml for maximum microbial growth (Mupangwa *et al.*, 2000). Animals in this study given *cassia* hay, although having higher intakes than those reported by Mupangwa *et al.* (2000), had lower rumen ammonia levels of 8.15 mg NH₃-N /100ml than those reported by Mupangwa *et al.* (2000), which were 15 mg NH₃-N /100ml. The CP concentration of *C. rotundifolia* (182 g/kg DM) was much higher than *C. sturtii* (122 g/kg DM) which could explain the difference in rumen ammonia concentration.

Although *S. microphylla* had a lower CP concentration than *M. sativa* and the *C. rotundifolia* reported by Mupangwa *et al.* (2000), it resulted in the highest rumen ammonia concentration indicating that its protein was more degradable than *M. sativa*. The high rumen ammonia concentrations in the absence of readily fermented energy source could result in energy limiting microbial growth and a significant loss of legume protein N in net transfer to the small intestines, thus resulting in increased urinary N excretion and reduced N retention by the animals (Mupangwa *et al.*, 2000).

Snyman (2006) reported values for rumen NH₃-N concentration of *A. nummularia* of between 10.64 and 13.41 mg/100ml. The CP concentration of *A. nummularia* varied between 215-243 g/kg DM which was much higher than *C. sturtii* and *S. microphylla*, therefore the *A. nummularia* probably had a lower protein degradability resulting in the lower rumen ammonia concentration. Ahn *et al.* (1988) reported rumen NH₃-N concentrations for *C. rotundifolia* of 14 mg/100ml, which is higher than those reported for *C. sturtii* in this trial. The CP concentration was again higher (138 g/kg DM) resulting in higher rumen ammonia concentrations.

In the feed analysis, *C. sturtii* had the lowest protein concentration, which could possibly be the reason for the lowest rumen $\text{NH}_3\text{-N}$; however, the difference between the measured and predicted apparent CP digestibility and the low rumen $\text{NH}_3\text{-N}$ concentration of *C. sturtii* may indicate that the protein degradation in *C. sturtii* was abnormal. This shows that polyphenolic tannins or similar compounds, which restrict degradation of proteins in other legumes, were possibly present (Ahn *et al.*, 1988; Komlong *et al.*, 2001). Low DMI and apparent digestibility of *C. sturtii* could be attributed to the depressed microbial activity due to lower ruminal $\text{NH}_3\text{-N}$, which could hamper fibre utilization in ruminants (Misra *et al.*, 2006).

Although there was also a difference between the measured and predicted apparent CP digestibility for *S. microphylla* and *M. sativa*, the rumen $\text{NH}_3\text{-N}$ concentration is within the optimum range, therefore protein degradation may be normal in these species.

The ammonia N in the rumen liquor is the key intermediate in microbial degradation and synthesis of protein. If the diet is deficient in protein, or if the protein has a lower degradability, the concentration of ammonia will be low (5mg/100ml) and the growth of rumen organisms will be slow, therefore slowing down carbohydrate metabolism. If the protein is highly rumen degradable, ammonia will accumulate in the rumen and optimum concentrations (8.5 – 30 mg/100ml) will be exceeded. Ammonium is then absorbed into the blood, carried to the liver and converted to urea, the greater part then being excreted in the urine and wasted. If the food has a low protein concentration and the concentration of ammonia N in the rumen is low, the amount of nitrogen returned to the rumen as urea from the blood may exceed that absorbed from the rumen as ammonium. This net gain in 'recycled' nitrogen is converted to microbial protein, which means the quantity of protein reaching the intestine may be greater than that in the food. By these means the ruminant is able to conserve nitrogen. The rumen microbes have a 'levelling effect' on the protein supply. They supplement in quantity and quality, the protein of foods such as low quality roughages. (McDonald *et al.*, 2002). The possible effect of recycled nitrogen will be discussed later in the microbial protein section.

If rumen $\text{NH}_3\text{-N}$ levels decrease below 80mg N/l, fibre digestibility may be depressed and digestibility is reduced well below 10mg N/l (Leng, 1997). All three diets never fell below the "low" level of rumen $\text{NH}_3\text{-N}$ (5mg/100ml) which means that the dietary protein and rumen protein degradation was sufficient to maintain microbial activity and carbohydrate breakdown in the rumen (Komlong *et al.*, 2001).

3.3.2 Volatile Fatty Acids

Acetic-, propionic- and butyric acids are the end products of rumen fermentation. These VFA are the energy source supplied to the animal from forages. Acetate and butyrate can only be used for growth if there is enough propionate and glucose (Hovell and Greenhalgh, 1978).

The volatile fatty acid concentrations are presented in Table 3.8.

Table 3.8 Volatile fatty acid concentrations in the rumen of sheep receiving *C. sturtii*, *S. microphylla* and *M. sativa*

Parameter	Species			s.e.
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	
Acetate (mmol/100ml)	5.39 ^a	6.19 ^a	7.56 ^b	0.489
Propionate (mmol/100ml)	1.45 ^a	1.34 ^a	1.60 ^a	0.106
Butyrate (mmol/100ml)	0.38 ^a	0.47 ^a	0.65 ^b	0.035
Valeric acid (mmol/100ml)	0.09 ^a	0.13 ^{ab}	0.11 ^a	0.008
Total VFA (mmol/100ml)	7.31 ^a	8.13 ^{ab}	9.91 ^b	0.614
Acetate:Propionate	3.75 ^a	4.61 ^{bc}	4.71 ^b	0.194

^{ab} Column means with the same superscript do not differ significantly ($P > 0.05$) (Duncan's multiple test range)
 s.e. Standard error

Sheep receiving *C. sturtii* had the lowest total VFA concentration and was significantly different from *M. sativa* which had the highest. *S. microphylla* had a similar total VFA concentration to both *C. sturtii* and *M. sativa*.

C. sturtii had the lowest proportion of acetate but did not differ significantly compared to *S. microphylla*, while both were significantly different to *M. sativa*, which was the highest. The propionate concentration for all three forages did not differ significantly. *S. microphylla* had the highest fibre concentration, therefore leading to higher acetate concentrations than *C. sturtii* but not higher than *M. sativa*, suggesting the fibre of *S. microphylla* is less digestible. This is supported by the low apparent NDF digestibility for *S. microphylla*.

However, referring to Table 3.6, ME intake may also have an effect on microbial activity in the rumen. The ME intake of *S. microphylla* was 2.89 MJ/day compared to that of *M. sativa* of 8.57 MJ/day.

The high NDF degradability of *M. sativa* shows a high fibre digestibility. Together with sufficient ME intake, is a resultant high acetic acid concentration. The NDF digestibility and ME intake of *C. sturtii* and *S. microphylla* did not differ significantly which corresponds to the non-significant difference between the acetate concentration of the two forages.

The proportion of VFA differs, where mature fibrous forages give rise to a mixture containing more acetate (70%), while less mature/young forages have a higher proportion of propionate (McDonald *et al*, 2002). All three forages had a high proportion of acetate in total VFA mixture, 73%, 76% and 76% for *C. sturtii*, *S. microphylla* and *M. sativa* respectively.

Lower rumen acetate concentrations in *C. sturtii* and *S. microphylla* compared to *M. sativa*, and no significant difference in propionate concentrations indicates that *C. sturtii* and *S. microphylla* may possibly contain similar amounts of readily available carbohydrates. This effect was also reported in the trial conducted by Misra *et al*. (2006). Although the difference is not significant, the propionate concentration from *C. sturtii* and *S. microphylla* is slightly lower than that of *M. sativa*. This corresponds well to the lower ME intake and DM/NDF digestibility of *C. sturtii* and *S. microphylla* (see Table 3.4 and 3.6). If the propionate decreases there will be a decrease in the usage of metabolisable energy for production (Hovell and Greenhalgh, 1978).

The butyrate followed the same trend as acetate. Rumen butyric acid tends to increase with an increase in fibre in the diet (McDonald *et al*, 2002), which was not the case in this trial. *M. sativa* had the lowest CF concentration, but had the highest butyrate concentration, corresponding well to the higher NDF degradability (see Table 3.11).

Snyman (2006) reported ranging values on three different sampling days for acetate (2.82 – 4.68mmol/100ml), propionate (0.51 – 1.04mmol/100ml) and butyrate (0.33 - 0.60mmol/100ml) for *A. nummularia* which were slightly lower than those reported for these species. Van Niekerk (1997) reported values on *M. sativa* for acetate (13.8mmol/100ml), propionate (4.7mmol/100ml) and butyrate (2.1mmol/100ml). These values are much higher than those reported in this study. The higher acetate may be due to higher fibre digestion. The low propionate concentrations found in this study can only be attributed to low intakes, which reduces ME intake (Hovell and Greenhalgh, 1978).

The relative concentrations of the VFA are assumed to represent their relative rates of production, but this may be misleading if they are absorbed at different rates. The total concentration of VFA in the rumen varies widely according to the animal's diet and the time since the previous meal, and it is normally in the range of 7 – 15 mmol/100ml. In ruminants, energy for maintenance is derived mainly from the VFA (McDonald *et al*; 2002).

Even though the total VFA concentrations fall into the normal range, they are at the bottom of that range. These low concentrations may have been due to low intakes.

Tannins have been found to reduce total VFA production (Snyder *et al.*, 2007). In this trial *C. sturtii* had a significantly lower concentration which could indicate tannin presence.

3.4 Nitrogen balance and microbial protein

3.4.1 Nitrogen balance

Nitrogen consumed in the feed and that excreted in the urine and faeces is used to determine the nitrogen balance in the animal, thus facilitating an evaluation of protein sources. When nitrogen intake is equal to nitrogen output, the animal is in nitrogen equilibrium: When intake exceeds output it is in a positive balance and when output exceeds intake it is in a negative balance. These balance trials are susceptible to some errors which may include inadequate adaption to feed, collection and storage of urine and faeces and preparation of samples for analysis (McDonald *et al*, 2002).

The nitrogen retention of the animals is presented in Table 3.9.

Table 3.9 Nitrogen retention in sheep given *C. sturtii*, *S. microphylla* and *M. sativa*

Parameter	Species			s.e.
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	
N intake (g/day)	5.59 ^a	7.71 ^a	24.02 ^b	2.293
Faecal N (g/day)	3.57 ^a	3.84 ^a	8.73 ^b	0.796
Urine N (ml/day)	0.05 ^a	0.07 ^a	0.13 ^b	0.010
N retention	1.97 ^a	3.80 ^a	15.17 ^b	1.594

^{ab} Column means with the same superscript do not differ significantly ($P>0.05$) (Duncan's multiple test range)
 s.e. Standard error

Nitrogen intake was highest for *M. sativa* and was significantly different from *C. sturtii* and *S. microphylla*. The same trend followed for faecal and urinary nitrogen outputs as well as nitrogen retention. The nitrogen retention for all species was positive with *C. sturtii* being the lowest. These values compare well to the CP concentration of the three forages with *C. sturtii* the lowest and *M. sativa* the highest.

Ahn *et al.*, (1988) reported a nitrogen retention value for *C. rotundifolia* and lucerne hay of 5.4 and 24.6g/d respectively which was higher than reported here, possibly due to higher intakes and CP concentration. The sheep fed *C. rotundifolia* hay gained body weight (0.95g/day) compared to those in this trial eating *C. sturtii* which lost weight (Ahn *et al.*, 1988).

The low nitrogen retention of *C. sturtii* reflects in the live weight losses of those animals eating *C. sturtii*. The only animals to gain body weight were those in the treatment group of *M. sativa*.

The reduced N retention of the *C. sturtii* and *S. microphylla* compared to *M. sativa* could be attributed to the low acceptance of this feed as indicated by low intakes. Nitrogen retention was primarily a function of N intake (Chriyaa *et al.*, 1997). A similar trend was observed in the trial conducted by Chriyaa *et al.* (1997), where the N retention of a black wattle diet was lower than one supplemented with either old man saltbush or lucerne hay.

Abou El Nasr *et al.* (1996) reported acacia and saltbush hays had negative nitrogen retention, possibly due to low CP intake and live weight losses.

Snyder *et al.*, (2007) reported the high tannin concentration in Black Locust lead to an increase in faecal N which could be directly attributable to the ability of tannins to form protein complexes resistant to degradation. In this trial there was no significant increase in faecal N compared to *M. sativa*, therefore if tannins are present in *C. sturtii* or *S. microphylla*, the effect was not seen in faecal N concentration.

3.4.2 Microbial protein

The simple and non-invasive procedure to determine the daily synthesis of microbial protein of determining either total urinary excretion of purine derivatives (PD), i.e. the sum of allantoin, hypoxanthine, xanthine and uric acid (Fujihara *et al*, 1987) or of allantoin alone (Dewhurst and Webster, 1988), which was considered for this trial, but the laboratory did not have the methods or apparatus to determine hypoxanthine/xanthine, so it was not possible to determine the total urinary excretion of purine derivatives. As seen in Table 3.10, allantoin concentration was determined. Further research is needed to determine the other components. However the method used in the trial has been described by several authors which revealed a close relationship between microbial nucleic acids reaching the small intestine and the urinary excretion of purine derivatives, especially allantoin (Antoniewicz *et al*, 1980 and McAllan & Smith, 1973).

The urinary excretion of allantoin, microbial nitrogen supply and efficiency of microbial nitrogen synthesis are presented in Table 3.10.

Table 3.10 Urine allantoin (mg/d) concentration in urine, microbial nitrogen supply (g/day) and efficiency of microbial N supply (g/kg DOMI) of sheep eating *C. sturtii*, *S. microphylla* and *M. sativa*

Parameter	Species			s.e.
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	
Allantoin (mg/d)	960.9 ^a	835.48 ^a	1540.99 ^b	169.95
Microbial N supply (g/day)	7.54 ^a	7.37 ^a	8.06 ^b	0.161
Efficiency of microbial N supply (g/kg DOMI)	1.22 ^a	1.31 ^a	3.23 ^b	0.317

^{ab} Column means with the same superscript do not differ significantly ($P > 0.05$) (Duncan's multiple test range)
 s.e. Standard error

The daily urinary allantoin elimination did not differ between *C. sturtii* and *S. microphylla* but was significantly different and higher for *M. sativa*. This result follows the trend observed by Chen *et al*. (1992), where the excretion of purine derivatives (in this case allantoin) increases with increasing intake.

The amount of microbial nitrogen supplied to the animal (g/day and g/kg DOMI) followed the same trend as allantoin.

Tebot *et al.*, (2002) reported that allantoin excretion was greater for a normal protein diet (108.75 g CP/day) than for a low protein diet (46.88 g CP/day) which is similar to this trial, with *M. sativa* having a higher protein intake and therefore higher allantoin excretion than both *C. sturtii* and *S. microphylla*.

Mupangwa *et al* (2000) reported an increase in microbial protein supply (2.7 – 4.53g/day) as the dietary protein intake was increased from 5.75 to 27.7 g/day. This was probably due to an increased intake of degradable N and more fermentable organic matter made available for microbial fermentation in the rumen of sheep. Increased OM intake and apparent digestibility have been reported to increase microbial production (Clark *et al*, 1992). This corresponds well to all values in this trial with *M. sativa* having a higher CP concentration and OM digestibility as well as a significantly higher DOMI. The efficiency of microbial N supply had a linear correlation with DOMI. The increased microbial N production can be attributed at least partially to the larger amount of energy supplied by the larger quantity of OM fermented in the rumen (Mupangwa *et al.*, 2000).

Although total urinary purine derivative excretion was used, Mupangwa *et al.* (2000) reported a similar trend with *C. rotundifolia*, a low nitrogen intake and microbial N supply.

Chen *et al.*, (1992) reported that the efficiency of microbial N supply is expected to increase with DM intake, which was the case in this study. The same allantoin method used in this study was used by Chen *et al.*, (1992), however reported values for microbial N supply, which were slightly lower, 5.7 g microbial N/day for an intake of 444 g DOMI/day, than the results reported in this trial. There is no clear explanation for the differences, but factors relating to the quality of the forage may play a role in explaining these differences.

Using the relationship between ME intake (MJ/day) and microbial N production (Microbial N (g/day) = ME intake x 1.34), the calculated microbial N supply (g/day), from the diets was 3.3, 3.9 and 11.5 g/day for *C. sturtii*, *S. microphylla* and *M. sativa* respectively. The values obtained in this study are higher than the predicted values for microbial N supply (Mupangwa *et al.*, 2000), however they do follow the same trend.

3.5. Rumen degradability

Rumen degradability was determined by estimating the disappearance of DM and NDF from the rumen, using rumen cannulated animals and placing small samples of food in Dacron bags into the rumen. This is called the nylon bag/*in sacco* technique (McDonald *et al*; 2002).

The parameters of digestibility are constants which may be fitted by a least squares procedure, where the disappearance is regressed on time. The intercept on the y -axis is represented by a , which is the part of the DM which is considered to be the soluble fraction; b is the difference between a and the asymptote and represents the part which is degraded more slowly and c is the rate of disappearance of the potentially degradable fraction b . The extent of degradability depends on the time which the material remains in the rumen (retention time). As the time of incubation increases, the fraction of material remaining in the rumen decreases (McDonald *et al*; 2002).

Table 3.11 represents the DM and NDF degradation parameters of the three forages.

Table 3.11 Ruminal degradation parameters of dry matter and neutral detergent fibre in, *C. sturtii*, *S. microphylla* and *M. sativa*

	Species			
Parameter	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	s.e.
Dry matter degradation				
a (g/kg)	287.6 ^c	326.8 ^b	436.2 ^a	0.413
b (g/kg)	284.9 ^a	185.8 ^b	247.8 ^a	1.018
c (%/h)	0.077 ^a	0.065 ^a	0.074 ^a	0.005
ED (g/kg)	511.7 ^b	468.1 ^c	629.5 ^a	0.080
NDF Degradation				
a (g/kg)	20.0 ^c	92.6 ^b	259.0 ^a	0.027
b (g/kg)	290.2 ^a	219.2 ^b	245.2 ^{ab}	0.911
c (%/h)	0.058 ^a	0.051 ^a	0.061 ^a	0.004
ED (g/kg)	233.4 ^b	249.2 ^b	438.1 ^a	0.289

^{ab} Column means with the same superscript do not differ significantly ($P > 0.05$) (Duncan's multiple test range)

a = soluble fraction (immediately degradable); b = slowly degradable/insoluble fermentable fraction; c = rate of disappearance of potentially degradable b fraction; ED = Effective degradability at a rate constant ($K=0.02/\text{hr}$)

s.e. Standard error

M. sativa had significantly higher a-values for both DM and NDF degradation compared to the two shrub species at a rate constant of 0.02/h. *C. sturtii* had a higher b-value for DM degradation compared to *S. microphylla* which shows that *S. microphylla* DM component was most readily soluble (Ikhimioya *et al.*, 2005). For NDF, however, the b-values didn't differ among the species. Species had also no effect on the c-values of both DM and NDF. Therefore all species appear to have a similar potential source of energy for use by micro-organisms in the rumen (Ikhimioya *et al.*, 2005). Effective DM degradability of *C. sturtii* and *S. microphylla* was similar while that of *M. sativa* was significantly higher. The effective NDF degradability for *C. sturtii* and *S. microphylla* was similar and *M. sativa* again had a significantly higher NDF degradability.

The rumen DM degradability for all three species showed a similar trend but much higher values than the apparent DM digestibility represented in Table 3.4. The rumen NDF

degradability values were almost identical to those reported in Table 3.4 for apparent NDF digestibility.

M. sativa had the highest concentration of VFA and proportion of propionate, so the higher DM degradability may be attributed to a higher cellulolytic activity in the rumen (Van Soest, 1982).

There is very little or no research that has been done on the rumen DM or NDF degradability of *S. microphylla* and *C. sturtii*, so there are few comparisons.

Degradability is known to decrease with an increase in NDF concentration and also depends on which fraction makes out most of the NDF, if it is hemicelluloses, then a decrease in NDF degradability is due to an increase in NDF concentration. However if the ADF fraction (cellulose and lignin) makes out the larger portion of NDF, then a lower NDF degradability is not due to higher NDF concentration but rather the ADF fraction (particularly the lignin), which causes most of the indigestibility (McDonald *et al.*, 2002; Chriyaa *et al.*, 1997).

The NDF concentration as well as the amount of hemicelluloses in *S. microphylla* (136 g/kg) is higher compared to *C. sturtii* (94 g/kg), therefore a lower DM degradability can be expected. This result is also consistent with those reported by Abdulrazak *et al.* (2000) and Gurbuz (2007). *S. microphylla* also contains more ADF and lignin than *C. sturtii*, therefore should lead to a lower NDF degradability, however, although not significantly higher, *S. microphylla* has a higher NDF degradability than *C. sturtii*, which could possibly again be explained by the distribution of the lignin, where lignin in the former is restricted to certain areas in the plant, whereas the lignin in *C. sturtii* is possibly more widely distributed, therefore having a greater inhibitory effect on degradability (McDonald *et al.*, 2002).

It is known that the degradation of a feed is directly correlated to the rumen microbial environment and population. When the rumen is favourable (optimum pH, NH₃-N and correct microbial composition), degradability will increase (McDonald *et al.*; 2002). Certain rumen parameters, such as rumen ammonia concentration and VFA contribution for *S. microphylla* are more optimal than *C. sturtii*, therefore leading to an improved DM degradability of *S. microphylla*.

Chriyaa *et al* (1997) reported a 65.8% DM degradability for *A. nummularia* and 53.3% for lucerne hay (*M. sativa*) incubated for 72 hours in the rumen of sheep, while that reported by Snyman (2006) for *A. nummularia* varied from 73.61 to 74.10%.

Snyman (2006) reported values for *A. nummularia* NDF degradability varying from 59.78 to 60.98%. The values for this forage crop are well above those reported for *C. sturtii* and *S. microphylla* but compare well with that of *M. sativa*. The NDF concentration of *A. nummularia* was reported to be much lower, therefore supporting the higher DM and NDF degradability values.

Ikhimoioya *et al.* (2005) reported effective DM degradability of leaves from some indigenous multipurpose tree species from Nigeria which are used as feed resources during the dry season. The values ranged from 72.67% to 44.33% at a rate constant of $k=0.02$. *S. microphylla* and *C. sturtii* had a higher effective DM degradability than some of these tree species.

Khazaal *et al.* (1994) pointed out that the *in situ* technique should be used with caution when estimating the nutritive value of feeds possibly containing phenolics (tannins etc.), because the potential negative effect of these compounds on rumen microbial fermentation is unlikely to be detected by *in situ* methods. *In vitro* methods such as IVOMD and gas production measurements are more reliable in detecting inhibitory compounds in feeds because these compounds are likely to affect the activity of rumen microbes in a closed system (Khazaal *et al.*, 1994). However, the *in situ* DM degradation may reflect the potential of the forage for species possessing the ability to detoxify tannins, such as goats and some game species (Van Soest, 1984). Although the tannin concentration was not determined in this trial, Ventura *et al.* (2004) reported that *C. sturtii* did contain tannins (0.63g catequins/kg DM) so the effect of these on the DM and NDF degradability may need further investigation. It was also mentioned in Chapter 1 that *S. microphylla* and possibly *C. sturtii* are known to contain various anti-nutritional factors which may also contribute to the lower DM and NDF degradability in this trial.

Again further research is necessary to determine the effect of these. The addition of tannin binding compounds such as polyethyleneglycol (PEG) and polyvinylpyrrolidone (PVP) to the forages may provide a better measure of the effects of tannins on the digestibility of nutrients.

The low soluble (a) DM values in these forages reveals that they do not have a high potential for being good sources of more nutrients for microbial growth as both Clark *et al.*, (1992) and Gomes *et al.*, (1994) reported a strong positive relationship between DM intake and microbial growth. *C. sturtii* and *S. microphylla* had ED values for both DM and NDF, much lower than *M. sativa*, thus cannot be considered as good feeds for ruminants.

3.6. Rumen kinetics

Results and parameters from the rumen evacuation trial are represented in Table's 3.12.

Table 3.12 Kinetics of NDF; rate of intake (Ki), rate of passage (Kp) and rate of digestion (Kd) (% per hour) and % NDF digested and passing from the rumen

Parameter	Species			s.e.
	<i>C. sturtii</i>	<i>S. microphylla</i>	<i>M. sativa</i>	
Ki %/hour	1.287 ^a	2.127 ^a	4.130 ^b	0.224
Kp %/hour	0.919 ^a	1.241 ^a	1.538 ^a	0.055
Kd %/hour	0.370 ^a	0.886 ^a	2.595 ^b	0.258
% NDF digested in rumen	27.58 ^a	34.52 ^b	62.65 ^c	12.327
% NDF passing from rumen	72.41 ^a	65.48 ^b	37.35 ^c	12.327

^{ab} Column means with the same superscript do not differ significantly ($P>0.05$) (Duncan's multiple test range) s.e. Standard error

The rate of intake and rate of digestion for *C. sturtii* and *S. microphylla* did not differ significantly, while that of *M. sativa* was the highest and significantly different. The rate of passage for all three species was similar. The equations used to determine the rates in this trial were those described by Robinson *et al.* (1987) and Mertens (1993).

The percent NDF digested in the rumen differed significantly between all three species with *C. sturtii* being the lowest and *M. sativa* the highest. The percent NDF passing from the rumen also differed significantly between all three species, however this time *C. sturtii* being the highest and *M. sativa* the lowest, which corresponds well to the values for NDF digested in the rumen.

The apparent NDF digestibility shown in Table 3.4, of *C. sturtii* is lower than *S. microphylla*, therefore it was expected to see the lower NDF digestion in the rumen is lower. The decrease in degradation is then associated with a decrease in the rate of digestion (Kd) and passage (Kp) (Ngwa *et al.*, 2002).

Rumen evacuation derived rates of digestion of fibre are highly correlated with *in vivo* digestibility of fibre (Tamminga *et al.*, 1989; Khalili, 1993), lending validity to this technique in estimating the rate of fibre digestion. In this trial the comparison of ED values in Table 3.11 and % NDF digested in Table 3.12 are similar.

When a food particle enters the rumen, it can only leave by one of two mechanisms, either by fermentative digestion or passage. The extent of digestion of potentially digestible fraction (percent NDF digested in the rumen) depends on its degradability and on the length of time the feed is exposed to breakdown in the rumen (rate of passage). The animals' requirements are met from the digested component of intake (Osuji *et al.*, 1993a) and the rate of passage and digestion affects the feed digestibility, intake as well as the fermentation end products (Firkins *et al.*, 1998).

The higher NDF degradability and voluntary intakes of *M. sativa* compared to those from *C. sturtii* and *S. microphylla* could result in lower rumen retention time and greater turnover of particulate matter from the rumen of sheep. The results above show that *C. sturtii* had the highest percent NDF passing from the rumen with the lowest NDF digested in the rumen. The retention time was longer (slower rate of passage, Kp), however this didn't improve NDF digested in the rumen (Firkins *et al.*, 1998). Therefore there may be other possible factors affecting digestibility of this forage.

Intake is more related to the rate of digestion than the digestibility (McDonald *et al.*, 2002), therefore rapid digestion should increase intake, which is the case for *M. sativa*.

C. sturtii and *S. microphylla* had a lower rate of digestion, with a longer retention time therefore intake may be depressed.

The rate of digestion and intake for *S. microphylla* was higher than *C. sturtii* but it has a higher NDF and lignin (ADL) concentration possibly leading to an assumption that the distribution of lignin is having an effect on rate of digestion (McDonald *et al.*, 2002).

Although the NDF concentration affects the rate of digestion and intake, the physical form and palatability could also have an effect. Further grinding of the forages and addition of a palatable energy source could improve the nutritional value of the forages.

There is very little data to support that found in this trial therefore, further research is necessary to compare these values found in this trial, and to possibly explain the rumen kinetics of the three forages over a longer period in more detail. Factors affecting the accuracy and precision of the kinetics of digestion and passage need to be considered before digestion models based on compartment analysis can be evaluated properly (Firkins *et al.*, 1998).

Chapter 4

Summary and Conclusions

The aim of this study was to determine the nutritional differences between two drought resistant leguminous forage species in comparison with the well-known forage legume, lucerne. Chemical composition, certain rumen parameters and digestibility were measured.

The mean CP concentration differed between species with *C. sturtii* having the lowest CP and *M. sativa* the highest. The nitrogen retention for all species was positive with *C. sturtii* being the lowest. These values compare well to the CP concentration of the three forages with *C. sturtii* the lowest and *M. sativa* the highest concentration.

The macro-mineral concentrations (Ca, P and Mg) of *C. sturtii* and *M. sativa* were similar and had a higher concentration than *S. microphylla*.

The trace mineral concentrations of all three plants varied. Iron concentration of all three plants differed, with *M. sativa* having the lowest concentration and *C. sturtii* the highest. The copper concentrations in *M. sativa* and *C. sturtii* were similar, while that of *S. microphylla* was slightly lower. The zinc concentrations in *M. sativa* and *C. sturtii* were similar, while that of *S. microphylla* was slightly higher. Manganese concentration of all three species differs, with *C. sturtii* being the lowest and *S. microphylla* the highest.

The apparent DM digestibility of *S. microphylla* is significantly lower than *M. sativa* while it did not differ significantly from *C. sturtii*. The apparent CP digestibility of all three species did not differ significantly, however that of *M. sativa* is numerically higher. With regards to the NDF digestibility, *C. sturtii* and *S. microphylla* differ significantly to *M. sativa* with lower NDF digestibility values. The apparent OM digestibility followed the same trend as that of apparent DM digestibility. Effective DM degradability of *C. sturtii* and *S. microphylla* was similar while that of *M. sativa* was significantly higher. The effective NDF degradability for *C. sturtii* and *S. microphylla* was similar and *M. sativa* again had a significantly higher NDF degradability.

The mean CF concentration differed between species with *S. microphylla* having the highest CF while *M. sativa* had the lowest. The NDF and ADF levels of the samples followed the same trend as CF. *C. sturtii* and *S. microphylla* had a lot of woody material (stems) and this material

was included in the feed. Some leaf material was lost in the drying process as well. This may explain the high fibre values compared to that of the *M. sativa*. The ADL concentration of *S. microphylla* was higher than both *C. sturtii* and *M. sativa*.

S. microphylla had the highest fibre concentration, therefore leading to higher acetate concentrations than *C. sturtii* but not higher than *M. sativa*, suggesting the fibre of *S. microphylla* is less digestible. This is supported by the low apparent NDF digestibility for *S. microphylla*. The high NDF degradability of *M. sativa* shows a high fibre digestibility. Together with sufficient ME intake, is a resultant high acetic acid concentration. Lower rumen acetate concentrations in *C. sturtii* and *S. microphylla* compared to *M. sativa*, and no significant difference in propionate concentrations indicates that *C. sturtii* and *S. microphylla* may possibly contain similar amounts of readily available carbohydrates. This corresponds well to the lower ME intake and DM/NDF digestibility of *C. sturtii* and *S. microphylla*.

The average intake was very different between species, with *C. sturtii* being the lowest and *M. sativa* the highest. The animals consuming either *C. sturtii* or *S. microphylla* tended to lose body weight during the experimental period, while those eating *M. sativa* gained body weight. Voluntary intake parameters of *C. sturtii* and *S. microphylla* were lower and differed significantly between *M. sativa*. The DM intake of *M. sativa* was higher than both *C. sturtii* and *S. microphylla*.

Increased OM intake and apparent digestibility have been reported to increase microbial production (Clark *et al*, 1992). This corresponds well to all values in this trial with *M. sativa* having a higher CP concentration and OM digestibility as well as a significantly higher DOMI.

The higher NDF degradability and voluntary intakes of *M. sativa* compared to those from *C. sturtii* and *S. microphylla* could result in lower rumen retention time and greater turnover of particulate matter from the rumen of sheep. *C. sturtii* had the highest percent NDF passing from the rumen with the lowest NDF digested in the rumen. The retention time was longer (slower rate of passage, Kp); however this didn't improve NDF digested in the rumen. Therefore there may be other possible factors affecting digestibility of this forage.

Intake is more related to the rate of digestion than the digestibility, therefore rapid digestion should increase intake, which is the case for *M. sativa*. The rate of digestion and intake for *S. microphylla* was higher than *C. sturtii* but it has a higher NDF and lignin (ADL) concentration

possibly leading to an assumption that the distribution of lignin is having an effect on rate of digestion (McDonald *et al.*, 2002).

In conclusion; both *C. sturtii* and *S. microphylla* had a higher concentration of all fibre components, thus decreasing intake significantly compared to *M. sativa*. The low nitrogen retention of *C. sturtii* reflects in the live weight losses of those animals eating *C. sturtii*. The only animals to gain body weight were those in the treatment group of *M. sativa*. The reduced N retention of the *C. sturtii* and *S. microphylla* compared to *M. sativa* could be attributed to the low acceptance of this feed as indicated by low intakes.

Both *C. sturtii* and *S. microphylla* had a lower nutritional value compared to *M. sativa*. The effect of all fibre parameters, digestibility values and rumen parameters correspond well to the relative voluntary intake parameters. Low intake is the biggest factor affecting the decisions to supplement with these forages during dry periods. If these two leguminous fodder species were to be used as maintenance feed, some other supporting source of energy would need to be supplied in order for these sheep to be maintained over a long period.

The effect of anti-nutritional factors present in *C. sturtii* and *S. microphylla* on the digestibility of forages and nutrient contribution from forages needs to be studied to determine if these play a role in reducing the nutritional value.

Chapter 5

Critical evaluation

Many different parameters were evaluated in this trial and it may be valuable to analyze each parameter individually in a trial, to assess its value as a contributor to the nutritive value of these plants. For example; the extensive loss of legume protein as ammonia in the rumen of animals consuming *S. microphylla* or *C. sturtii*, occurs in the absence of a readily fermentable energy source, which can result in a reduction of undegraded dietary protein flowing out the rumen. The efficiency of microbial production from rations based on forage legumes can be limited by the lack of a readily available energy source and the addition of such sources may assist in giving increased ammonia utilization and microbial protein production.

The overall results of the three feeds indicate the factors which affect the intake, palatability and degradability, and the difference between the three species can be seen. From this study the factors identified can be used to determine whether the two “treatment” feeds were suitable in supplying enough nutrients to the animal for maintenance during dry periods. It is clear that *M. sativa* still remains the most applicable legume forage of the three studied, when it comes to maintenance requirements. There are certain factors which may contribute to *S. microphylla* and *C. sturtii* being unable to provide the necessary nutrients for maintenance.

An evaluation of the tannin concentration as well as levels of other substances found by other authors to be potentially deleterious in *S. microphylla* and *C. sturtii* as mentioned in Chapter one is necessary.

A larger number of animals per treatment, or more repetitions per treatments, may also provide more accurate results and a better statistical analysis and understanding of the factors studied.

After a thorough literature research, it seems there is some valuable information in the microbial protein synthesis section of trial. It is a very interesting topic with a lot of research already conducted. The lack of proper analytical methods to analyze for total purine derivatives and possibly sample error was a limitation in this trial to determine the effects significantly.

From the research a recommendation can be made that it may be necessary to include another type of forage with *C. sturtii* and *S. microphylla*, such as combining them with lucerne to maintain animals for longer periods. It may also be beneficial to supplement with an energy source, which will improve intakes and possibly support some level of production. Although the chemical composition shows a sufficient CP, VFA concentration etc, other factors such as the high lignification and possible high tannins and other anti-nutritional factors, such as those reported by other authors on *S. microphylla* and *C. sturtii*, may affect the intake and digestibility and may also cause toxicity if fed for long periods of time.

In summary, both *S. microphylla* and *C. sturtii* had higher concentrations of all fibre parameters, CF, NDF, ADF and ADL. These nutrients contributed to the lower digestibility of the two forages as well as lower intakes which affected the nutritive value of the forages for maintenance of sheep. This is clear when comparing the voluntary intakes of all nutrients. *M. sativa* had significantly higher values for all these parameters. ME intake is also an important parameter when considering nutrient value. Again, both *S. microphylla* and *C. sturtii* have ME intake values significantly lower than *M. sativa* and also below the requirement for maintenance. These are all key factors in contributing to the effectiveness of the forages in providing enough nutrients for maintenance.

Chemical analysis is essential for understanding the nutritional potential of the forages, although it may not be sufficient. *In situ* and *in sacco* digestion as well as measuring rumen parameters, more accurately determines the nutritional value. In this trial we did not analyse for or predict the potency of anti-microbial or other anti-nutritional factors which may, together with the basic nutrient limitations mentioned above, play a significant role in whether these legume forages have the potential to sustain animals.

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