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*

	Species		
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*
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

	Species		
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*
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 it's 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 homestatically, 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*

	Species		
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*
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	*C. sturtii*	*S. microphylla*	*M. sativa*	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*

	Species			
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*	s.e.
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:

DM intake (g/kg $W^{0.75}$) = 1.76 DM digestibility – 44.5 (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:

ME (MJ/kg DM) = 0.016 (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*

	Species			
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*	s.e.
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 (NH_3-N) and the three important volatile fatty acids (VFA), acetate, propionate and butyrate.

3.3.1 Rumen Ammonia (NH_3-N)

The rumen NH_3-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*

	Species			
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*	s.e.
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 NH_3-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 NH_3-N /100ml than those reported by Mupangwa *et al.* (2000), which were 15 mg NH_3-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 NH_3-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 NH_3-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 NH_3-N; however, the difference between the measured and predicted apparent CP digestibility and the low rumen NH_3-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 NH_3-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 NH_3-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 NH_3-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 NH_3-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*

	Species			
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*	s.e.
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*

	Species			
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*	s.e.
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*

	Species			
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*	s.e.
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	*C. sturtii*	*S. microphylla*	*M. sativa*	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/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

	Species			
Parameter	*C. sturtii*	*S. microphylla*	*M. sativa*	s.e.
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).