Effect of type and level of carbohydrate supplementation on intake and digestibility of *Atriplex nummularia* cv. De Kock by sheep.

By

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DECLARATION

I declare that this dissertation,
for the degree MSc (Agric) at the University of Pretoria,
has not been submitted by me for a degree at any other University.

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LIST OF ABBREVIATIONS

ADF	-	Acid detergent fibre
ATP	-	Adenosine triphosphate
Cl	-	Chloride
CO_2	-	Carbon dioxide
CP	-	Crude protein
D	-	Potential fermentable
DM	-	Dry matter
DMD	-	Dry matter digestibility
DMI	-	Dry matter intake
g	-	Gram
H_2SO_4	-	Sulphuric acid
ha	-	Hectare
K_c	-	Rate of clearance
K_d	-	Rate of degradation
kg	-	Kilogram
K_p	-	Rate of passage
L	-	Litre
ME	-	Metabolisable energy
mg	-	Milligram
mL	-	Millilitre
mm	-	Millimetre
mM	-	Millimole
N	-	Nitrogen
Na	-	Sodium
NaCl	-	Sodium chloride
NDF	-	Neutral detergent fibre
NH_3	-	Ammonia
NRC	-	National Research Council

ºC	-	Degree Celsius
OM	-	Organic matter
PEP	-	Phospoenolpyruvate carboxylase
PIR	-	Potential intake rate
S	-	Soluble
sp	-	Species
ssu	-	Small stock unit
U	-	Undegradable fraction
VFA	-	Volatile fatty acids
VFI	-	Voluntary feed intake

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SUMMARY

THE EFFECT OF TYPE AND LEVEL OF CARBOHYDRATE SUPPLEMENTATION ON INTAKE AND DIGESTIBILITY OF *ATRIPLEX NUMMULARIA* CV DE KOCK FED TO SHEEP.

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The aim of this study was to investigate the effect of different carbohydrate sources, with rapid and medium fermentabilities, on the digestibility and rumen fermentability of *Atriplex nummularia* cv. De Kock fed to sheep. Maize was used as the medium fermentable carbohydrate source and barley as the rapid fermentable carbohydrate source. The trial ran in four sequential experimental periods using two groups of animals. The two groups stayed constant during the entire trial, one group receiving the maize treatment and the other the barley treatment. During each experimental period the groups of animals received different levels of the two carbohydrate sources. Each experimental period consisted of a digestibility trial and a rumen fermentation trial.

Four different levels of supplementation were used, namely 0, 15%, 30% and 45%. Measurements included dry matter intake, water intake, percentage dry matter digestibility, percentage neutral detergent fibre digestibility, rumen pool sizes, rumen pH, rumen ammonia nitrogen and rumen volatile fatty acid production.

Supplementation of *A. nummularia* cv. De Kock with an energy source tended to increase feed and water intake. The tendency of energy sources to increase dry matter and neutral

detergent fibre digestibilities diminished when the level of supplementation was raised from 15% to 30% and from 30% to 45%. The results suggest that maize and barley supplementation at a level of 15% resulted in the highest incremental increase in dry matter and neutral detergent fibre digestibilities of *A. nummularia* cv. De Kock.

The results also indicated that energy supplementation tended to increase dry matter intake and rumen fill. Rumen pools of dry matter, neutral detergent fibre and total nitrogen all increased with an increase in the level of supplementation. The tendency of energy supplementation to increase the different rumen pools diminished as the level of supplementation increased from 30% to 45% for both the energy sources. The results also indicate that the rumen environment was more favourable for fibre degradation when maize was used as an energy source.

In the final phase it is reported that ruminal ammonia nitrogen increased as the rate of supplementation was increased from the 0% to the 15% level. The drop in rumen ammonia nitrogen as the rate of supplementation was increased to 30% indicates an improvement in microbial protein synthesis. Ruminal pH decreased as the rate of supplementation increased with barley supplemented animals having lower rumen pH values. The decrease in rumen pH supports the increase in volatile fatty acid production as the level of energy supplementation was increased.

It is concluded that an energy supplement of maize or barley will increase the intake and digestibility of *A. nummularia* cv. De Kock and that a level of 30% should be optimal for microbial protein synthesis without significantly affecting fibre degradation.

Chapter 1

1.1 General introduction

Southern Africa is largely arid and semi-arid with large variations in rainfall and soil conditions. Periodic and prolonged droughts and a large variation in the quality of natural pastures places large constraints on livestock production in the region.

The genus *Atriplex* (saltbush), of the family Chenopodiaceae, has a large genetic diversity and can adapt and survive in difficult conditions. *Atriplex* species has the ability to flourish on a wide range of soil and climatic conditions and can produce large quantities of green biomass (up to 18t/ ha/ year; De Kock, 1980) during the summer and autumn months, without irrigation, due to its deep root system.

Atriplex species have been proved to be very useful drought crops for bridging the periods when natural pastures do not satisfy the nutritional requirements of small stock (Jacobs & Smit, 1977). The nutritional value of *Atriplex* species has previously been well investigated (Wilson, 1966; Hassan *et al.,* 1979). It is characterized by high digestible crude protein and mineral content. The value of *Atriplex* material as a production feed for small stock may, however, be limited by poor palatability and acceptability, which will limit intake.

The synchronization of energy and nitrogen supply to the rumen is one of the most promising approaches to improve efficiency of rumen fermentation (Krishnamoorthy *et al.,* 1991). The crude protein content of *Atriplex* leaves, of up to 200 g/ kg (Wilson, 1966), which is sufficient for production purposes of small stock can be misleading because proportionally up to 0.60 of the chemical fraction may be non-protein nitrogen (Benjamin *et al.,* 1992), which is not necessarily utilized by small stock as a nitrogen source unless readily available energy material is present in the rumen during fermentation and digestion. It is reported in the literature that sheep consuming *Atriplex*

material had a negative nitrogen balance, which became positive when their diets were supplemented with a grain source (Hassan & Adbel-Aziz, 1979).

The aim of this dissertation was to quantify the effect of different carbohydrate sources and levels of supplementation on the digestion of *A. nummularia* cv. De Kock fed to Merino wethers.

Chapter 2

Literature review

2.1 Introduction

In this literature review the characteristics of saltbush and its use as a source of feed and nitrogen for animals are discussed. The advantages and disadvantages of this potential fodder source and, in particular, the effects of different carbohydrate sources (rapid fermentable and medium fermentable) on rumen characteristics and animal production will be emphasized.

2.2 Saltbush

2.2.1 Distribution of saltbush

The dicotyledonous family, *Chenopodiaceae* is one of the 11 families in the order *Chenopodiales*. This plant family contains a number of shrubs and small tree species, nearly all of which are halophytic (Willis, 1973 as quoted by Atiq-ur-Rehman, 1995). Chenopods are found throughout the semi-arid regions of south central Asia, southern Australia, western-north America and to a lesser extent in western South America and the Mediterranean basin (Atiq-ur-Rehman, 1995). The family contains a number of genera including the genus *Atriplex* (saltbush), which has hundreds of species distributed throughout the arid and semi-arid parts of the world, including the western United States, the Middle East, Australia, Africa and Siberia (Goodin, 1979; Davis, 1981; Dennis, 1981).

Atriplex species are well adapted to a wide range of climatic and soil conditions (De Kock, 1980). They can be cultivated successfully in arid and semi – arid areas with a mean annual rainfall of 250 mm and above, where the temperature does not fall below – 12°C (Steynberg & De Kock, 1987, Fair, 1989). Although most *Atriplex* species are

susceptible to frost (Shepherd *et al.,* 1991 as cited by Verschoor, 1992), *A. nummularia* is very tolerant of cold and frost (De Kock, 1980).

2.2.2 Characteristics of saltbush

Halophytes can survive on soils with high electrolyte concentrations, either by salt exclusion or salt accumulation. Saltbush is a salt accumulator (Glenn & O'Leary, 1985; Koheil *et al.,* 1992). Greenway & Osmond (1969), found that *A. nummularia* grows more vigorously at high (100 – 200 mM) than at low (1 – 10 mM) salt concentrations. Due to the high salt concentration in roots and leaves, plants maintain a high osmotic value in the cells, which is a physiological adaptation of the saltbush to moisture stress, thus making it drought resistant (De Kock, 1980).

Atriplex nummularia has a well-developed suberous tap root system together with horizontal basal root development (De Kock, 1980). The highest root concentration is found within 1m of the soil surface and lateral roots can be found up to 10m away from the plant (Jones & Hodgkinson, 1970).

Another characteristic of saltbush (*Atriplex*) is that it is one of very few genera in which two types of photosynthetic carbon fixation, termed C3 and C4 types, are displayed in related species. *A. spongiosa*, which is common in semi-arid Australia, is a typical example of a C4 type, whereas the coastal species, *A. hastata*, which is widespread throughout the world, is representative of the C3 type (Osmond, 1969; West, 1969). Osmond (1969) described the features of the C3 and C4 type of carbon metabolism in *A. hastana* and in *A. spongiosa* in Table 2.1. *A. nummularia* is a high producing species with a C4 type of photosynthetic carbon fixation (Jones & Hodgkinson, 1970).

Salisbury & Ross (1991) give an equation for photosynthesis:

$C0_2 + 2H_2O + 12$ photons $\rightarrow$ $(CH_2O) + O_2 + H_2O$

Photosynthesis and transpiration involve the nett flux of CO_2 and water vapour between plant leaves and the surrounding area. Water use efficiency of a species is, therefore, dependent upon the rate of nett photosynthesis relative to its transpiration.
Slayter (1969) demonstrated that the efficiency of water use in *A. spongiosa* (C4) was substantially greater than that in *A. hastata* (C3) mainly because of the high rate of photosynthesis but partially due to the lower rate of transpiration.

Table 2.1 Features of the C3 and C4 type of carbon metabolism in saltbush species (adapted from Osmond, 1969)

	***A. hastata* (C3 type)**	***A. spongiosa* (C4 type)**
Structure	1. Uniform spongy mesophyll 2. Uniform chloroplast type	1. Mesophyll/bundle-sheath complex 2. Specialized chloroplast types
Biochemistry	1. 3-C products of $^{14}CO_2$ photosynthesis 2. High carboxydismutase 3. Low PEP (Phospoenolpyruvate carboxylase) 4. No PEP synthetase 5. High glycolate oxidase	1. 4-C products of $^{14}CO_2$ photosynthesis 2. Low carboxydismutase 3. High PEP (Phospoenolpyruvate carboxylase) 4. PEP synthetase 5. Low glycolate oxidase
Gas exchange	1. High intracellular resistance to CO_2 2. Low O_2 enhancement of nett photosynthesis 3. CO_2 evolution in the light photorespiration	1. Low intracellular resistance to CO_2 2. No low O_2 enhancement of nett photosynthesis 3. No photorespiration

A further characteristic feature of saltbush is the presence of bladder-like epidermal hairs on their leaves. The salt content in these bladders is about half of the total salt in the leaf (Pallaghy, 1969). These bladders are common on the surface of young leaves. When collapsed they form a mat over the surface of the leaves (West, 1969). According to West (1969) the functions of these bladders in saltbush are to act as a screen against the intensity of excessive light, as well as an insulation against excessive transpiration and as a water storage tissue, and as a medium to allow the rapid absorption of atmospheric moisture into the mesophyll of the leaf.

Almost all species of *Atriplex* are dioecious – male and female flowers are carried on different plants. A few monoecious species occur amongst the annual species and in the tetraploid form of *A. canescens* (Barrow, 1987). It is reported that in an *Atriplex* population, up to 40% of the plants can change their sexual phenotype (Strawbridge *et. al.,* 1997). Changes of sexual phenotype have been influenced by extreme environmental conditions such as drought, irrigation and extreme temperatures as well as when the plants mature. This may influence the male to female ratio in a plantation (Maywald *et al.,* 1998).
Saltbush is representative of plants, which can adapt and survive in high salt and water stress conditions.

2.3 The value of saltbush as a forage reserve

The value of this drought resistant fodder shrub as a feed source in arid and semi–arid environments is well known. This is due to its ability to stay green and leafy under extreme drought and temperature regimes (Wilson, 1966).
Atriplex nummularia is well adapted to a wide range of climatic and soil conditions (De Kock, 1980; Verschoor, 1992). The type of soil in which the plant grows also influences the salt concentration in the shrub. Plants established on very alkaline soils are generally less palatable to animals than plants established on more favorable soils (Steynberg & De Kock, 1987).

Jones & Hodgkinson (1970) reported that *Atriplex* species were of considerable value to livestock in areas subject to drought; The following reasons were enumerated:

- the capacity for production during feed shortage is high;
- the water requirement is low, indicating a high efficiency of production in terms of rainfall;
- the root system is deep penetrating and capable of using moisture which has reached the subsoil;
- the protein and phosphoric acid contents are high; and
- several species produce considerably higher yields than lucerne under field conditions.

Four criteria are important for a forage species: i) grazing and recovery; ii) yield; iii) chemical composition; and iv) nutritive value. If saltbushes are to be considered as a forage source, they must be evaluated with these factors in mind (Atiq-ur-Rehman, 1995).

2.3.1 Grazing and Recovery

This shrub can be grazed any time of the year. New plantings of *A. nummularia* should be grazed from the second season so that the plants develop a bushy growth habit. Plants, which are not grazed, are inclined to develop beyond the reach of sheep and edible material can not be reached by stock (De Kock, 1980).
The grazing capacity of *A. nummularia*, as with all other forage species, is dependent on moisture. Barnard *et al.* (1992) researched the grazing capacity of three different shrub species, *A. nummularia, A. canescens* (four-winged salt bush) and *Chrysanthemoides monilifera* subsp. *pisifera* (West Coast Bitou). Their results showed that over a seven-year period, the mean grazing capacities were 1.6, 1.0 and 1.7 ssu/ha, respectively. These grazing capacities were evaluated in the Strandveld of the Cape West Coast, South Africa, where the capacity of the natural veld was 5 ha/ssu.

The recovery and survival of saltbush after grazing is affected by the extent and frequency of grazing. Some saltbush species can withstand severe grazing, for example *A.*

nummularia, and recover from complete leaf loss by producing leaves along the main stems. This species can also recover from being completely cut off just above ground level (Leigh and Wilson, 1969). However, other *Atriplex* species are susceptible to heavy grazing (Leigh & Wilson, 1969; Clark, 1983; Vallance, 1989). It is unlikely that any shrub can tolerate extended periods of extreme grazing. On the other hand, moderate grazing may improve the vigour of individual plants by removing terminal buds, which encourages the development of lateral shoots so that a more compact and leafy bush can develop (Grice & Muir, 1988). It is safer to base stocking rates on the condition of the vegetation rather than on the condition of the animals, as is often the tradition.

2.3.2 Yield

An important characteristic of *Atriplex* species is their high production of green, succulent feed under relatively poor moisture conditions. Dry matter yields of *A. nummelaria* Lindl. and *A. lentiformis* (Torr.) S. Wats. were found to be comparable to that of lucerne by Glenn & O'Leary (1985). Watson *et al.* (1986) reported that forage yields and quality vary with species, harvest treatments and phenological stage at the time of harvest. Nutritive value is linked directly with leafiness and thus forage quality is associated with changes in the stem and leaf components of harvested material.

Plant density can also have an influence on the amount of edible biomass produced by the plants. Higher densities have a higher production of leaf biomass per unit area, a lower branch diameter and an increased proportion of edible biomass in the standing biomass, relative to lower densities (Benjamin *et al.,* 1995).

Table 2.2 illustrates the variability in biomass yield per hectare of *A. nummularia*, during six years of growth, and emphasises that biomass yield can vary widely between years.

Table 2.2 Leaf biomass yield from *A. nummularia* during six years of growth at Grootfontein, Middelburg, with an average annual rainfall of 350 mm (adapted from De Kock, 1980)

Year	Dry mass (ton/ha)	Green mass (ton/ha)
1	2.05	7.78
2	2.59	10.37
3	4.75	18.14
4	4.00	12.53
5	1.94	5.40
6	2.16	8.00
Mean	2.19	10.37

In a study conducted in Australia by Goodin (1979), as cited by Verschoor (1992), no significant differences in production were found between plants irrigated with brackish and fresh water. This highlights the fact that the quality of water available to *Atriplex* spp. does not have a great influence on the production of these shrubs.

The production of *A. nummularia* in the Eastern Cape, South Africa, was determined by Du Toit (1991), as cited by Verschoor (1992). An annual dry matter production of between three and four ton/ha was achieved. Strydom (1991), as cited by Verschoor (1992), reported a dry matter yield of seven ton/ha in the Senekal district, Free State, South Africa.

2.3.3 Chemical composition

An important characteristic of *Atriplex* species is that it has a high nitrogen (N) and crude protein (CP) content. The CP content of saltbush leaf matter is either comparable to or higher than other annual and perennial grasses (Atiq-ur-Rehman, 1995). It is very likely

that there is variation in chemical composition between saltbush species, and within species, as a result of change in season and the soil in which the shrubs are growing (Grice and Muir, 1988).

Table 2.3 Apparent crude protein (N × 6.25) concentration in different species (% dry matter) (adapted from Atiq-ur-Rehman, 1995)

Species	**Crude protein (%)**	**Reference (cited by Atiq-ur-Rehman, 1995)**
A. vesicaria	15	(Leigh and Mulham, 1967)
A. nummularia	17-18	(Wilson, 1966)
A. ungulata	17.5	(Wilson, 1966)
Kochia (Bluebush)	14	(Wilson, 1966)
Danthonia caespitosa (Perennial grass)	8	(Leigh and Mulham, 1967)
Parapholis incurva (Annual grass)	6	(Leigh and Mulham, 1967)
Medicago polymorpha (Annual herb)	18	(Leigh and Mulham, 1967)

The high ash concentration of saltbush (20 – 38%), which is principally sodium chloride (NaCl), may be a nutritional disadvantage to animals (Wilson, 1966). The high concentration of salt in *Atriplex* diets increases the demand for fresh water for livestock. Limited water supply can lead to a decrease in feed intake and result in a loss in live weight.

The amount of salt consumed by sheep on an *Atriplex* diet can vary with the season in which it is harvested and with the change in the ratio of leaf to stem. The leaves of *Atriplex* species contain higher amounts of Na and Cl than stem, probably because of the presence of bladder cells on the leaf surface (Wilson, 1966).

Leaves of saltbush plants may also contain high levels of oxalate. Davis (1981) reported oxalate levels of nine % and six % in *A. nummularia* and *A. vesicaria* species respectively. Davis (1981) also reported that young seedlings of saltbush contain oxalates in toxic amounts and that the concentration of oxalates decreases with the age of the plants.

Van Niekerk *et al*. (2004b) reported oxalic acid concentrations for *A. nummularia* of 3.26% and 3.51%. These concentrations are lower than that recorded by Wilson (1966) of 5.8%. It could be that Wilson (1966) used younger plants. There were no cases of oxalate poisoning in sheep reported by Wilson (1966). This may be because sheep prefer grazing mature plants rather than young seedlings and the diets of sheep are unlikely to ever be 100% *Atriplex* under grazing conditions. Ruminants can, under normal conditions, consume large amounts of oxalate containing plant material without apparent ill effects, unless there is mismanagement of drinking water (Leigh, 1986).

Atriplex species generally have low tannin concentrations. Davis (1981) reported 6 mg/g tannin on an air dry basis in *A. nummularia*. This level of tannin is below the reported level of 20 mg/g dry matter that will result in rejection of feed by grazing animals (Donnelly & Anthony, 1969 as cited by Atiq-ur-Rehman, 1995).

The NRC (1988) considered a phosphorus concentration of 0.16 – 0.37% to be satisfactory for production in sheep. Grice & Muir (1988) reported that older plants of *A. lentiformis* and *A. nummularia* have low levels of phosphorus. These authors suggested that supplements of phosphorus would be necessary for animals relying on these plants as their sole source of feed.

Davis (1972) reported that *Atriplex* species could accumulate selenium if grown on seleniferous soil. Of the 15 species tested, Davis (1972) found that *A. canescens* accumulated 204 mg/kg when grown on soils with 18 mg/kg selenium, while *A. lentiformis* grown in the same soil had only 7 mg/kg. Other species varied between these extremes.

2.3.4 Nutritive value

The nutritive value of a forage species is defined as the concentration of nutrients contained in that forage species (Ulyatt, 1973). The nutritive value of *Atriplex* varies with species, material sampled and season of growth. The co-efficients of dry matter digestibility, neutral detergent fibre (NDF) and nitrogen, determined by pen-feeding trials, for some of the *Atriplex* species commonly grown in Western Australia are presented in Table 2.4.

Table 2.4 The co-efficients of digestibility of saltbush species determined in pen feeding trials (Adapted from Atiq-ur-Rehman, 1995)

Species	**Co-efficient of digestibility**			
	DM	**NDF**	**N**	
A. undulata	0.53	0.26	0.75	(Warren *et al*., 1990)
A. lentiformis	0.62	0.41	0.81	(Warren *et al*., 1990)
A. amnicola	0.57	0.41	0.70	(Warren *et al*., 1990)
A. cinerea	0.60	0.45	0.70	(Warren *et al*., 1990)
A. undulata	0.33	-	0.56	(Pol, 1980)
A. rhagodioides	0.33	-	0.51	(Pol, 1980)

The low digestibilities reported by Pol (1980), are probably due to the high proportion of stem in the plant material evaluated. Pol (1980) used saltbush that was cut and stems up to 10 mm in diameter were included, whereas in the work of Warren *et al*. (1990), leaf and stem material of less than six mm diameter were included.

As indicated in Table 2.2 and 2.3, the crude protein and digestible crude protein concentrations in *Atriplex* species were high (Leigh, 1986). However, Weston *et al*. (1970) reported that the high crude protein values can be misleading as the crude protein in *A. nummularia* was extensively degraded to ammonia in the rumen of sheep. There was also a high loss of nitrogen in the urine which may have been due to inefficient

utilization of ammonia by the rumen micro-organisms. Thus, the true protein value of an *A. nummularia* diet may be lower than that indicated by the apparently digestible crude protein value.

Weston *et al*. (1970) reported that *Atriplex* diets differed from other herbage diets in two aspects of digestion in sheep. First, the proportion of total organic matter digestion in the stomach was 33% with *Atriplex*, as compared to the range of 55% to 67% found with other herbage diets. Second, with *Atriplex* species only 43% of total digestion of cell wall constituents took place in the stomach compared to 75% to 100% with other diets. These authors also found that the protein of the saltbush was extensively degraded to ammonia in the rumen and the ruminal absorption of fatty acids was impaired as calculated as the difference between the production rate and the rate of transfer of volatile fatty acids (VFA) to the omasum in the digesta. The explanation for the impaired absorption of VFA is not clear. These authors found that there was ample time for absorption of the acids. Ruminal pH was 0.3 – 0.9 units higher with the *Atriplex* diet that with other herbage diets. This would tend to reduce the absorption rate of volatile fatty acids. However, it was suggested that this alone was unlikely to account for more than a small proportion of the discrepancy.

In a pen feeding trial, Hassan & Abdel-Aziz (1979) evaluated the nutritional values of Rhodes grass, Napier grass and saltbush (*A. nummularia*) for sheep. These authors reported that the digestible energy supplied was a limiting factor to sheep production. Colomer & Passera (1990) stated that the lignin values of *Atriplex* spp. were higher than those of grasses. As a result, shrubs such as *Atriplex,* are often inferior to grasses with respect to energy values because of the higher cellulose to lignin ratio in their cell walls (Colomer & Passera, 1990).

The crude protein levels of *Atriplex* species are generally high, but high ash- and NDF (neutral detergent fibre) levels, as well as the presence of mineral imbalances, limit the use of this shrub as sole fodder source (Colomer & Passera, 1990).

Warren *et al*. (1990) reported that *A. undulata,* fed alone, was consumed at a lower level than other saltbush species when used in a pen feeding trial for 21 days. However, these authors found that the intake of an *A. undulata* diet mixed with 50% oaten hay was

almost twice as much as for other diets including oaten hay alone. Sheep were able to maintain weight on the hay/ *A. undulata* diet, while sheep on the other diets of *Atriplex* alone lost weight.

The recovery of *Atriplex* species from grazing, in addition to the yield, chemical composition and nutritive value, suggest that some *Atriplex* species may have a definite value as a forage, and could play an important role in sheep nutrition during dry seasons. There are, however, problems associated with the feeding of *Atriplex,* as a sole feed, as described in this section. These problems can, however, be overcome for example by supplementing *Atriplex* with dry pasture and stubble, or by providing the animals with salt free water.

2.3.5 Palatability in saltbush species

Palatability, the qualitative measure of taste and/or odour (Colebrook *et al*. 1985 as cited by Atiq-ur-Rehman, 1995), is one of the important factors that influence the selection of feed by animals under grazing conditions. Kenney & Black (1984) quantified and simplified the Arnold & Dudzinski (1978) approach. These authors argued that the selection of any particular feed by sheep is dependent upon three main factors: i) the potential intake rate (PIR) which is dependent largely on the physical characteristics of the feed, such as the feed's ease of fracture, size of particles and water content; ii) accessibility, which depends upon height, density and position in the sward relative to other components; and iii) acceptability, which is known to be a function of taste, odour and surface characteristics of the component, and which can be modified by experience and degree of satiation of the animal.

The palatability of *Atriplex* varies from species to species, and within a species depending upon the maturity of plant and the area where it is grown and even between individual plants in any population. Graetz (1978) conducted an experiment on the pattern of influence of sheep grazing *A. vesicaria* and reported a marked difference in grazing within three sexual classes, males, females and steriles. Graetz (1978) found that there

was preferential grazing pressure on female bushes as compared to the male bushes. This author also observed that this differential selection against male bushes was most likely because the flowers are borne terminally in the male plant as compared to the female plant with axillary flowers and that the bladders on saltbush fruits have a much higher salt content than does the foliage. Maywald *et al*. (1998) found that herbivores preferred to graze male saltbush shrubs during late spring, and that no sex based preference was apparent in the winter. These authors suggested that differences in physiological vigour and/or chemistry could have influenced relative palatability of the sexes over time.

Jacobs & Smit (1977) reported that acceptability differed only within *A. nummularia* and that this difference was not found within *A. canescens*, *A. brewerii* and *A. lentiformis*. Out of 506 plants of *A. nummularia* species, the first 20 plants in which the leaves were completely stripped off by Merino ewes, were regarded as the most palatable plants, and the last 20 plants which were not grazed or stripped off completely, were called unacceptable plants. These authors argued that low potassium, high phosphorous content, leaf compactness, leaf thickness, and the degree of creasing of the leaf were the mean contributors in the acceptability of *A. nummularia* plants. Data of McKell (1989) corresponds with that of Jacobs & Smit (1977). These authors found that high chloride and low crude fibre content reduce the acceptability of plants. These authors concluded that the season and/or age of the plants influence the acceptability of *A. nummularia*.

The fact that animals select one plant species and even an individual plant within a species during grazing, suggests that inherent differences exist between and within species. Understanding the reasons for animal's preference for some saltbush species and for individual plants within a species, is therefore important in the breeding and selecting of the preferred species.

2.4 Feeding value of saltbush

Ulyatt (1973) stated that the feeding value of forage is determined by factors including chemical composition, voluntary feed intake, digestibility and the efficiency of utilization of digested nutrients for maintenance and production. The feeding value of *Atriplex* species can vary over a wide range and it is generally characterized as a poor quality feed. The most important factors that contribute towards the wide variability in feeding value of saltbush are probably voluntary feed intake and digestibility.

2.4.1 Voluntary feed intake of *Atriplex* species

Animal production can be influenced by increasing intake or by making digestion and metabolism more efficient. The voluntary intake of a feed is defined as the amount of feed eaten during a period of time when it is offered *ad libitum*. The mechanism of intake control is a very complex phenomenon. This phenomenon has been extensively reviewed in the literature by Campling (1970); Baumgardt (1970); Ulyatt (1973); Freer (1981) and Grovum (1984). In general, it is considered that intake is controlled by centres in the hypothalamus. The original concept of the feed centre (lateral hypothalamus) and the satiety centre (ventromedial hypothalamus) has been modified (Weston, 1966). It has been suggested that probably peptides were involved in the interface between the energy balance regulator and controller of feed intake (Ribeiro, 1989). Orskov & Ryle (1990) suggested that some of the following factors could initiate hunger or inhibit eating.

Initiation of hunger	Inhibitation of eating
Metabolic demand unsatisfied	Metabolic demand satisfied
Palatable food	Unpalatable food
Social stimuli	Social stimuli
Endocrine stimuli	Gut distention Disturbance, fear, pain
Pharmacological stimuli	Nausea
	High temperatures

Grovum (1984) stated that palatability is of lesser importance as a determinate of intake when life is threatened by starvation. Beyond palatability effects, daily intake of food can be increased or decreased by long-term controls. In spite of the obvious importance to production, very little is known about how these long term intake effects function, except that the effects of season (low intakes in winter, high intakes in summer) may reflect photoperiod and hence be mediated, at least in part, by the pineal gland. Daily intake regardless of whether it is set at a high or low level by the long-term controls is normally consumed as a number of spontaneous discrete meals. Factors, which begin and end each meal, are referred to as short-term controls over intake (Grovum, 1984). These controls must operate by altering the activity of the hunger and satiety centers in the brain and are shown in Figure 2.1.

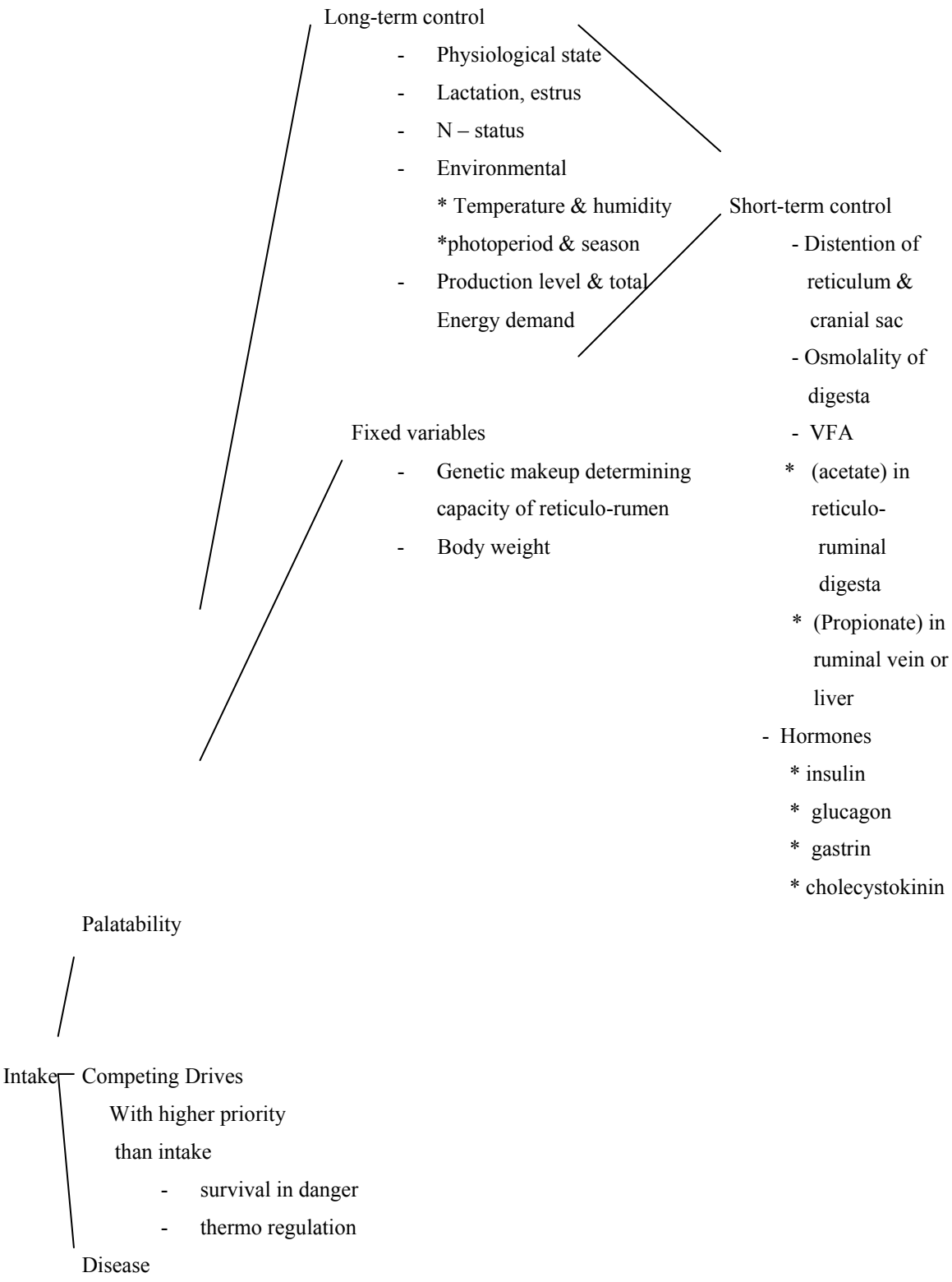

Figure 2.1. Determinants of intake in ruminants (adapted from Grovum, 1984)

Atiq-ur-Rehman (1995) stated that it is assumed that as with other forages, the level of intake for *Atriplex* in sheep is governed by: i) the amount of material in the reticulo-rumen; ii) it's rate of digestion; and iii) the rate of passage of digesta out of the reticulo-rumen. Therefore, the mechanical processes associated with digestion, like particle size reduction during eating and rumination, and rate of microbial fermentation, can be important factors determining the amount of *Atriplex* consumed by sheep.

When mature perennial plants such as *Atriplex* are grazed, the grazing behaviour of animals can change from grazing the whole plant when it is immature to where the proportions of different fractions of plant in the diet will depend upon the availability of feed and the composition of each plant part. Differences in the proportions of the main morphological parts, particularly leaf and stem fractions, can influence the voluntary feed intake (Casson *et al*., 1996).

Poppi *et al*. (1981) reported that voluntary intake of leaf fractions is always higher than that of stem fractions, and it was suggested that the higher intake of leaf is associated with a short retention time in the rumen rather than with differences in the digestibility (Hendricksen *et al*., 1981). Particle size is one of the major factors that influence the passage through the rumen. It has been suggested that particulate matter must be reduced to 1.0 or 1.18 mm before it can pass through the reticulo-ruminal orifice (Poppi *et al*., 1981).

Intake of animals can also vary with environmental conditions, age, body size, sex and physiological state of animals. In addition, voluntary intake varies widely between animals. Therefore, intake comparisons of poor quality feeds such as *Atriplex*, under field conditions might be inconclusive because of plant, animal and environmental variables. The comparisons of intake among different species of *Atriplex* can, therefore, be of value only when they are conducted under standard conditions, where some of the variables can be either eliminated or cancelled out. Therefore, the indoor pen feeding trial has become the best option to determine and compare intake of different shrubs.

2.4.1.1 Pen feeding trials

Few pen feeding trials have been conducted to determine the feeding value of saltbush and the data available for the intake of *Atriplex* species are quite variable. It is difficult to compare results from different studies because of conditions and in particular, different morphological fractions of the diets were either not described or differed markedly. In Table 2.5, data from different pen feeding trials are summarized for voluntary intake, crude fibre/ lignin, digestibility and performance of sheep in terms of live weight change.

Table 2.5 Summary of some main pen feeding trials conducted to determine the nutritive value of *Atriplex* species (Adapted from Atiq-ur-Rehman, 1995)

Species	DMI/kg$^{0.75}$ (g/kg)	Duration (days)	Description of diet	*In vivo* DMD (%)	Crude fibre (%)	Live weight change (g/day)	Country	Reference
A. nummularia	34	21	Leaves + little stems	68	-	+57	Australia	Wilson (1966)
A. nummularia	33	21	Leaves + little stem	74	-	+57	Australia	Wilson (1966)
A. nummularia	117	238	Leaves + stem (24h sun cured)	-	-	+9	Libya	Le Houerou (1991)
A. nummularia	41	21	Leaves + succulent stem	62	32	-	Egypt	Hassan *et al.*, (1979)
A. nummularia	39	49	Leaves + succulent stem	72	27	-80	Egypt	Hassan & Adbel-Aziz (1979)
A. nummularia	55	50	Edible biomass summer	64 (*in vitro*)	10	-	Spain	Correal *et al.*, (1990)
			Edible biomass spring	72 (*in vitro*)	13	-		
A. vesicaria	49	21	Leaves + 30% stem	54	-	+43	Australia	Wilson (1966)
A. vesicaria	46	21	Leaves + 30% stem	52	-	-57	Australia	Wilson (1966)
A. vesicaria	-	-	Leaves	66 (*in vitro*)	11	-	Australia	Malcolm *et al.*, (1988)
	-	-	Twigs	39 (*in vitro*)	44	-		
A.ungulata	26	21	Whole top of plant	58	-	-14	Australia	Wilson (1966)
A. undulata	33	21	Leave + stem (5-6mm)	53	13 lignin	-183	Australia	Warren *et al.*, (1990)
A. undulata	37	20	Leaves + stem (up to 10mm)	33	16lignin	-	Australia	Pol (1980)
A. lentiformes	34	21	Leaves + stem (5-6mm)	62	8 lignin	-211	Australia	Warren *et al.*, (1990)
A. amnicola	52	21	Leaf + stem (5-6mm)	57	10 lignin	-251	Australia	Warren *et al*., (1990)
A. amnicola	-	-	Leaves	72 (*in vitro*)	13	-	Australia	Malcolm *et al* (1988)
	-	-	Twigs	38 (*in vitro*)	43	-		
A. canescens	143	196	Leaf + stem (24h sun cured)	-	-	0	Libya	Le Houerou (1991)
A. rhagodiodes	59	120	Leaf + stem (up to 10 mm)	33	14 lignin	-	Australia	Pol (1980)

The reasons for the wide range of variability in intake may be explained by both plant and animal factors. The plant factors may be: i) the preference/palatability differences among *Atriplex* species; ii) morphological differences in *Atriplex* diets fed to animals, especially the percentage of stem and diameter of stem in the diets; and iii) time of year the *Atriplex* was harvested.
Animal factors, such as age, body condition, genotype, nutrition prior to the experiment and health might also have contributed to the variation in intake and digestibility of diets in these studies.

2.4.1.2 Field grazing trials

Ulyatt (1973) suggested that feed intake accounts for at least 50% of the variation observed in the feeding value of forages. Graetz (1978) conducted a grazing trial and reported that the diet selected by sheep varied greatly throughout the measurement period. The natural *Atriplex* and bluebush rangeland pasture used in the investigation conducted by Graetz (1978), contained different classes of plants including perennial shrubs, sub-shrubs, green grasses, dry grasses and green annual forbs. Graetz (1978) reported that not one class of plant was dominant in all the samples that was taken, yet *A. vesicaria*, green grasses, dry grasses and green annual forbs comprised almost the complete diet on some occasions. This author also found that because of the foraging behaviour of sheep, diet quality varied much less than the quality of feed on offer. This study highlights the importance of animal's selectivity and the relative proportions of different plant fractions in the diet influenced by plant, animal and environment interactions.

Some of the studies conducted to determine the nutritive value, defined as an animal response per unit intake of *Atriplex*, are summarized in Table 2.6. The table highlights that heterogeneity of *Atriplex* plantations and that is probably one of the major limitations in the determination of nutritive value.

Table 2.6 Summary of field grazing trials conducted to determine the nutritive value of saltbush (adapted from Atiq-ur-Rehman, 1995)

Species	Rainfall (mm)	No. of sheep	Stocking rate (sheep/ha)	No. of bushes/ ha	Biomass (t/ha)		Duration	Animal performance	Reference (cited by Atiq-ur-Rehman, 1995)
					Saltbush	understorey			
Mixed	400	40	5.7	733	0.98*	0.42*	7 years	Dry ewes maintained live weight, pregnant ewes covered their reproductive req. without supplement	Otal *et al.,* 1991
A. vesicaria community	303	6	1/1.7 ha ½.5 ha	-	0.25-.075	0.03-0.65	4 years	No difference between stocking rates. Animals gain weight & growth differs from year to year.	Wilson, 1966
A. nummularia	-	4	6.0	506	**	--	25 days	Maintained live weight	Jacobs & Smit, 1977
A. canescens	-	4	1.7						
A. brewerii	-	4	2.2						
A. lentiformis		4	2.2						
A. nummularia	290	35	--	-	9.9	5.8	10 weeks	Maintained live weight	Benjamin *et al.,* 1992
A. canescens									
Cassia sturtii									
A. vesicaria & cotton bush community	337	36	2.5 1.2 0.6	-	1.3 1.3 1.3	0.7 0.7 0.6	3 years	At 0.6 wether/ha animals maintained productivity	Wilson, 1966

- * Dry matter basis
- ** 3 years old fully grown

The heterogeneity of *Atriplex* plantations results from the presence of understorey, different sizes of shrubs, spatial distribution of edible material on shrubs and variability in chemical composition of edible material within and between species.

2.4.2 Water requirements of animals utilizing *Atriplex* species

Animals grazing halophyte pastures, such as *Atriplex,* ingest large quantities of sodium and potassium chloride as compared to animals grazing non-halophyte pastures. Beal & Budtz-Olzen (1968) as cited by Atiq-ur-Rehman (1995) assessed the importance of urinary and feacal routes of excretion in maintaining stable sodium and potassium levels in sheep. These authors found that 88% of sodium and 89% of potassium was excreted through urine and there was a strong correlation ($r^2 = 0.92$) between intake and excretion of these elements. Large quantities of salts ingested will be excreted in the urine and this will increase the water requirements of animals consuming saltbush. In Table 2.7 the water consumption of sheep on saltbush diets in pen feeding trials is presented.

Table 2.7 Effect of total ash and sodium in various diets on water consumption by sheep

	Daily intake			
	Ash (g)	Sodium (g)	Water (L)	
A. nummularia	130	51	6	Hassan & Abdel-Aziz (1979)
A. undulata	99	28	6	Warren *et al*. (1990)
A. lentiformis	100	28	5	Warren *et al*. (1990)
A. amnicola	193	46	8	Warren *et al*. (1990)
A. barclayana	236	-	10	Benjamin *et al*. (1992)
Oaten hay	29	0.3	2	Warren *et al*. (1990)

The data in Table 2.7 indicate a large variation in the sodium content of different saltbush species. The high level of sodium in leaves is important for the plants to withstand

drought and soil salinity. Sharma *et al*. (1972) as cited by Atiq-ur-Rehman (1995) found that both sodium and chloride concentrations in leaves of *A. nummularia* and *A. vesicaria* increased progressively with a corresponding decrease in soil water content. This indicates that the season (rainy season or dry season) in which the saltbush is grazed or harvested may influence the water requirements of animals eating that saltbush.

According to Atiq-ur-Rehman (1995), in addition of the high water requirements of sheep, water quality is another important consideration for animals grazing saltbush. The tolerance of sheep to saline drinking water is less when grazing saltbush. Peirce (1966) as cited by Atiq-ur-Rehman (1995), found that a concentration of 1.0% NaCl in the drinking water had no adverse effects on sheep, but 2.0% NaCl was detrimental to all the sheep in the trial. The feed intake of animals receiving 2.0% NaCl declined and several of the animals became very emaciated and weak.

It can be concluded that the availability and quality of water is clearly an important factor in determining the performance of sheep on saltbush pastures.

2.5 Energy supplementation

2.5.1 Introduction

The reasons for producers to consider supplementation of ruminants on grazing or forage fed based diets include: i) correction of a nutrient deficiency in the forage; ii) increasing the carrying capacity of the pasture or stretching forage supplies; iii) providing a carrier for growth promoting additives; iv) aiding in the prevention or treatment of potential health problems; and v) enhancement of animal management (Fahey, 1994).

Energy supplementation, to meet animal requirements and production demands, is often practiced during periods of summer dormancy and in winter months. Sources of supplemental energy vary widely and include grains, readily digestible fibre sources, and high quality forages. Intake and digestibility can be reduced or unaffected by energy supplementation. The effect of energy supplementation on forage intake varies depending on the quantity and quality of the supplements that are fed. A forage intake reduction appears to be primarily related to the form and source of supplemental energy (whole vs. processed; starch vs. rapid digestible fibre) (Mould *et al*., 1983).

Lower levels of energy supplementation have been shown to increase the utilization of grazed forage. Reductions in ruminal pH, often cited as the major cause of reduced fibre digestion, may not always explain reductions in intake and digestibility associated with energy supplementation (Chase & Hibberd 1987; Caton & Dhuyvetter, 1997).

As the concentrate percentage in a diet increases, the efficiency of energy use for both gain and maintenance increases (NRC, 1988). This could compensate for the reduction in forage intake and marginal changes in total digestible OM intake that often occur when supplementing a diet with energy.

When forage is supplemented with grain-based concentrates, the intake and digestion usually follow the pattern shown in Fig. 2.2.

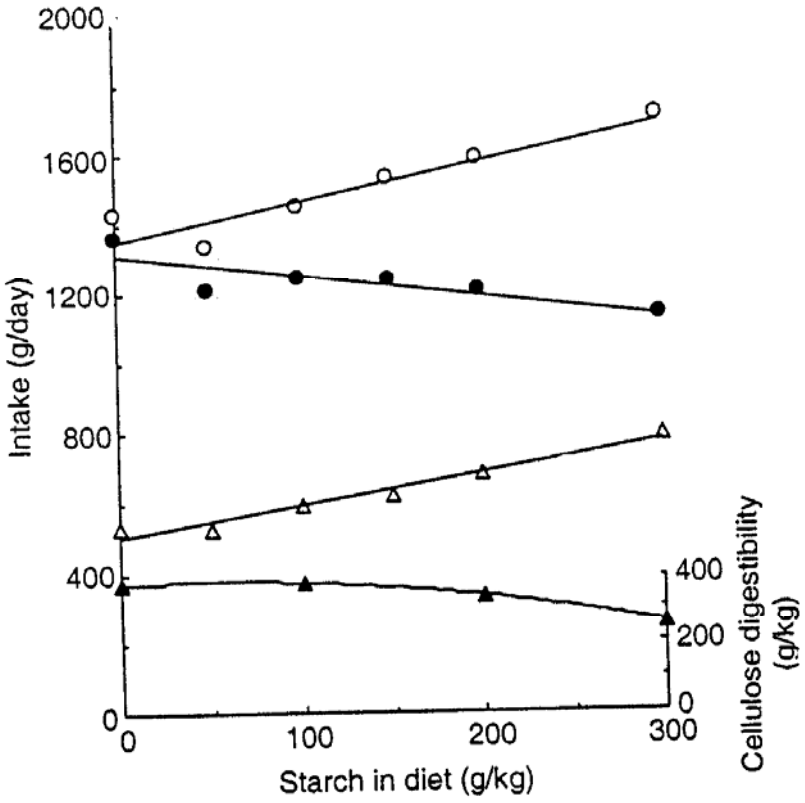

Fig 2.2. Voluntary intake of total dry matter (DM) (o), straw (•) and digestible organic matter (Δ) and cellulose digestibility (▲) by sheep given ground oat straw and various levels of starch (Mulholand *et al.*1976 as cited by Dixon & Stockdale, 1999).

Intake of total dry matter (DM) and of ME increased with consecutive levels of starch supplement, but intake of the straw and digestion of the fibrous components of the straw decreased (Dixon & Stockdale, 1999).

2.5.2 Characteristics of carbohydrates

Carbohydrates are the most important source of energy for rumen microbes, and their behaviour in the rumen varies greatly among sources. These differences are not limited to

structural carbohydrates versus non-structural carbohydrates, but to origin and ruminal behaviour patterns. Unique behavioural patterns result in different proportions of starch escaping undegraded from the rumen (Owens *et al.*, 1986; Rooney & Pfugfelder, 1986), resulting in a variable supply of starch for enzymatic digestion in the small intestine.

Carbohydrates may be divided, according to their chemical nature, into two major groups, sugars and non-sugars. The simplest sugars are the monosaccharides, which are divided into sub-groups: trioses ($C_3H_6O_3$), tetroses ($C_4H_8O_4$), pentoses ($C_5H_{10}O_5$), hexoses ($C_6H_{12}O_6$) and heptoses ($C_7H_{14}O_7$), depending on the number of carbon atoms present in the molecule. Monosaccharides may be linked together, with the elimination of one molecule of water at each linkage, to produce di-, tri- or tetra-polysaccharides containing respectively two, three, four or larger numbers of monosaccharides. Polysaccarides, also called glycans, are polymers of monosaccharide units. They are classified into two groups, the homoglycans, that contain only a single type of monosaccharide unit, and the heteroglycans, which on hydrolysis yield mixtures of monosaccharides and derived products (McDonald *et al*., 2002).

Polysaccharides in animal feeds are a complex group of components differing widely in physical properties and physiological activity. Polysaccharides may be classified as structural polysaccharides or storage polysaccharides. Structural polysaccharides are complex carbohydrates, which can be divided into pectins, cellulose and hemicellulose. Storage polysaccharides include starch, fructans and cell wall storage polysaccharides (Åman & Graham, 1990; De Visser, 1990).

The chemical structure of polysaccharide polymers or complexes can affect both the rate and extent of digestion and can be used to control and optimize nutrient assimilation in different parts of the gastro-intestinal tract (Åman & Graham, 1990). Monomers are very often combined to form disaccharides, such as maltose, lactose and saccharose, which are important feed carbohydrates (oligosaccharides).
The rate of digestion of carbohydrates is the major factor controlling the energy available for microbial growth; in addition, rate of digestion of total carbohydrates is directly related to the proportion of starches, pectins, and sugars. Proteins affect both total

fermentation and production of microbial DM per unit of carbohydrate fermented (Hoover & Stokes, 1991).

In ruminants, structural-polysaccharides and rumen degradable starches are fermented in the rumen and, through the action of the microflora, becomes available to the host animal as volatile fatty acids (VFA). The amount of volatile fatty acids produced depends on the substrate availability of both the structural polysaccharides and rumen degradable starch. Starch resistant to rumen degradation becomes, if digested, available to the host animal as glucose after hydrolysis and absorption in the small intestine (Nocek & Tamminga, 1991).

The microbial population involved in the digestion of various carbohydrates changes due to concentrations of and attachment to the substrates. The availability of carbohydrates are affected by the ease of solubilization, whereby sugars, starches and pectins would be expected to undergo the most rapid fermentation. The chemical structure of polysaccharides also affects the rate of degradation. For the less soluble polysaccharides, extent of lignification, acetylation, and phenolic esterfication is negatively related to digestion (Hoover & Stokes, 1991).

2.5.3 Degradation of carbohydrates

Carbohydrate and starch degradation may be influenced by several factors such as its origin, the amylose/ amylopectin ratio, the size of starch granules, amylose-lipid complexes, and type of crystallinity (Behall *et al*., 1988). According to Moran (1982), the degradation rate of starch is a function of surface area of the granules, nature of the starch and extent of crystallinity.

The rate of starch degradation influences the fermentation pattern (Murphy *et al.,* 1982), which in turn determines the amount of energy extracted by the micro-organisms in the rumen and this affects the availability of different nutrients for rumen micro-organisms

(Nocek & Russel, 1982). This could be important in the utilization of non-protein nitrogen and rumen degradable protein as a nitrogen source for microbial synthesis. On the other hand, large amounts of easily degradable starch may inhibit the degradation of cell wall material, either directly, as a competitive substrate, or indirectly, through causing a reduced pH (Hoover, 1986).

Cone *et al.* (1992) reported results that showed that differences in starch degradability between different starch samples are not necessarily caused by chemical properties but likely also by physical properties. In trials, conducted by Cone *et al.* (1992), it was found that differences in starch degradation were much more pronounced when α-amylase was used to degrade the starch than with rumen fluid. However, the order in degree of degradation in both cases was about the same. Cone *et al*. (1992) determined the degree of starch degradation using purified starch with 100 mg α-amylase after 4 h incubation at 39°C, and with rumen fluid from a concentrate fed cow after 6 h incubation at 39°C. The results reported by these authors for maize and barley were 20.2 % and 28.1 % for the amylase incubation and 19.7 % and 14.9 % for the rumen fluid incubation respectively.

Cone & Wolters (1990) suggested that starches containing high amylose content are degraded faster by α-amylase than starches having high amylopectin content. Although there are differences in chemical properties between amylose and amylopectin, the differences in physical properties are much more pronounced and may have much more influence on the degree of degradability (Cone *et al.,* 1992). The physical properties of starch, as measured with differential scanning calometry, have been shown to correlate in a linear way with degradability (Cone *et al.,* 1992).

Differences in enzymatic degradation of starch can be caused by several factors such as amylose and amylopectin content (Behall *et al.,* 1988), crystallinity, particle size and the presence of enzyme inhibitors (Cone, 1991). Starch granules contain components other than amylose and amylopectin and possibly need non-amylolytic enzymes for full degradation (Cone, 1991).

2.5.3.1 Protein: Energy ratio

Synchronization of energy and nitrogen supply to the rumen is one of the most promising approaches to improve the efficiency of ruminal fermentation (Krishnamoorthy *et al*., 1991).

Protein degradation in the rumen often exceeds carbohydrate availability, and protein wastage occurs (NRC, 1988). This is possibly what happens when *A. nummularia* is fed to animals. Conversely, protein degradation may be too slow to support optimal ruminal digestion of carbohydrates. Both these conditions depress microbial protein synthesis. Provision of available energy and protein at coordinated rates should allow microbes to obtain simultaneously ATP and NH_3 and/or amino acids, needed for cell synthesis. This will result in better utilization of nutrients in the rumen as well as increasing the supply of microbial protein to the small intestine (Oldman, 1984).

The sources of protein and energy have a significant effect on the utilization of N and energy in the rumen, the production of VFA, and the flow of nutrients to the small intestine (McCarthy *et al.,* 1989).

Nitrogen and energy sources must be available at the same time for optimal microbial protein synthesis in the rumen. Large differences occur between feedstuffs in the percentage of starch that is resistant to rumen degradation. Table 2.8 gives an example of starch degradations assuming a passage rate of 6%/h.

Table 2.8 Nylon bag incubations concerning starch degradability and resistance to rumen degradability in compound feedstuffs (De Visser *et al.*, 1990)

Feedstuff	Starch (g/kg DM)	S (%)	D (%)	Rate of degradation Kd (5) of D/h)	Effective rumen resistant starch (%)
Sorgum	652	32	67	3.6	41
Maize	676	27	73	4.0	42
Barley	561	62	38	24.2	7
Wheat	654	68	32	17.5	8

S = Soluble fraction, D = potentially degradable fraction.

Assuming rumen outflow rate of 6%/ h

The assumption in the above mentioned experiment of a single passage rate irrespective of feed type might be criticized, as the types of basal feed influence the passage rate. For example, feeding *A. nummularia* as the roughage source could give a faster passage rate, as intake of this roughage source is correlated to a higher water intake (De Visser *et al.*, 1990).

Atriplex nummularia can be viewed as an unbalanced roughage source, in which it has a relatively high nitrogen content and a low energy value. If the nitrogen components and rumen degradable carbohydrates are out of balance, the capacity of the rumen to digest slowly degradable NDF is reduced, due to a lack of energy (Van Straalen & Tamminga, 1991).

2.5.4 Associative effects

Associative effects occur when digestion of one feed is not independent of other feeds and are detected when the combination of two feeds shows a non-linear response (Hart, 1987). It can be defined as non-additive (synergistic or antagonistic) effects of two or more feedstuffs on diet utilization or animal performances.

Intake of metabolisable energy (ME) when forages and grains are fed together to ruminants may, due to digestive and metabolic interactions, be lower or higher than expected from feeding these components separately. These interactions, or associative effects, are due primarily to changes in intake and/or the digestibilities of the fibrous components of forage. Effects on voluntary feed intake (substitution effects) are usually much larger than on the digestibility of fibrous components, although the changes in forage intake may be a consequence of changes in the rate of digestion of the fibrous components (Dixon & Stockdale, 1999).

Positive associative effects, where grains increase voluntary intake and/or digestion of forage, are usually due to the provision of a limiting nutrient (e.g. nitrogen, phosphorus) in the grain, which is deficient in the forage. Negative associative effects, where grains decrease voluntary intake and/or digestion of forage, occur frequently and can cause low efficiency of utilization of grain (Dixon & Stockdale, 1999).

Bargo *et al*. (2002) defined substitution rate as the change in forage intake in kg dry matter per kg supplement fed. A negative substitution rate indicates that forage intake is reduced by supplement intake substituting for forage and a positive substitution rate indicates that forage intake is increased by supplementation. The rate of substitution of grain for forage is related to forage intake, forage digestibility, the proportion of grain in the diet, and the maturity of the animal. Substitution rates are usually high in ruminants consuming high intakes of forage of high digestibility, probably due to the metabolic mechanisms, which control voluntary intake and thus reduce forage intake. The rate of substitution is often low when animals are consuming forages of low to medium digestibility. The voluntary intake of such forages is most likely determined by the capacity of the rumen to accommodate and pass digesta through to the lower gastro-intestinal tract and the rate of forage fibre digestion in the rumen. Substitution is most likely to be determined by changes in these processes. Reduced rate of fibre digestion in the rumen is often due to low rumen pH and/or insufficiency of essential substrates for rumen microbes.

Negative associative effects can be alleviated by ensuring supply of essential microbial substrates, feeding management, and modification of grain to minimize their adverse effects on fibre digestion, while ensuring satisfactory digestion of the grain and efficient microbial protein production (Dixon & Stockdale, 1999).

Positive associative actions can be illustrated by the inclusion of some grain in combination with forage, causing a more aggressive growth of rumen micro-flora and a better fermentation of the carbohydrate portion of the forage and an increase in dry matter intake (Chandler, 1992).
Negative associative effects can be seen in the depression of fibre degradation when a source of readily fermentable carbohydrate is included in the diet. Joaning & Johnson (1979) and Teeter *et al*. (1980) identified associative effects when using maize as carbohydrate source and found that the negative associative effects were due more to a decrease in starch digestion than to a decrease in cellulolysis.

In the work of Mould *et al.* (1983) it was found that the depression in digestibility of hay, supplemented with barley, could largely be avoided if the rumen pH was maintained above the level inhibitory to cellulolysis (6.0 – 6.1). This may be achieved either by giving fibrous roughage in the long or chopped form in sufficient amounts to stimulate rumination and salivation, or by adding the concentrate supplement in such a way as to minimize the risk of reducing the rumen pH to 6.0 or below.

2.5.5 Effect of energy supplementation on ruminal digestion.

When readily fermentable carbohydrates are added to a forage diet, fibre digestion is depressed. Of the several theories advanced to explain the depressing effects of readily fermentable carbohydrates on fibre digestion (El-Shazly *et al.,* 1961; Mertens & Loften, 1980), the following have received the most attention: a preference by rumen microbes for readily fermentable carbohydrates rather than fibre components; a decrease in ruminal pH caused by rapid readily fermentable carbohydrate fermentation with a resulting

depression in fibre degradation; and competition for essential nutrients resulting preferential proliferation of readily fermentable carbohydrate digesting microbes.

The choice of energy supplementation fed to steers consuming medium quality grass hay can alter forage digestion and rate of forage crude protein degradation (Carey *et al*., 1993). Forage intake was decreased by all sources of energy (barley and maize) been used, which indicates that reductions in forage intake in conjunction with energy supplementation should be considered when formulating diets.

Rapid fermentation characteristics of starch supplements often exceed the ability of ruminants to maintain a stable rumen pH (Orskov & Frazer, 1975). As pH decreases, cellulolytic bacterial function is often impaired and fibre digestion decreased. Hoover, (1986) stated that a moderate depression in rumen pH, to approximately 6.0, results in a small decrease in fibre digestion, but numbers of fibrolytic organisms are usually not affected. Further decreases to 5.5, or 5.0, result in depressed growth rates and decreased fibrolytic microbes, and fibre digestion may be completely inhibited.
It appears that reductions in ruminal pH to the 5.8 to 6.2 range that are cyclic and of short duration, cause a moderate transient depression in fibre digestion. This depression may be alleviated by controlling pH through buffering or feeding strategy, resulting in only marginal reductions in ruminal organic matter digestion associated with the presence of readily fermentable carbohydrates. Further pH reduction for longer periods will cause a wash out of rumen microbial species associated with fibre digestion, severe reduction in fibre and organic matter digestion, and decreases in microbial dry matter production (Hoover, 1986).

Several studies reported that grain supplementation reduced total tract DM and OM digestibilities (Zorrilla-Rios *et al.,* 1989). Others have noted increased, or no, effect on total tract digestibility in response to grain supplementation (Freeman *et al.,* 1992; Matejovsky & Sanson, 1995). Feeding a mixture of rapidly digesting starch grains (high moisture corn, wheat, barley) and slowly digesting starch grains (grain sorghum and dry corn) may reduce the incidence of acidosis and change the site of starch digestion in the

gastrointestinal tract (Axe *et al*., 1987; Britton & Stock., 1986 as cited by Mendoza *et al.,* 1999).

Digestibility responsiveness to energy supplementation may depend on protein level. In situations in which CP is limiting, energy supplementation alone could theoretically aggravate the CP deficiency and result in reduced intake, digestibility and performance (Sanson *et al.,* 1990).

Working with sheep, De Kock, (1980) reported that low levels of maize supplementation (7.8% of DM intake) actually increased forage intake of sheep utilizing *A. nummularia*. However, with higher levels of maize supplementation (greater than 23% of DM intake) forage intake was reduced compared with that of control sheep. Others have reported that low levels of energy supplementation to sheep consuming forage-based diets have increased intake (Matejovsky & Sanson, 1995 as cited by Caton & Dhuyvetter, 1997).

Low quality roughages, with fibre of low digestibility or a high undegradable fraction of fibre are more sensitive to a decrease in ruminal pH and show a more pronounced decrease in DM intake than roughages with higher quality fibre (Malestein & Van't Klooster, 1986; De Visser *et al*., 1990).

2.5.6 Effects of energy supplementation on ruminal fermentation

According to the results obtained by Bach *et al.* (1999), energy supplementation reduced rumen pH, rumen NH_3-N flow, and ruminal NH_3-N concentration and increased bacterial-N (as a percentage of N intake). These authors found that the supplementation of lush pasture with maize and soybean hulls resulted in the highest microbial N flow (as a percentage of N intake), compared to supplementation with beet pulp and grass-legume pasture. The results of these authors showed that true DM and OM digestion (percentage) was greater in diets supplemented with energy than in pasture only diets. The increases in digestion can be explained by the higher content of digestible OM in the energy sources than in the pasture. These authors also observed values for NDF digestibilities, which indicate that cracked maize tended to have a negative effect on the NDF digestibility of a

diet containing pasture and cracked maize. Grant & Mertens, (1992) reported that the decrease in NDF digestion when supplements, rich in starch are fed, is related to a decrease in rumen pH.

The pH in the rumen is a consequence of a number of factors. Fermentation produces VFA, which reduce pH when production is faster than absorption from the rumen. Thus, rumen pH is often decreased due to fermentation of rapid fermentable carbohydrates, following their ingestion, and the decrease tends to be linearly related to the level of rapid fermentable carbohydrates in the diet (Franklin *et al.,* 1981; Kennedy & Bunting 1992). Salivary secretions tend to maintain rumen pH. The amount of saliva secreted depends on the physical characteristics of the forage, but also varies widely between animals (Franklin *et al.,* 1981). Forages, particularly legumes in an immature stage of growth, have some buffering capacity. *A. nummularia,* having a high mineral content, may thus also have some buffering capacity to a certain degree, which will keep the rumen pH stable under relative low levels of grain supplementation.

There are important differences between carbohydrate sources in their effects on nitrogen metabolism in the rumen. Differences between starch and sugars appears to relate to the influence of the carbohydrate on the microbial population of the rumen, as was indicated by the differential effects of the carbohydrate sources on the number of total protozoa. Differences between sugars appear to depend, in part, on the rates of sugar fermentation and the associated reduction in rumen pH (Chamberlain *et al.,* 1985).

The use of concentrates high in ruminal degradable starch negatively influences rumen fermentation conditions, which may reduce NDF degradation, especially from roughage. These negative effects may be reduced by decreasing the amount of substrate available for rumen fermentation by increasing the amount of rumen resistant starch, or by changes in the feeding system (McCarthy *et al*., 1989).
Robinson *et al. (*1986) confirmed findings by De Visser (1984), as cited by De Visser *et al*. (1990), in which they fed two levels of concentrates, with a starch content between 150 and 450 g/kg DM, and showed that the total amount of feed negatively influenced

conditions for cellulolytic activity (increase in H^+ concentration four hours after feeding). These effects were more pronounced when feeding concentrates high in rumen degradable starch. These authors reported that the diurnal pattern of ruminal pH was negatively influenced and showed a significantly longer period of values below 6.0. De Visser *et al*. (1990) found that NDF accumulated in the rumen as concentrates high in rapid rumen degradable starch were included into diets.

Several researchers (Malestein & Van't Klooster, 1986; Herrera-Saldana & Huber, 1989) have reported that an increase in rumen resistant starch content of concentrates, as compared to rumen degradable starch, positively influenced rumen fermentation conditions, depicted by a higher ruminal pH, reduced total volatile fatty acid concentrations and an increase in the acetic: propionic acid ratio.

2.5.6.1 Volatile fatty acid production

Large differences occur in the undegradable fraction between feedstuffs (roughage and concentrate ingredients), which influences the amount of substrate available for VFA production in the rumen (McCarthy *et al.,* 1989).
The rapid and extensive ruminal fermentation of starch results in high concentrations of VFA in ruminal fluid. High concentrations of VFA in ruminal fluid depress ruminal fluid pH to values that approach 6.0. Barley-based diets gave a lower ruminal fluid pH than corn-based diets (McCarthy *et al.,* 1989).

The pattern, in which VFA's are produced and present in the rumen, is a reflection of substrate composition. Murphy *et al.* (1982) reported important differences in VFA patterns produced from structural carbohydrates or non-structural carbohydrates, which can be explained as reflecting differences in the rate of carbohydrate degradation.

Increasing the proportion of concentrates in the diet reduces the cellulolytic activity of the microbial population in the rumen and causes a shift towards amylolytic activity. This

results from an increase in the amount of easily fermentable carbohydrate substrate (sugars, rumen degradable starch) causing a rapid fermentation in the rumen, increasing the total concentration of volatile fatty acids and thus reducing the pH of the rumen fluid (De Visser, 1990).

Tamminga (unpublished) as cited by De Visser *et al.* (1992), stated that total rumen fluid contents increased sharply after feeding, resulting in a dilution of the VFA and causing the pH to decline. In addition, the rate of absorption from the rumen increases with higher VFA concentrations.

In comparing the different molar concentrations of volatile fatty acids between carbohydrate sources, Casper & Schingoethe (1989) found that cows fed different carbohydrate sources had similar molar concentrations of acetate. Herrera-Saldana & Huber, (1989) reported that cows fed barley-based diets had the lowest concentration of butyrate and highest concentration of propionate, compared to maize-based diets. This may be related to the more rapid degradation rate of barley starch than of maize starch.

Similar results were reported by McCarthy *et al.* (1989), where they found that when barley replaced corn in a diet, the ruminal fermentation of starch increased and the degradation of fibre decreased which resulted in higher molar percentages of propionate and lower percentages of acetate. These authors also found that molar percentage of butyrate, isovalerate and valerate were not affected by the energy source.

2.5.6.2 Rumen ammonia nitrogen

Wilkins (1981), as cited by Chamberlain *et al*. (1985), stated that animals receiving grass or silage based diets had high ruminal ammonia concentrations. This suggests that there is a scope to increase the extent of fixation of ammonia into microbial protein through an increase in the supply of energy (*i.e.* ATP) from rumen fermentation. The addition of foods rich in fermentable carbohydrate to roughage diets can result in reduced ruminal concentrations of ammonia (Wilkins, 1981 as cited by Chamberlain *et al*., 1985). The extent of reduction depends on the type of carbohydrate in the supplement (Chamberlain

et al., 1985). Sometimes there is little or no reduction in ammonia concentration in response to supplements of barley or maize starch (Thomas *et al*., 1980), and presumably only a small effect on the content of bacterial protein in the duodenal digesta.

Less NH_3 will be available to rumen microbes when the degradation of feed in the rumen is slow. Robinson *et al.* (1987) reported a tendency towards lower amounts of microbial protein in the rumen content when decreasing the starch in the diet.

There are important differences between carbohydrate sources in their effects on nitrogen metabolism in the rumen. Differences between starch and sugars appear to relate to the influence of the carbohydrates on the microbial population of the rumen (Chamberlain *et al*., 1985). Differences between sugars appear to depend, in part, on the rates of sugar fermentation and the associated reduction in rumen pH.

Blackburn (1965), as cited by Chamberlain, *et al.* (1985), pointed out that maximal microbial fixation of nitrogen in the rumen will occur when the production of ammonia, from the breakdown of dietary nitrogen sources, is closely synchronized with the release of energy from the fermentation of dietary carbohydrates.

Chamberlain *et al.* (1985) found that, when supplementing with barley, rumen ammonia concentration was reduced and that the total amount of protozoa increased, but that the time of supplementation had no effect.

2.5.7 Comparison between different carbohydrate sources

The inclusion of concentrates in diets changes total dry matter intake, particle size distribution of the rumen content and often the chemical composition of the diet. Changes in the chemical composition of diets can be reflected in the degradability of organic matter, fermentation pattern and kinetics of particle digestion and passage in the rumen (De Visser *et al*., 1990).

The effects of carbohydrates in concentrate mixtures (starch vs. cell wall constituents) and in rate of rumen degradation (rapid vs. slow) on ruminal characteristics were studied by De Visser *et al*. (1992) using four Dutch Friesian dairy cows. Results obtained by De Visser *et al*. (1992) comparing the effects of maize and barley on ruminal fermentation characteristics are reported in Table 2.9.

Table 2.9 Rumen pH and concentrations of volatile fatty acids, lactic acid and ammonia (mMol/L), between diets containing barley and maize as carbohydrate sources in diets fed to Dutch Friesian dairy cows (De Visser *et al*., 1992)

	Barley based diets	Maize based diets	SED
DM intake (kg/day)	24.0	23.1	0.7
pH mean	5.73	5.76	0.037
pH range	0.28	0.27	0.019
Total VFA	142	142	3.62
Total VFA range	14	13	1.14
Lactate mean	1.13	1.70	0.34
Lactate range	1.43	2.66	0.86
Ammonia mean	7.46	8.36	0.96
Ammonia range	4.45	4.71	0.32
Acetate mean	86	85	2.75
Acetate range	7	6	0.52
Propionate mean	35	35	2.07
Propionate range	5	4	0.59
Butyrate mean	17	18	0.53
Butyrate range	2.5	2.7	0.42

SED = Standard error of difference

* No significant differences were found between barley and maize based diets.

In this experiment the animals were fed a total mixed ration and this may have slowed the intake of rapidly digestible organic matter enabling the animals to consume large amounts of feed without having a too strong negative effect on rumen fermentation.

In the same experiment De Visser *et al*. (1992) calculated the rumen degradability of different carbohydrate sources using the nylon bag incubation technique. The results of the rumen degradability study are shown in Table 2.10.

Table 2.10 The soluble (S), potentially fermentable (D), undegradable fraction (U) (g/kg) and rate of degradation (k_d) (%/h) of organic matter, nitrogen, neutral detergent fibre and starch of barley and maize based diets fed to Dutch Friesian dairy cows (De Visser *et al*., 1992)

	Ingredient	
	Barley	**Maize**
Neutral detergent fibre		
S fraction	0	17
D fraction	678	936
U fraction	322	47
K_d	9.44	2.25
Starch		
S fraction	115	50
D fraction	885	949
U fraction	0	1
K_d	25.83	8.66

The results reported by De Visser *et al.* (1992) show that the rate of NDF and starch degradation was highest for the barley-based diets when compared to maize-based diets. During the experiments done by De Visser *et al.* (1992) an increase in the proportions of

rapidly fermentable carbohydrates (i.e. starch and sugars) reduced feed (energy) intake and as a result reduced production, especially when feeding roughage and concentrates separately.

De Visser *et al*. (1992) examined the effects of different carbohydrate sources on the rumen kinetics of Dutch Friesian cows. These authors found that in comparing barley-based diets with maize-based diets, cows fed barley-based diets had a significant higher dry matter intake. The results reported by these authors are presented in Table 2.11.

Table 2.11 Total dry matter intake and daily mean rumen pool sizes of dry matter, organic matter, starch and cell wall constituents of Dutch Friesian cows fed diets based on different carbohydrate sources (De Visser *et al*., 1992)

	Barley	**Maize**
DM intake (kg)	22.7[a]	21.1[b]
Body weight (kg)	612	610
DM pool/kg body weight (g)	19.9	18.9
Total rumen contents		
Non dry matter (kg)	67.2	64.0
Dry matter (kg)	12.2	11.5
Total ingesta (kg)	79.4	75.5
% DM	15.3	15.2

Values with different superscripts are significantly different ($p<0.05$)

Table 2.11 Total dry matter intake and daily mean rumen pool sizes of dry matter, nitrogen, and organic matter, starch and cell wall constituents of Dutch Friesian cows fed diets based on different carbohydrate sources (De Visser *et al*., 1992) (Continued)

Rumen pool sizes	Barley	Maize
OM (kg)	11.2	10.6
Starch (g)	424[a]	610[b]
NDF (kg)	6.9	6.4
ADF (kg)	3.8	3.6
IADF (kg)	1.2	1.1
Rumen pool sizes of large particles (> 2mm)		
OM (kg)	5.5	5.1
NDF (kg)	4.5	4.2
ADF (kg)	2.5	2.4
IADF (kg)	0.6	0.6

Values with different superscripts are significantly different ($p<0.05$)

The rumen pool of OM, NDF, ADF and IADF did not differ significantly between the two carbohydrate sources. Maize-based diets had a significant higher rumen starch pool when compared to barley-based diets. This could possibly be explained by a lower k_d of maize starch as compared to starch originating from barley. The similar composition of

rumen pool of large particles (> 2 mm) is strongly related to the roughage part of the basal diet fed in both treatments (De Visser *et al*., 1992).

In other experiments Casper & Schingoethe (1989) stated that different bacterial communities colonize starch granules from different sources. These authors reported higher concentrations of ruminal carbohydrates for cows fed barley when compared to maize. This could possibly be due to a reduction of microbial uptake of carbohydrates because of different starch degradation moieties for cows fed barley and maize diets, respectively.
Stern *et al.* (1978), reported that as the concentration of starch increased *in vitro*, the concentration of NH_3 decreased with an increase in microbial protein synthesis. These results were supported by Casper & Schingoethe (1989), who reported that ruminal NH_3 concentrations were lower for cows fed barley than for cows fed maize.

McCarthy *et al.* (1989) stated that feeding a maize-based diet increased N intake compared with feeding a barley-based diet because of a greater total feed intake, but the quantity of total N that passed to the duodenum was not altered by the source of energy. Microbial N passing to the duodenum was greater with the barley-based diets than that of the maize-based diet. The larger quantity of OM truly digested in the rumen when barley was fed compared to maize may have increased the availability of energy for microbial growth.

McCarthy *et al.* (1989) reported that cows fed maize-based diets consumed more DM, OM, and starch than cows fed barley-based diets. However, the average true ruminal digestibility of OM and apparent ruminal digestibility of starch were 1.5 and 1.3 kg/ cow per day greater, respectively, when barley-based diets were fed than when maize-based diets were fed. These authors also stated that the flow of OM and starch to the duodenum was increased when maize replaced barley in the diet. A larger amount and percentage of starch was apparently digested post-ruminally when maize based diets were fed compared to barley-based diets. This resulted in a larger amount and percentage of OM apparently digested post-ruminally. The mean apparent total tract digestibility of starch

was 3.6 percentage units greater for barley-based diets than for maize based diets. Total tract apparent digestibility coefficients for OM were not affected by source of energy, however, because of the greater OM intake, cows fed corn based diets digested a greater quantity of OM daily than did cows fed barley based diets.

McCarthy *et al.* (1989) reported that intake, ruminal, postruminal and total tract digestibility of ADF were not affected by the source of energy. Replacing maize with barley in diets of ruminants increased ruminal fermentation of starch and OM but may have decreased ruminal degradation of fibre.

2.6 Conclusion

This review of literature has highlighted the special characteristics of *Atriplex* species that qualifies them to be a very useful source of fodder for ruminants in arid and semi-arid regions. *Atriplex*, if used as a source of green forage for animals, can be advantageous in reducing grazing pressures on pastures and veld. The limited data available from animal studies to evaluate the potential of *Atriplex* as an animal feed point to opportunities for its use. However, these studies indicate that animal preferences can vary between different *Atriplex* species. Breeding and selection of palatable *Atriplex* species would be beneficial to animal production.

There are opportunities to combine *Atriplex* with cereal straws or carbohydrate sources to reduce the impact of the negative factors of *Atriplex*, such as salt, on voluntary feed intake, and thus improve animal production.

Literature reviewed reported that feeding diets varying in carbohydrate composition and rate of degradation (starch and cell wall constituents) influenced the rate of rumen fermentation, the amount of starch escaping rumen fermentation and the balance between energy and nitrogen available for rumen microbial growth. As a result rumen fermentation patterns can be changed, and this can have an effect on animal production.

Atriplex species provides a potential feed source for sheep in arid and semi-arid regions that can sustain maintenance requirements and with the correct grain supplementation sustain a certain level of animal production.

Chapter 3

General material and methods

3.1 Introduction

The following section outlines the management of animals, diets, feeding procedures and general experimental techniques.

The experiment was carried out as a split plot design. All the animals were randomly divided into two groups so that five animals each received the different treatments within a group. The trial was run in four sequential experimental periods. Each experimental period lasted for 24 days. The first 14 days were allowed for adaptation to the diets, while the last 10 days were used for the collection of data and samples.

3.2 Animals and their management

Ten mature Merino wethers were used throughout the trial. All the animals were equipped with rubber rumen cannulae with an internal diameter of 100 mm. Prior to placement in individual metabolism cages the animals were drenched for internal parasites, injected with a vitamin A, D & E complex and had their hooves clipped. The animals were also weighed at the start and end of each experimental period.

The animals were placed in metabolism cages fitted with individual feed and water troughs and they were kept under natural lighting. The metabolism cages were also equipped with urine pans for the collection of urine. During the experimental period the animals were fitted with harnesses and nylon canvas bags for total collection of faeces. Throughout the experimental periods all the animals' cannulae were cleaned and disinfected regularly to avoid infestation with maggots. The wool around the cannulae was also clipped regularly.

Sick or "off feed" animals were removed from the experimental period and treated immediately. The experiments were conducted at the Hatfield Experimental Farm of the University of Pretoria in South Africa. The ethics committee approved the experiment: EO 010621-010.

3.3 Experimental treatments

3.3.1 Collection of material

Atriplex nummularia cv. De Kock material, used in experiments, was collected from Grootfontein Research Centre in the Eastern Cape, South Africa. The material was harvested with a mechanical weed eradicator and by hand, sun dried and sorted into edible and non-edible material. Edible material was defined as leaf and stems with a diameter of less than 6 mm. After sorting, the edible component was milled using a hammer mill with a 25 mm sieve.
The crude protein (CP), ash concentration and organic matter (OM) concentration were determined according to AOAC (2000) and neutral detergent fibre (NDF) concentration according to the method of Van Soest & Wine (1967). The following values were found for CP, ash, OM and NDF respectively 10.29 g/kg DM, 14.93 g/kg DM, 75.94 g/kg DM and 60.86 g/kg DM.

Afgri, (Kaalfontein) and Southern Associated Maltsters in Alberton, South Africa supplied the maize and barley respectively.

3.3.2 Experimental diets

The basal diet consisted of *A. nummularia* cv. De Kock. In addition to the control diet of 100% *Atriplex*, 15%, 30% and 45% of maize and barley was added to the basal diet on a dry matter basis. To prevent particle selection all the diets was offered as total mixed rations.

During periods when the animals were not on dietary treatments, they were fed a lucerne/ *Atriplex* mixture (15% *Atriplex*) *ad lib.* outside of metabolism cages.

3.4 Feeding and collection of samples

3.4.1 Digestibility trial

All the animals were used in the digestibility trial, *i.e.* five per group. The animals were weighed at the start and finish of the each experimental period. Individual feed intakes were recorded daily by weighing the feed offered as well as the orts. The quantity of feed offered each day was adjusted to ensure that the feed troughs contained feed throughout the day. Samples of feed offered and orts, which were collected every morning, were pooled for proximate analysis (A.O.A.C., 2000).

All faecal output during the experimental period was recorded and a 10% sample of the daily output was stored at – 10°C until the end of the collection period. At the end of the collection period, the total faeces collected for each sheep were mixed and sub-sampled. Feed and faecal samples were dried at 60°C and ground through a 1 mm sieve before chemical analyses (Mahgoub *et al*., 2000).

The urine output was measured daily and collected in bottles that contained 25 ml of 10% H_2SO_4 to prevent loss of urinary ammonia. Urine aliquots (10% of daily output) were pooled, frozen at –10°C and kept for analyses (Mahgoub *et al*., 2000).

The animals received fresh water daily on an *ad lib.* basis and the remaining water was measured back and recorded before feeding commenced the next morning. Water consumption of individual animals was recorded daily and it was corrected for evaporation losses. Temperature fluctuations during the period of data collection were also recorded daily.

3.4.2 Rumen fermentation trial

Rumen samples were collected at the end of the digestibility trial. Rumen fermentation pattern was determined for 24 hours by taking 9 samples as described by De Visser *et al*. (1992). The samples were taken at 5:00, 7:00, 10:00, 14:00, 16:00, 19:00, 22:00, 2:00 and 5:00 hours respectively, and immediately analysed for pH with a pH meter.

At each sampling time about 100 mL of rumen fluid was drawn with the aid of a rubber tube closed at the end with a 0.5 mm screen using suction provided by a 100 mL syringe. Rumen fluid was filtered through two layers of cheesecloth before sub-sampling.

Sub-samples (10 mL) were taken and stored for analyses of volatile fatty acids (VFA) and ammonia (NH_3-N) as described by Robinson *et al*. (1986). The samples, for ammonia determinations, were preserved with 2 ml of a 0.5 M H_2SO_4 solution and stored at –10°C pending analysis for ammonia-N. Samples for volatile fatty acid analysis were preserved with 1 mL 10% NaOH and frozen at -10°C pending determination of concentrations of volatile fatty acids.

3.4.3 Rumen kinetics trial

Three animals out of every group were used for measuring rumen ingesta mass and kinetics. Rumen content of liquid and dry matter were estimated from ingesta, manually removed at 11:00, 21:00 and 01:00 on three consecutive days, while the animals had free access to feed and water (Robinson *et al*., 1987).

All rumen contents that could be removed by hand were emptied into a 50 L insulated cooler box with a lid and continuously flushed with CO_2. This material, referred to as mat, was weighed and sub-sampled (about 500 g regardless of total content). Two sub-samples were taken at each sampling time. Material not removable by hand was bailed into a similar 50 L insulated cooler box covered with a lid into which a large funnel had

been fitted. The cooler box was continuously flushed with CO_2. This material, referred to as bailable liquids, was weighed and sub-sampled roughly in proportion to volume (about 500 mL). The liquids were then returned to the rumen followed by mat.
All the samples were frozen and rumen DM and non-DM was calculated for each time of incubation (Robinson *et al*., 1987).

The first of the two samples taken at each sampling time was freeze dried and analysed for ash concentration, N and NDF. The second was washed through a sieve with a pore size of 2 mm under cold tap water until the water that washed out was clear. The residue after washing was freeze-dried and analysed for ash concentration, N and NDF. Total rumen pool and pool of particles > 2 mm (OM and NDF) were measured, while the pool of particles < 2 mm was calculated by subtraction.

3.5 Parameters

The following variables were studied:

3.5.1 *Atriplex* material and diets

- Dry matter (DM) concentration
- Nitrogen (N) and crude protein (CP) concentrations
- Neutral detergent fibre (NDF) concentration

3.5.2 Rumen fermentation parameters

- Rumen pH
- Rumen NH_3-N concentration
- Rumen VFA concentration

3.5.3 Rumen kinetics parameters

- DM concentration
- N concentration

- NDF concentration

3.5.4 Derived parameters

The following parameters were calculated from the variables measured in section 3.5.1 to 3.5.3

3.5.4.1 Feed and rumen samples

- Dry matter intake (DMI)
- Nitrogen intake
- Molar proportions of VFA
- Apparent DM digestibility
- Apparent NDF digestibility
- Diurnal rumen pH
- Diurnal rumen ammonia-N concentration

3.5.4.2 Faeces

- Dry matter concentration

3.6 Analytical methods and derivation of parameters

3.6.1 Dry matter concentration

Samples of diets, faeces and digesta were dried at 100°C for 24 hours in a forced draught oven in aluminum foil containers. Dry matter of milled samples was determined in porcelain crucibles. Dry matter concentration was calculated as recommended by A.O.A.C. (2000).

3.6.2 Nitrogen and crude protein concentration

Nitrogen concentrations of diets, faecal and digesta samples were determined using a LECO System Model CHN – 1000. Nitrogen concentration of the urine samples was

determined by the macrokjehdahl method (A.O.A.C., 2000). A block digestor was used for the digestion of the samples and a Tecator Kjeltec System Model 1002 for the distillation.
Crude protein (%) of the samples was calculated as follows: % N × 6.25.

3.6.3 Neutral detergent fibre (NDF) concentration

The NDF concentration of diets, faeces and digesta samples were determined with neutral detergent solution (NDS) on a Tecator Fibertec system as outlined in the application note (Van Soest & Wine, 1967)

3.6.4 Rumen pH

A pH meter was used to determine the pH of the rumen fluid immediately after the sample was drawn from each animal at each sampling time. The electrodes were rinsed with distilled water between measurements of different animals and the pH meter was calibrated with standards before each sampling time.

3.6.5 Rumen NH_3-N concentration

The concentration of NH_3-N was determined from filtered rumen fluid samples taken at each sampling time. A Technicon autoanalyser was then used to determine the concentrations (mg/ 100-mL) after appropriate dilutions (A.O.A.C., 2000).

3.6.6 Rumen VFA concentration and molar proportions of VFA

A modified derivatization technique of Püttman *et al*. (1993) for high performance liquid chromatography (HPLC) with a Phenomenex LUNA C18 column and a UV detector was used for the analyses of volatile fatty acids.

3.6.7 Rumen kinetics

Kinetics of rumen OM, N and NDF clearance, passage and digestion were calculated as:

- rate of clearance (k_c) = ((feed intake, $kg.d^{-1}$)/(average rumen pool, kg))/ 24

- rate of passage (k_p) = (NDF intake, kg.d^{-1})/(average rumen NDF pool, kg))/ 24
- rate of digestion (k_d) = (K_c – K_p)

The kinetics of rumen OM and N of the large particle fraction were also calculated using the same equations. The clearance of the large particles was calculated with the assumption that the concentrate part of the diet only consisted of small particles (< 2 mm). The NDF of the saltbush component was assumed to be part of the large particle fraction. As a result of this assumption the large particle cell wall fraction (NDF) of the rumen contents can only originate from the saltbush component of the diets (De Visser *et al*., 1992).

3.7 Statistical analysis

An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between treatments and different levels of supplementation. Least square means and standard errors (SE) were determined. Significance of difference (5%) between least squares means was determined by using Bonferroni's test (Samuels, 1989).

Chapter 4

The effect of supplementing different energy sources, at different levels, on the digestibility of *Atriplex nummularia* cv. De Kock fed to sheep

4.1 Introduction

Atriplex nummularia has proved to be a very useful drought tolerant crop for bridging the periods when natural pasture does not satisfy the nutritional requirements of small stock (Jacobs & Smit, 1977). Information on the voluntary feed intake and performance of sheep on diets containing *Atriplex* is, however, limited. The voluntary feed intake of sheep on pure *Atriplex* diets ranges from 500 to 2500 g DM/ day and the usefulness of *Atriplex,* as a sole diet is questionable (Hassan & Abdel-Aziz, 1979; Le Houerou, 1991). High salt content of *Atriplex* species of up to 20% (Leigh, 1986), is considered to be one of the major factors that affect the voluntary feed intake of sheep and it also increases the need for a large quantity of drinking water (up to 13 mL/ g DM/ day) as compared to stubbles of oaten hay (2 mL/ g DM/ day of oaten hay; Warren *et al*., 1990).

According to McCarthy *et al.* (1989) there are two approaches to increasing the availability of nutrients for production from ruminants: 1) Increase the quantity and improve the ratio of rumen fermentation end products and 2), supplement nutrients that will escape rumen fermentation and pass to the small intestine for absorption. Energy and amino acids are the two nutrients most likely to limit animal production (Overton *et al*., 1995). The relationship between energy and nitrogen requirements is complex (Clark & Davies, 1983). Establishing an optimum ratio of nitrogen to energy for ruminants is complicated because there are two requirements to be met: One for the ruminal microbes and another for the host animal. The source of protein and energy has a significant effect on the utilization of nitrogen and energy in the rumen, the production of volatile fatty acids and the flow of nutrients to the small intestine (McCarthy *et al*., 1989).

The protein content of *A. nummularia*, estimated from Kjeldahl nitrogen, and digestible crude protein content of *Atriplex* species is high (Leigh, 1986). Van Niekerk *et al*.

(2004a) reported crude protein values for *A. nummularia* of 208 g/kg DM and 234 g/kg DM. However, Weston *et al.* (1970) reported that these high crude protein values could be misleading, as the protein in *A. nummularia* was extensively degraded to ammonia in the rumen of sheep. These authors also reported a high loss of nitrogen in the urine, which may have been due to inefficient utilization of ammonia by the rumen microbes. According to Chamberlain *et al.* (1985) there is a possibility to increase the extent of fixation of ammonia into bacterial protein through an increase of energy (ATP) from rumen fermentation. The addition of concentrates to roughage diets can result in reduced ruminal concentrations of ammonia. The extent of such a reduction depends on the type of carbohydrate supplement (Chamberlain *et al.*, 1985).

Rapid fermentation characteristics of starch supplements often exceed the ability of ruminants to maintain a stable ruminal pH and as the pH decreases, cellulolytic bacterial function is often impaired and fibre digestion decreased (Carey *et al*., 1993). A decline in ruminal pH is usually as a result of the production rate of volatile fatty acids from ruminal fermentation being faster than the absorption rate from the rumen. The decrease in pH tends to be linearly related to the level of rapid fermentable carbohydrates in the diet (Franklin *et al*., 1981; Kennedy & Bunting, 1992). Salivary secretions tend to maintain rumen pH. The amounts of various types of saliva secreted depend not only on the physical characteristics of the forage, but also vary widely between animals (Franklin *et al*., 1981). Forages, particularly legumes in an immature stage of growth, have some buffering capacity. *A. nummularia,* having a high mineral content (Atiq-ur-Rehman, 1995), may also have some buffering capacity, which will keep the rumen pH stable under relatively low rates of grain supplementation.

The aim of this experiment was to quantify the influence of type and level of carbohydrate supplementation on the digestibility, ruminal fermentation characteristics, pH and ruminal ammonia concentrations of *A. nummularia* cv. De Kock fed to sheep.

4.2 Materials and methods

The trial was conducted at the Hatfield Experimental Farm of the University of Pretoria, South Africa. Ten mature, rumen fistulated, Merino wethers were used in the trial. The animals were randomly allocated to two groups of five animals per group, each group receiving a different treatment during each experimental period. The trial ran for four sequential experimental periods and the two groups of animals stayed constant for each experimental period.

The *Atriplex* material used in the trial was harvested from an established stand. The stand was established in the early 1990's and since establishment sheep have grazed it heavily, as a drought fodder, during winter. The material was harvested a month after the animals were withdrawn from the stand. The harvested material consisted mainly of material that was not selected by sheep during the grazing season and with a diameter of less than 6 mm.

The basal diet consisted of *A. nummularia cv*. De Kock. In addition to the control diet of 100% *Atriplex*, 15%, 30% and 45% of milled maize as well as barley was added to the basal diet on a dry matter basis for each of the experimental periods. Maize was selected as the medium fermentable carbohydrate source and barley as the rapid fermentable carbohydrate source. To prevent particle selection all the diets was offered as total mixed rations.

During each experimental period the animals were adapted to the experimental diets for 14 days before the collection period of 10 days commenced for the digestibility trial and there after a 24 hour collection period for the fermentation trial. The animals were injected with a vitamin A, D & E supplement at the start of each period and each animal's initial and final weight was recorded for each sequential experimental period. All the animals were kept in individual metabolism cages for the duration of the experimental period. Individual feed intakes were recorded daily by weighing the feed offered as well as the orts. The quantity of feed offered each day was adjusted to ensure that the feed

troughs contained feed throughout the day. Samples of feed offered and orts were collected every morning during each collection period and pooled for proximate analysis (A.O.A.C., 2000).

All faecal output during each collection period was recorded and a 10% representative sample of the daily output was stored at -10°C until the end of each collection period. At the end of the collection period, the total faeces collected for each sheep were mixed and sub-sampled. Feed and faecal samples were dried at 60°C and ground through a 1 mm sieve before chemical analyses (Mahgoub *et al*., 2000).

The animals received fresh water daily on an *ad-lib* basis and the remaining water was measured back and recorded before feeding commenced the next morning. Water consumption of individual animals was recorded daily for each collection period and it was corrected for evaporation losses. Maximum and minimum temperature fluctuations during each period of data collection were also recorded daily.

Rumen fermentation patterns were determined for 24 hours by taking nine samples of each animal within the two groups as described by De Visser *et al*. (1991). The samples were taken at 5:00, 7:00, 10:00, 14:00, 16:00, 19:00, 22:00, 2:00 and 5:00 hours respectively, and immediately analysed for pH with a pH meter.

At each sampling time approximately 100 mL of rumen fluid was drawn with the aid of a rubber tube closed at the end with a 0.5 mm screen using suction provided by a 100 mL syringe. Rumen fluid was filtered through two layers of cheesecloth before sub-sampling.

Sub-samples (10 mL) were taken and stored for analyses of volatile fatty acids (VFA) and ammonia-N (NH_3-N) as described by Robinson *et al*. (1986). The samples for ammonia analysis were preserved with 2 mL of a 0.5 M H_2SO_4 solution and stored at –10°C pending the analysis. Samples for volatile fatty acid analysis were preserved with 1 mL 10% NaOH and frozen at -10°C pending the determination of concentrations of volatile fatty acids.

4.2.1 Analytical methods and derivation of parameters

4.2.1.1 Dry matter concentration

Samples of diets and faeces were dried at 100°C for 24 hours in a forced draught oven in aluminium foil containers. Dry matter of milled samples was determined in porcelain crucibles. Dry matter concentration was calculated as recommended by A.O.A.C. (2000).

4.2.1.2 Neutral detergent fibre (NDF) concentration

The NDF concentration of diets and faeces samples were determined with neutral detergent solution (NDS) on a Tecator Fibertec system using the procedure described by Van Soest & Wine (1967).

4.2.1.3 Apparent digestibilities

The apparent dry matter digestibility (DMD) for each experimental diet was calculated using the formula described by McDonald *et al*. (2002):

$$\frac{\text{Nutrient consumed} - \text{Nutrient in faeces}}{\text{Nutrient consumed}}$$

4.2.2 Statistical analysis

An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between treatments and different levels of supplementation. Least square means and standard deviations (SD) were determined. Significance of difference (5%) between least squares means was determined by using Bonferroni's test (Samuels, 1989).

4.3 Results and discussion

4.3.1 Voluntary feed intake

The daily dry matter intake on a g/kg live weight basis is presented in Table 4.1.

Table 4.1 Dry matter intake of sheep fed *Atriplex nummularia* cv. De Kock supplemented with two energy sources at different levels

	Treatments		
	Supplementation Level (%)	**Maize**	**Barley**
Intake/ kg W (g/day)	0	$23.5^{a}{}_{1}$ (±9)*	$27.9^{ab}{}_{1}$ (±10)*
	15	$23.6^{a}{}_{1}$ (±5)	$21.2^{a}{}_{1}$ (±4)
	30	$33.5^{ab}{}_{1}$ (±7)	$37.9^{b}{}_{1}$ (±9)
	45	$38.6^{b}{}_{1}$ (±5)	$25.8^{a}{}_{2}$ (±2)

* Values in brackets designates standard deviation

[a,b] Column least square means with common superscript do not differ significantly

[1,2] Row least square means with common subscript do not differ significantly

The results in Table 4.1 indicate that supplementation of both energy sources tended towards an increase (not significant) in dry matter intake when compared to the control. The intake of the control diets ranged between 23.5 g/kg W and 27.9 g/kg W, which is lower than intakes found from different pen feeding trials as can be seen in Table 4.2.

Table 4.2 Summary of pen feeding trials conducted to determine the nutritive value of *Atriplex* species (Atiq-ur- Rehman, 1995)

Species	DMI/ kg W (g/d)	Description of diet	*In vivo* DMD (%)	Country	Reference
A. nummularia	34	Leaves + little stem	68	Australia	Wilson (1966)
A. nummularia	33	Leaves + little stem	74	Australia	Wilson (1966)
A. nummularia	41	Leaves + succulent stem	62	Egypt	Hassan *et al.* (1979)
A. nummularia	39	Leaves + succulent stem	72	Egypt	Hassan & Abdel –Aziz (1979)
A. nummularia	55	Edible biomass summer	64	Spain	Correal *et al.* (1990)
A. nummularia	-	Edible biomass spring	72	Spain	Correal *et al.* (1990)
A. nummularia	117	Leaves + stem	-	Libya	Le Houerou (1991)
A. nummularia	-	-	47-57	-	Warren & Casson (1993)

DMI = Dry matter intake

DMD = Dry matter digestibility

The reason for the wide variability of intake may be explained by both animal and plant factors. The plant factors may include: i) the preference/ palatability differences between *Atriplex* species and within species; ii) morphological differences in *Atriplex* diets fed to animals, especially the percentage of stem and the diameter of stem material and iii) the time of the year the plant material was harvested. The level of intake of *Atriplex* by sheep is governed by: i) the amount of material in the reticulo-rumen; ii) its rate of digestion; and iii) the rate of passage of digesta out of the reticulo-rumen (Atiq-ur-Rehman, 1995).

Therefore, the mechanical processes associated with digestion, like particle size reduction during eating and rumination, and the rate of microbial fermentation can be important factors determining the amount of *Atriplex* consumed by sheep (Atiq-ur-Rehman, 1995).

The fact that the *Atriplex* material used in this trial was harvested from an old established stand after it has undergone heavy grazing by sheep on an annual basis, could partly explain the lower intake of pure *Atriplex* found in this trial when compared to the literature. The crude protein and neutral detergent fibre concentration of the diets used in the present trial are presented in Table 4.3.

Table 4.3 The mean crude protein (CP) and neutral detergent fibre (NDF) concentration of *Atriplex nummularia* cv. De Kock supplemented with two energy sources at different levels

	Supplementation level (%)	Maize	Barley
CP (%)	0	10.29	10.29
	15	9.92	10.05
	30	10.62	10.64
	45	13.65	13.66
NDF (%)	0	60.9	60.9
	15	53.2	54.6
	30	47.6	51.8
	45	37.8	38

Van Niekerk *et al*. (2004) reported CP values for *A. nummularia* ranging from 20.8% to 23.4% and NDF values ranging from 33.2% to 40.7%. This suggests that the *Atriplex* material used in the present trial was of lower quality.

Significant differences occurred in intake at the 45% maize and 30 % barley inclusion levels compared to the lower supplementation levels. These results are supported by Hassan *et al*. (1979) who found that the intake of *Atriplex* material increased significantly when supplemented with barley at levels of up to 100 g/d (10% of total intake per day). These authors also found no further appreciable increase in intake when the levels of supplementation increased to 150 g/d (15% of total intake per day).

The incremental increase in intake decreased as the supplementation level was increased to 45 % of dry matter when compared to the 30 % level of both energy sources as can be seen in Table 4.1. Animals that were supplemented with maize tended towards a higher intake (not significant) than animals that received barley as energy supplement. Similar results was found by McCarthy *et al*. (1989) when cows fed maize based diets consumed more dry matter than cows fed barley based diets. The higher intake of supplemented diets could have been as a result of the higher palatability and digestibility of the two energy sources.

The daily *Atriplex* intake on a g/kg live weight basis during each experimental period is presented in Table 4.4.

Table 4.4 Daily *Atriplex nummularia* cv. De Kock intake of sheep during each experimental period on a dry matter basis

	Treatments		
	Supplementation Level (%)	**Maize**	**Barley**
Intake/ kg W (g/day)	0	$23.5^{a}{}_{1}$ (±9)*	$27.9^{a}{}_{1}$ (±6)*
	15	$20.1^{a}{}_{1}$ (±5)	$17.9^{a,b}{}_{1}$ (±4)
	30	$23.5^{a}{}_{1}$ (±5)	$26.6^{a}{}_{1}$ (±7)
	45	$21.25^{a}{}_{1}$ (±3)	$14.17^{b}{}_{1}$ (±2)

* Values in brackets designates standard deviation

[a,b] Column least square means with common superscript do not differ significantly

[1,2] Row least square means with common subscript do not differ significantly

The results presented in Table 4.4 show that although the total DM intake tended to increase as the level of supplementation increased, the *Atriplex* intake from the maize supplemented diets stayed fairly constant and the *Atriplex* intake from the barley supplemented diets showed a significant decrease from the control to the 45% supplemented diet. The animals receiving the maize supplemented diets tended to have higher roughage intakes when compared to the barley supplemented animals (not significant). The higher and more constant intake of *Atriplex* material in the maize supplemented animals suggests a more stable rumen pH in the animals receiving the maize supplement.

4.3.2 Water intake

The daily water consumption of sheep in the present study is indicated in Table 4.5.

Table 4.5 Water intake of sheep fed *Atriplex nummularia* cv. De Kock supplemented with different energy sources at different rates (mL/ kg live mass per day)

Rate of supplementation (%)	Maize	Barley
0	166.98 $^{a}{}_{1}$(26.79)	192.56 $^{a}{}_{1}$ (26.79)
15	199.17 $^{a}{}_{1}$ (23.96)	174.58 $^{a}{}_{1}$ (26.79)
30	112.39 $^{a}{}_{1}$ (26.79)	165.80 $^{a}{}_{1}$ (23.96)
45	138.18 $^{a}{}_{1}$ (26.79)	104.33 $^{a}{}_{1}$ (26.79)

* Values in brackets designates standard deviation

[a,b] Column least square means with common superscript do not differ significantly

[1,2] Row least square means with common subscript do not differ significantly

The daily water consumption decreased (non significant) as the rate of supplementation increased, except for the 15% maize supplementation where there was a non significant increase. As the rate of supplementation increased the concentration of *Atriplex* in the diets decreased. The decrease in the concentration of *Atriplex* could explain the tendency for water consumption to decrease with an increase in rate of supplementation.

Atriplex species have a high mineral and salt content. The high salt content, especially in the leaves, has a water retention function and helps these plants to survive in arid environments. *Atriplex nummularia* contains mainly sodium and potassium salts. Beal & Budtz-Olzen (1968) found that 88% of the sodium, and 89% of the potassium, was excreted through urine. These authors also found a strong correlation ($r^2 = 0.92$) between the intake and excretion of these elements. This means that large quantities of salts ingested will be excreted in urine and this will increase water requirements of sheep consuming *Atriplex* pastures.

The water consumption in the present study of 9L/sheep/day was slightly higher than that reported by Warren & Cason (1993) of 8L/sheep/day. Water consumption of sheep on *Atriplex* diets in a number of pen feeding trials is presented in Table 4.6.

Table 4.6 Effect of total ash and sodium in various diets on water consumption of sheep

	Daily intake			
	Ash (g)	**Sodium (g)**	**Water (L)**	
A. nummularia	130	51	6	Hassan and Abdel-Aziz (1979)
A. undulata	99	28	6	Warrren *et al*. (1990)
A. lentiformes	100	28	5	Warrren *et al*. (1990)
A. amnicola	193	46	8	Warrren *et al*. (1990)
A. barclayana	236	-	10	Benjamin *et al*. (1992)
Oaten hay	29	0.3	2	Warren *et al*. (1990)

Water intake of sheep fed dried *Atriplex* was found to be 3 to 4 L/sheep/day higher when compared to sheep fed fresh material (Warren & Cason, 1993). This could explain the slightly higher water intake found in this study compared to previous studies. It has also been reported that the sodium levels of *A. nummularia* can vary with season (Sharma *et al*., 1972). These authors found that sodium and chloride levels of *A. nummularia* increase with a decrease in soil moisture. This indicates that the season in which *Atriplex* is grazed or harvested, may influence the water requirements of the animals consuming it. The material used in the present trial was harvested from a very arid region in South Africa, and this may also have played a role in the slightly higher water intake found in this trial.

The water intake of animals is also related to the climatic conditions under which the animals are kept. During this trial the daily temperature and humidity was recorded but no significant differences were found.

4.3.3 Dry matter digestibility

The digestibility of a feed is defined as that proportion of the feed which is not excreted in the faeces. It is a useful first approximation of nutritive value and has been used as an index of both nutritive and feeding value of herbage. The dry matter digestibility of sheep fed *Atriplex nummularia*, supplemented with two energy sources with different rates of fermentabilities, and at different rates of supplementation is presented in Table 4.7.

Table 4.7 Dry matter digestibility of *Atriplex nummularia* cv. De Kock, based rations, with different levels of supplementation of two energy sources, fed to sheep

Rate of supplementation (%)	Treatments	
	Maize	Barley
0	$32.7^{a}{}_{1}$ (±3)	$35.6\ ^{a}{}_{1}$ (±13)
15	$54.7\ ^{a}{}_{2}$ (±7)	$61.3\ ^{a}{}_{2}$ (±5)
30	$55.7\ ^{a}{}_{2}$ (±14)	$62.3\ ^{a}{}_{2}$ (±8)
45	$65.8\ ^{a}{}_{2}$ (±7)	$67.0\ ^{a}{}_{2}$ (±6)

Column (1,2) and row (a,b) means with common scripts do not differ significantly ($p>0.05$)

*Values in brackets designate standard diviations

The percentage dry matter digestibilities of *A. nummularia* (0% supplementation) reported in this trial were lower than those that reported in literature (Wilson, 1966; Hassan *et al*. 1979; Hassan & Adbel-Aziz, 1979; Correal *et al*., 1990; Warren & Casson, 1993). Table 4.2 gives a summary of dry matter digestibilities found during pen feeding trials in the past. This Table illustrates the range (47% - 74%) of dry matter digestibilities found when feeding *Atriplex* to sheep. The lower digestibilities found in the present trial may be due to a higher stem to leaf ratio and differences in the amount of lignin found in the stems. The material used for this trial was also from older plants that had been grazed prior to collection so it is feasible that more fibrous and less palatable material was harvested for use in this trial (Table 4.3).

There was a significant difference in the percentage dry matter digestibility from 0% to 15%, 30% and 45% supplementation rate of both energy sources as indicated in Table 4.7. This increase in dry matter digestibility as energy supplementation is provided supports the results reported by previous authors (Orskov, 1982; McCarthy *et al*., 1989; Warren & Casson, 1993 and Overton *et al*., 1995). The increase in digestibility when diets are supplemented with energy sources can be explained by the higher content of digestible organic matter in the energy sources. As the rate of supplementation increased the incremental increases in dry matter digestibility decreased. This was probably due to negative associative effects occurring in the rumen.

There were no significant differences found between the two different energy sources, although barley supplementation tended to give a higher increase in dry matter digestibility. McCarthy *et al*. (1989) found that when feeding different starch sources (maize and barley) to cows the apparent ruminal digestibilities of starch were greater when barley was fed, than when maize was fed. However, the flow of organic matter and starch to the duodenum was increased when maize replaced barley in the diets. Maize starch is less rumen degradable than barley starch. Theurer (1986) compared the protein and starch digestibilities of maize and barley and found that both starch and protein were lower in maize than in barley. De Visser (1990) also compared the starch degradability of maize and barley. This author found that although maize had more starch than barley (676 g/kg DM vs. 561 g/kg DM) the rate of degradation (K_d) of maize starch was much lower (4.0 vs. 24.4) than that of barley. Maize starch also had a lower soluble fraction than barley (27% vs. 62%). De Visser (1990) also noted that maize contained a higher percentage of effective rumen resistant starch than barley (42% vs. 7%). Starch resistant to rumen degradation becomes, if digested, available to the host animal as glucose after hydrolysis and absorption in the small intestine (Nocek & Tammanga, 1991). The apparent digestibilities for the energy sources used in the present study may have been lower than those cited in literature because of an increased throughflow rate caused by an increased water intake due to the amount of *A. nummularia* in the diets.

4.3.4 Percentage NDF digestibility

The results obtained for NDF digestibility in the present study are presented in Table 4.8.

Table 4.8 Percentage NDF digestibility of *Atriplex nummularia* cv. De Kock fed to sheep supplemented with two energy sources

	Treatments	
Supplementation level (%)	**Maize**	**Barley**
0	$30.24_1{}^{a}$ (±6.5)	$28.21_1{}^{a}$ (±5.8)
15	$51.58_1{}^{a}$ (±5.8)	$61.57_1{}^{b}$ (±6.5)
30	$40.22_1{}^{a}$ (±5.8)	$51.54_1{}^{b}$ (±5.8)
45	$42.72_1{}^{a}$ (±5.8)	$41.56_1{}^{a,b}$ (±6.56)

Column (a, b, c) and row (1,2) means with common scripts do not differ (p>0.05)

* Values in bracket indicate standard deviations

Warren & Casson (1993) found that the percentage NDF digestibility for *Atriplex* species varied from 26% to 50%. The values found in the present study, 30.24% and 28.21%, fall within the range found by Warren & Casson (1993), but are much lower than the NDF digestibility for *Atriplex nummularia* found by Abou El Nasr *et al.* (1996) of 68.4%. The poor NDF digestibilities found in this study could be due to a higher lignin content in the plant material, which was harvested from an old stand after it had been heavily grazed. The poorer quality material harvested, which was not selected by the grazing sheep, could explain the lower NDF digestibilities of the unsupplemented *A. nummularia*.

Barley supplementation showed a significant increase in the NDF digestibility when supplementation was increased from 0% to 15% and from 0% to 30%. Maize supplementation increased the NDF digestibility from 0% to 15% and from 0% to 30% and 45% (not significantly). These increases may have been due to positive associative

effects occurring in the rumen. Significant positive associative effects of grain supplementation were noted for feedlot animals by Huck *et al.* (1998). With the 30% and 45% supplementation rates of both energy sources, there was a decline in NDF digestibility, relative to the 15% level (not significant), most probably due to negative associative effects in the rumen. De Visser (1990) stated that the use of concentrates high in ruminal degradable starch negatively influenced rumen fermentation conditions, which may reduce NDF degradation, especially that of roughage.

The ability of the microbial population within the rumen to digest fibre decreases when the amount and proportion of readily fermentable carbohydrate digested in the rumen increases (Hoover, 1986). Overton *et al.* (1995) found that the decrease in fibre degradation occurred because of a decrease in pH as the amount of readily fermentable carbohydrate in the diet increased. In the present trial there was a significant decrease in rumen pH as the level of supplementation was increased from the control to 30% and to 45% for both the energy sources (Table 4.10). The extent to which rumen cellulolysis is inhibited varies with the type and level of concentrate supplementation and the inhibition of fibre degradation can be partially alleviated if rumen pH is maintained at a level normally associated with the fermentation of an all roughage diet of 6.70 (Mould *et al.,* 1983). The results in Table 4.10 indicate that the pH decreased from 6.98 and 7.05 for maize and barley respectively at the control diet to 6.10 and 5.94 at the 45% level of supplementation.

Barley supplementation gave a higher incremental increase in NDF digestibility at the 15% and 30% supplementation rates when compared to maize supplementation. However, at the 45% supplementation level maize had a marginally higher NDF digestibility. McCarthy *et al.* (1989) found that the mean quantity and proportion of NDF digested in the rumen and the total tract digestibility coefficients for NDF were greater when maize based diets were fed, than when barley based diets were fed.

Mould *et al.* (1983) identified associative effects when using maize as a carbohydrate source, but these effects were due more to a decrease in starch digestion than to a

decrease in cellulolysis. The associative effects found when barley was used as a carbohydrate source were probably due more to a decrease in cellulolysis in the rumen. McCarthy *et al.* (1989) found that replacing maize with barley in ruminant diets increased ruminal fermentation of starch but may decrease ruminal degradation of fibre. The tendency of a decreased ruminal degradation of fibre when barley is supplemented compared to maize was also found at the 45% supplementation level in the present study.

4.3.5 Rumen ammonia-N

Protein is broken down into peptides, amino acids and ammonia. Rumen ammonia nitrogen (NH_3-N) is used by microbial organisms to produce microbial protein. Urinary excretion of more than 50% of the extra nitrogen intake for diets with a 50% and greater proportion of *Atriplex* suggests that rumen micro-organisms make poor use of the nitrogen in *Atriplex* diets (Atiq-ur-Rehman, 1995). Poor utilization of nitrogen by rumen micro-organisms can be due to the low carbohydrate content of *Atriplex* (Atiq-ur-Rehman, 1995). The amount of nitrogen required by micro-organisms is related to the amount of fermentable energy available in the rumen.

The results obtained in the present study for rumen NH_3-N concentration are presented in Table 4.9.

Table 4.9 Rumen ammonia-nitrogen (mg/100*mL*) of sheep fed *Atriplex nummularia* cv. De Kock, supplemented with two energy sources at different levels of supplementation

	Energy Source	
Supplementation level	**Maize**	**Barley**
0	7.28 $_1^a$ (±0.54)	7.23 $_1^a$ (±0.54)
15	8.12$_1^a$ (±0.41)	7.90 $_1^a$ (±0.46)
30	4.68 $_1^b$(±0.46)	5.24 $_1^b$ (±0.41)
45	8.36 $_1^a$ (±0.41)	5.26 $_2^b$ (±0.54)

Column (a, b) and row (1, 2) means with common scripts do not differ ($p>0.05$)

* Values in brackets designate standard deviations

The rumen NH_3-N concentration of pure *A. nummularia,* with 15% supplementation rate for both energy sources, was higher than that of the other supplementation groups, except for the 45% maize supplementation group where NH_3-N showed a sharp increase from the 30% supplementation rate. The relatively high rumen NH_3-N concentrations when compared to the 5mg/100mL reported by Satter and Slyter (1974) as a minimal concentration for optimum microbial protein synthesis may, be due to the high proportion of non-protein nitrogen (NPN) in *Atriplex* (47%; Benjamin *et al*., 1995), which is highly soluble in the rumen.

Weston *et al*. (1970) reported that protein in *A. nummularia* was degraded extensively to ammonia in the rumen, this could probably be due to the inefficient utilization of NH_3-N by rumen microbes because of the low fermentable energy content of *A. nummularia*.

The high NH_3-N concentration found with the 15% supplementation rate of both energy sources suggests that 15% energy supplementation did not supply enough fermentable energy to the rumen micro-organisms to make efficient use of the available NH_3-N.

The present results are supported by Bach *et al.* (1999) who found that energy supplementation reduced NH_3-N concentrations in the rumen. Low rumen NH_3-N concentrations suggest reduced fibre digestion (Bach *et al*., 1999). As the supplementation level increased from 15% to 30% the rumen NH_3-N concentration decreased (significantly) from 8.12 to 4.68 and 7.90 to 5.24 for maize and barley respectively. This decrease in NH_3-N could possibly be due to more efficient N-utilization due to more available fermentable energy as the rate of supplementation increased.

Cellolytic bacteria only use NH_3-N as a nitrogen source and low NH_3-N concentrations associated with energy supplemented diets could prevent greater growth of fibrolytic bacteria and thus fibre digestion due to a reduction in ruminal pH (Russel *et al*., 1992, cited by Bach *et al*., 1999). Mertens (1977) suggested that ruminal pH concentrations of < 6.2 could inhibit ruminal fibre digestion. Ruminal pH results for the present trial in Table 4.10 indicate that pH decreased as the level of supplementation increased from 6.98 to 6.10 and 7.05 to 5.94 for maize and barley respectively. The decrease in NH_3-N concentration, as the supplementation level was further increased, was the largest when the supplementation level increased from 15% to 30% for both energy sources. This could probably be because of an increased OM fermentation in the rumen, which supplied more energy for the utilization of NH_3-N in microbial protein synthesis.

Although there were no significant differences found between the two different energy sources, barley supplemented animals tended to have the lowest ruminal NH_3-N concentrations. McCarthy *et al*. (1989) also found that NH_3-N concentrations were higher for maize based diets than for barley based diets, but these authors also found that a larger amount of OM was digested in the rumen by barley-supplemented animals. Lower NH_3-N concentrations resulted in greater microbial protein flows to the small intestine of animals fed barley than of animals fed maize (McCarthy *et al*., 1989).

Starch escaping rumen fermentation results in lower energy supply to rumen microbes (Van Vuuren *et al.,* 1989). Maize starch is less degradable in the rumen when compared

to barley starch. This might be the reason why maize supplemented animals showed a tendency towards higher NH_3-N concentrations in the ruminal fluid.

Figure 4.1 illustrates the diurnal pattern of ruminal NH_3-N found in the present study. The figure illustrates that ruminal NH_3-N concentrations peaked approximately two hours after feeding and then started to decline until evening (feeding was done at 8:00 daily).

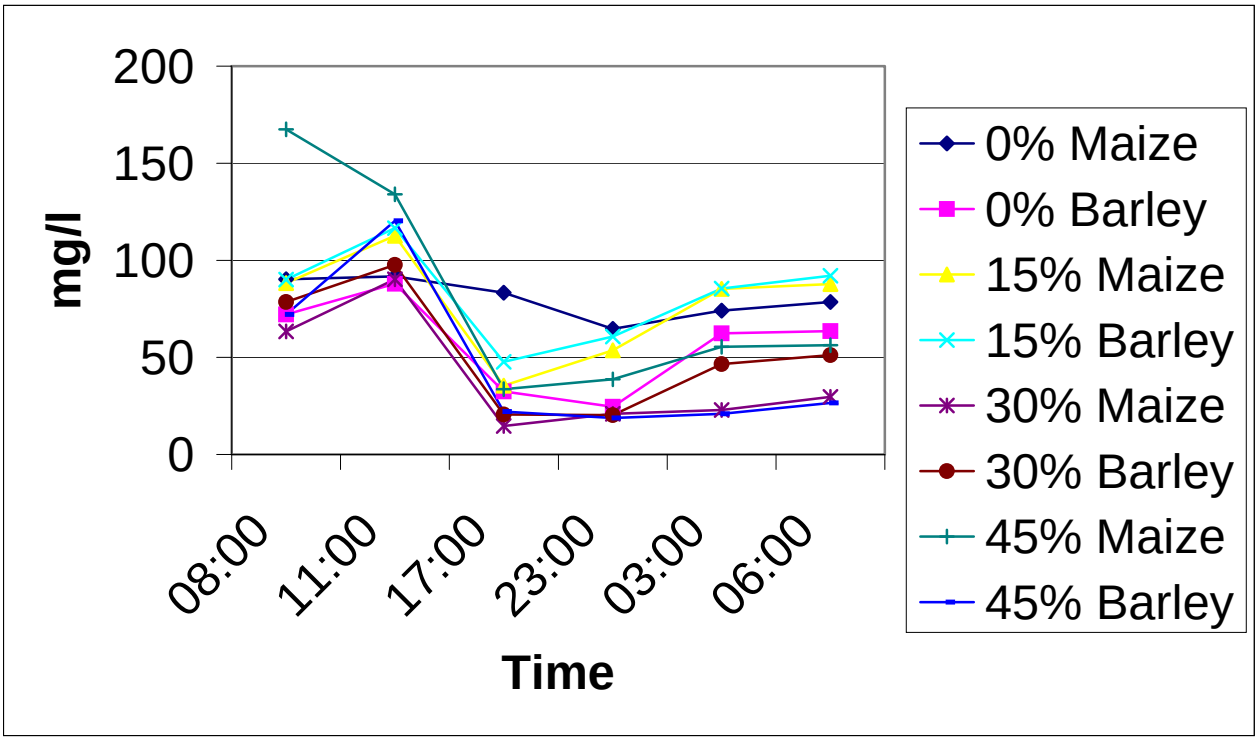

Figure 4.1 Diurnal rumen ammonia-N patterns of sheep fed *A. nummularia* cv. De Kock supplemented with two different energy sources at different levels

Robinson *et al.* (1986) and Casper *et al.* (1999) also found that ruminal NH_3-N concentrations of animals supplemented with energy sources peaked at two hours after feeding. The tendency of increasing levels of NH_3-N during the night has been observed previously by Robinson *et al.* (1986), and might reflect recycling of nitrogen to the rumen to maintain bacterial nitrogen requirements.

4.3.6 Ruminal pH

The ruminal pH of sheep fed *A. nummularia* cv. De Kock supplemented with two different energy sources at different rates is presented in Table 4.10.

Table 4.10 Ruminal pH of sheep fed *A. nummularia* cv. De Kock supplemented with two energy sources at different levels

	Energy Sources	
Supplementation rate of energy	**Maize**	**Barley**
0	6.98_1^{a} (±0.11)	7.05_1^{a} (±0.10)
15	6.77_1^{ab} (±0.10)	6.75_1^{ab}(±0.11)
30	6.50_1^{bc} (±0.11)	6.53_1^{b} (±0.10)
45	6.10_1^{c} (±0.10)	5.94_1^{c} (±0.10)

Column (a, b, c) and row (1, 2) means with common scripts do not differ ($p>0.05$)

* Values in brackets designate standard deviations

As the rate of supplementation increased ruminal pH decreased significantly for both maize and barley supplemented animals. There was a significant drop in rumen pH from the control group to 30% and from the control group to 45% supplementation rates with both energy sources. The decrease in rumen pH as the rate of supplementation was increased suggests a possible increase in ruminal acid concentration. These results are supported by the findings of Overton *et al.* (1995) who also found that as the proportion of starch in the diets was increased, ruminal pH decreased linearly.

Although there were no significant differences between the two energy sources, ruminal pH of animals receiving the maize supplement tended to be higher that those receiving barley supplementation (De Visser *et al*., 1992; Overton *et al*., 1995). De Visser (1990), who fed high starch concentrate supplements to animals, found that the total amount of feed negatively influenced conditions for cellulolytic activity (increase in H^+

concentration four hours after feeding). De Visser (1990) also reported that the effects were more pronounced when feeding concentrates high in rumen degradable starch. This could explain the lower ruminal pH values found when using the barley supplement.

The greatest effects of rumen degradable starch content were present at the highest levels of intake and supplementation. Robinson *et al.* (1986) found that, in particular, the diurnal pattern of ruminal pH was negatively influenced and they found a significant longer period of pH values below 6.0 when feeding diets high in ruminal degradable starch. The optimal pH for cellulolytic activity of bacteria in the rumen is near 6.8 (McCarthy *et al*., 1989), and ruminal fibre degradation is decreased as the pH of ruminal fluid declines, especially when it decreases below 6.0.

The diurnal patterns of ruminal pH for the present study are presented in Figure 4.2a and 4.2b. The pH remained relatively stable for a few hours after feeding before it started to decline. In the present study feeding occurred at 9:00. Tamminga (unpublished), as cited by De Visser *et al*. (1992), reported that rumen fluid contents increased sharply after feeding, resulting in the dilution of volatile fatty acids and buffering the pH to decline. The drop in ruminal pH after feeding indicates an increase in the rate of volatile fatty acid production, which cannot immediately be compensated for, by an increase in absorption. Ruminal lactic acid build up could also influence rumen pH of animals receiving carbohydrate supplements. Counotte & Prins (1981) reported that 60 – 80% of lactate produced in the rumen was metabolised by bacteria to acetic, propionic and butyric acids. Lactate utilization decreases as rumen pH declines towards 5 and lactate production might exceed it's utilization in the rumen. As lactic acid concentration increases and pH decreases, lactic acid concentration becomes an important influence on ruminal pH. Briggs *et al.* (1957) reported that lactic acid never accumulates in the rumen after feeding when ruminal pH varies from 5 to 7.5. In the present trial the influence of lactic acid on the ruminal pH of sheep receiving supplemented *A. nummularia* was not investigated, but the ruminal pH of sheep in this trial did not approach a level of 5 or less.

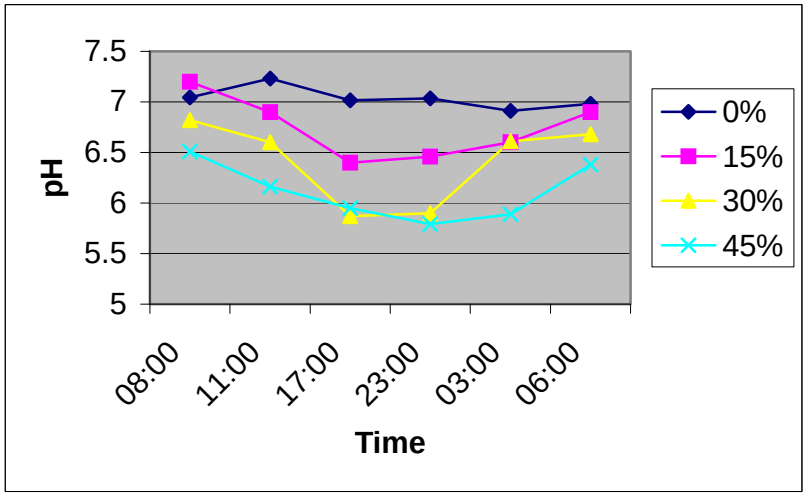

Figure 4.2a Diurnal ruminal pH pattern of sheep fed *A. nummularia* cv. De Kock supplemented with maize

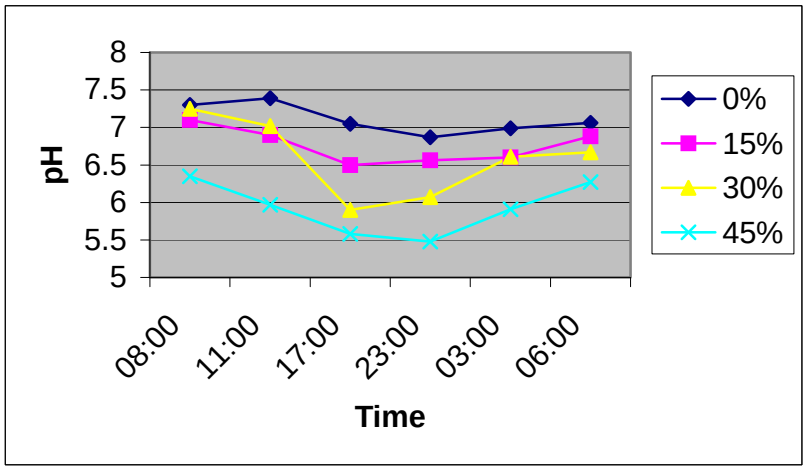

Figure 4.2b Diurnal ruminal pH pattern of sheep fed *A. nummularia* cv. De Kock supplemented with barley

In the present trial ruminal pH only declined below 6 in the 30% and 45% supplementation rates for both the energy sources. The ruminal pH dropped below 6 for six hours when feeding both energy sources at the 30% supplementation rate. The time that pH values were below 6 increased as the rate of supplementation was increased from 30% to 45% for both energy sources. The pH values spend the longest period below 6 for sheep receiving the 45% barley supplement (18 hours) when compared to the animals receiving the 45% maize supplement (10 hours). If the increased fluctuations in ruminal pH reflects changes in rumen fermentation and if such fluctuations leads to inefficiencies, as suggested by Johnson (1972), as cited by Robinson *et al*. (1986), then a tendency for a reduction in the rate of digestion, and the efficiency of microbial growth, at higher levels of energy supplementation, might have been expected.

4.3.7 Volatile fatty acid production

The following table shows the different levels of volatile fatty acids produced in the rumen of sheep fed *A. nummularia* cv. De Kock supplemented with two energy sources at different levels.

Table 4.11 Volatile fatty acid (mmol/ 100mL rumen fluid) production of sheep fed *A. nummularia* cv. De Kock supplemented with two energy sources at different levels of supplementation

Parameters	Rate of supplementation (%)	Maize	Barley
Total volatile fatty acids	0	12.89 $^{a}{}_{1}$(±1.30)	11.09 $^{a}{}_{1}$ (±1.30)
	15	21.55 $^{b}{}_{1}$ (±1.30)	22.45 $^{b}{}_{1}$ (±1.30)
	30	25.42 $^{b}{}_{1}$ (±1.30)	23.32 $^{b}{}_{1}$ (±1.30)
	45	32.49 $^{c}{}_{1}$ (±1.30)	30.71 $^{c}{}_{1}$ (±1.30)
Acetate	0	7.21 $^{a}{}_{1}$ (±0.96)	7.12 $^{a}{}_{1}$ (±0.96)
	15	10.20 $^{b}{}_{1}$ (±0.96)	11.97 $^{b}{}_{1}$ (±0.96)
	30	9.62 $^{b}{}_{1}$ (±0.96)	10.59 $^{b}{}_{1}$ (±0.96)
	45	14.0 $^{c}{}_{1}$ (±0.96)	12.99 $^{c}{}_{1}$ (±0.96)
Propionate	0	3.54 $^{a}{}_{1}$ (±0.45)	2.23 $^{a}{}_{1}$ (±0.45)
	15	7.41 $^{b}{}_{1}$ (±0.45)	7.78 $^{b}{}_{1}$ (±0.45)
	30	8.55 $^{b}{}_{1}$ (±0.45)	7.69 $^{b}{}_{1}$ (±0.45)
	45	10.13 $^{c}{}_{1}$ (±0.45)	10.29 $^{c}{}_{1}$ (±0.45)
Butyrate	0	2.12 $^{a}{}_{1}$ (±0.49)	1.74 $^{a}{}_{1}$ (±0.49)
	15	3.94 $^{a}{}_{1}$ (±0.49)	2.70 $^{a}{}_{1}$ (±0.49)
	30	7.25 $^{b}{}_{1}$ (±0.49)	5.04 $^{b}{}_{2}$ (±0.49)
	45	7.76 $^{b}{}_{1}$ (±0.49)	7.42 $^{c}{}_{1}$ (±0.49)

Column (a,b,c) and row (1,2) means with common scripts do not differ significantly ($p>0.05$)

Values in brackets designate standard deviations

Volatile fatty acids, acetate, propionate and butyrate are the results of anaerobic fermentation of roughages in the rumen of animals and represent the form in which most of the energy in roughages will be absorbed by animals (Van Niekerk, 1997).
On fibre based diets, as in this experiment, acetate will tend to dominate, while it will decline when an animal is fed on a grain based diet (Chandler, 1992).

In this experiment energy supplementation had a significant influence on the production of rumen volatile fatty acids. The 45 % supplementation level in both the energy sources resulted in the highest concentration of total volatile fatty acids. There were no significant differences found between the two energy sources for total volatile fatty acid production. As the level of supplementation increased there were significant differences found between the control group and the 15 %, 30 % and 45 % supplementation levels for both the energy sources. The increase in total volatile fatty acid production as the level of supplementation was increased corresponds with the results for rumen pH reported earlier which showed that rumen pH declined with an increase in the level of supplementation. There was also a significant increase in the total volatile fatty acid concentration when the level of supplementation was increased from 30 % to 45 % for both maize and barley.

There was a significant increase in the acetate concentration as the level of supplementation was increased from the control group to the 45 % supplementation level in both the energy sources. McCarthy *et al.* (1989) reported a decrease in the molar proportion of acetate and an increase in the molar proportion of propionate with an increase in the feeding of rumen degradable starch to animals. This was also found in the present trial where the molar proportion of acetate decreased from 55.9 % and 64.2 % for maize and barley respectively to 43.1 % and 42.3 %.

The molar proportions of the different volatile fatty acid produced in the rumen of sheep fed *A. nummularia* cv. De Kock supplemented with two energy sources at different levels are presented in Table 4.12.

Table 4.12 Molar proportions (%) of ruminal volatile fatty acids produced by sheep fed *A. nummularia* cv. De Kock supplemented with two energy sources at different levels

Parameters	Rate of supplementation (%)	Maize	Barley
Acetate	0	55.9	64.2
	15	47.3	53.3
	30	37.8	45.4
	45	43.1	42.3
Propionate	0	27.5	20.1
	15	34.4	34.7
	30	33.6	33
	45	31.2	33.5
Butyrate	0	16.4	15.7
	15	18.3	12
	30	28.5	21.6
	45	23.9	24.2

The concentration of propionate in the rumen increased as the level of supplementation of both the energy sources increased significantly compared to the control, from 27.5 % and 20.1 % for maize and barley respectively to 31.2 % and 33.5 % (Table 4.11). There was also a significant increase in propionate concentration as the level of supplementation was increased from 30 % to 45 % in both the energy sources. The molar proportion for propionate peaked at the 15 % supplementation rate for both the energy sources at 34.4 % for maize and 34.7 % for barley. There were no significant differences found between the two energy sources for ruminal propionate concentration. The molar proportions of propionate increased from the control group to all the levels of supplementation but the incremental increase decreased as the level of supplementation increased (Table 4.12).

The results for acetate and propionate agrees with results reported by previous authors, who also found that energy supplementation decreases the molar proportion of acetate and increases the molar proportion of propionate in the rumen (McCarthy *et al.,* 1989; Bodine *et al.,* 1999; Casper *et al.,* 1999).

The results for butyrate followed similar tendencies, as for propionate, in the present trial. The ruminal butyrate concentration increased as the level of supplementation increased from 0 % to 45 % in both the energy sources (Table 4.11). The butyrate concentration showed a significant difference between maize and barley at the 30 % level of supplementation. There were no significant differences between the 0 % and 15 % level of supplementation and the 30 % and 45 % levels of supplementation levels in both the energy sources. However, the present results show a significant increase in the butyrate concentration as the level of supplementation was increased from 15 % to 30 % in both the energy sources. These results are similar to results reported by Bodine *et al.* (1999) who reported that rumen butyrate concentration increased with an increase in energy supplementation.

Van Vuuren *et al.* (1990) reported that maize starch is less rumen degradable than barley starch. This might be a contributing factor to the higher rumen ammonia-N and pH values found in maize supplemented animals when compared to barley fed animals reported earlier. The lower rumen pH in barley supplemented animals suggests a higher total rumen volatile fatty acid concentration in barley supplemented animals. The results reported in Table 4.11 do not agree with this statement. Table 4.11 shows that barley supplemented animals had lower total rumen volatile fatty acids concentrations at both the 30 % and 45 % supplementation levels. Animals receiving the barley supplemented diets could possibly have had higher ruminal lactic acid concentrations which could explain the lower rumen pH values found for these animals. Sutton and Jones (1969) reported that the proportions of volatile fatty acids in the rumen are more closely related to rumen pH than to the type of ration. The diurnal pattern for rumen pH in the present experiment dropped below 6 for six hours when feeding both the energy sources at the 30 % supplementation rates. The time that pH values were below six increased as the level

of supplementation increased from 30 % to 45 %. However, the time that pH values were below 6 was higher for sheep receiving the barley supplement (18 hours vs. 10 hours). The lower total rumen volatile fatty acid concentration in the barley supplemented animals could be explained by greater fluctuations in ruminal pH. These fluctuations reflects changes in rumen fermentation and such fluctuations could lead to inefficiencies and a tendency for a reduction in the rate of digestion and efficiency of microbial growth at higher levels of supplementation.

Casper *et al.* (1999) reported that although barley had a greater rate of non structural carbohydrate degradability, it did not result in greater ruminal volatile fatty acid concentrations when compared to maize. These authors reported that this might have been due to greater ruminal liquid volumes of animals fed barley based diets and the greater rate of non-structural carbohydrate degradability might not have translated into greater volatile fatty acid production. In the present trial animals receiving the barley supplement had greater water intakes at the 30 % level of supplementation.

4.4 Conclusion

Supplementation of *A. nummularia* cv. De Kock with an energy source tended to increase intake of feed and decrease water intake. The tendency of energy sources to increase DM and NDF digestibility diminished when the supplemental level was raised from 15% to 30% and from 30% to 45%. These results suggested that barley and maize supplementation at a level of 15% gave the highest incremental increase in DM and NDF digestibility in *A. nummularia* cv. De Kock. Negative associative effects occurred in the rumen at supplemental levels of 30% and higher.

As *A. nummularia* cv. De Kock was supplemented with an energy source the ruminal ammonia nitrogen concentration increased from the control to the 15 % supplementation rates. There was a significant drop in the rumen ammonia nitrogen concentration at the 30 % supplementation rate for both the energy sources. Ruminal pH decreased as the

rates of supplementation increased from the control to 45 %. Barley supplemented animals had a numerical lower rumen pH. The drop in rumen pH supports the increase in rumen volatile fatty acid production as the level of energy supplementation increased. These results suggest that barley and maize supplementation, at a level of 30 %, is optimal for microbial protein synthesis without significantly affecting fibre degradation.

Chapter 5

The effects of two different carbohydrate sources on rumen kinetics and degradability of *Atriplex nummularia* cv. De Kock fed to sheep at different levels.

5.1 Introduction

Nutrient supply and, as a result, production in sheep is influenced by the capacity of the rumen to clear the ingested feed (rumen contents x clearance rate). Ingested feed can be cleared from the rumen either by microbial degradation or by passage to the lower gut (Bosch & Bruining, 1995). The rate of clearance of rumen contents has been shown to depend on the chemical and physical composition of the feedstuff, the rate of microbial degradation, particle size and pH in the rumen (Sutherland, 1987, as cited by Bosch & Bruining, 1995).

A major dietary factor in determining sheep production from *Atriplex* is the voluntary feed intake (VFI) of *Atriplex* diets. The rumen has been identified as the compartment in the alimentary tract associated with constraints on forage intake. This constraint relates to the rate of clearance of particulate matter, as affected by digestion and by transfer to the omasum. The digestion rate of fibre is usually less than 0.08/h (i.e. 8% of the fibre pool is digested per hour) and thus feed particles need to be retained in the rumen for a significant time period to permit adequate fibre degradation (Weston, 2000). Weston (2000) reported a direct relationship between forage intake and rate of clearance of organic matter from the rumen. This indicates that clearance rate can act as an intake constraint.

The rate of removal of organic matter from the rumen is determined by both the characteristics of the forage consumed and the physiological processes controlling digesta transactions in the rumen. Slow removal of organic matter from the rumen prevails with forages having a high resistance to degradation by chewing and a high content of components –generally fibre constituents- that are relatively slowly digested by the

rumen microbes (Weston, 2000). Ulyatt *et al.* (1986), as cited by Bosch *et al.* (1992), reported that microbial degradation hardly influences particle size.
It may, however, weaken the cell wall structure, so that particle breakdown during rumination is facilitated (Chai *et al.,* 1984). Bosch *et al*. (1992) stated that chewing during eating and rumination are the main factors involved in particle size reduction. Rumination time per kg DM ingested increases with an increase in cell wall content (Ulyatt *et al.,* 1986 as cited by Bosch *et al.,* 1992).

Studies with annual plants (grasses) have shown that the VFI of stem material is always lower than that of leaf fraction (Poppi *et al.*, 1981), probably because stem material is difficult to break down during mastication and rumination. In *Atriplex* species the impact of the stem fraction on VFI can be even more important, than with grasses, because of the degree of lignification of stems, which increases with the maturity of plants (Atiq-ur-Rheman, 1995). Lignin has a number of functions, which are essential for plants, the most important are in the structural integrity of plants and probably to act as a shield for cellulose and hemicellulose from microbial and enzymatic attack (Theander & Aman, 1984). The utilization of perennial plants by sheep, therefore, can be limited by stem and by its degree of lignification, which increases resistance to biodegradation in the rumen. The purpose of the present experiment was, however, to quantify the influence of type and level of carbohydrate supplementation on the digestibility and ruminal kinetics of *A. nummularia* cv. De Kock fed to sheep.

5.2 Materials and methods

The rumen kinetics trial was conducted at the Hatfield Experimental Farm of the University of Pretoria, South Africa. Six mature rumen canulated Merino wethers were used in the kinetics trial. The animals were randomly allocated to two groups of three animals per group, each group receiving a different treatment during each experimental period. The trial ran for four sequential experimental periods and the two groups of animals stayed constant for each experimental period.

The *Atriplex* material used in the trial was harvested from a stand established in the early 1990's. Since establishment it had been heavily grazed by sheep, as a drought fodder, during winter. The material was harvested a month after the animals were withdrawn from the stand and contained a high percentage of stems with a diameter of greater than 6 mm. The harvested material consisted mainly of material that was not selected by sheep during the grazing season.

The basal diet for the kinetics trial consisted of *A. nummularia cv*. De Kock. In addition to the control diet of 100% *Atriplex*, 15%, 30% and 45% of maize and barley was added to the basal diet on a dry matter basis for each of the four experimental periods. To prevent particle selection all the diets were offered as total mixed rations.

During each experimental period the animals were adapted to the experimental diets for 21 days before the collection period of 3 days commenced. The animals were injected with a vitamin supplement at the start of each period and each animal's initial and end weight was recorded for each sequential experimental period. Individual feed intakes were recorded daily by weighing the feed offered as well as the orts. The quantity of feed offered each day was adjusted to ensure that the feed troughs contained feed throughout the day. Samples of feed offered and orts were collected every morning during each collection period and pooled for proximate analysis (A.O.A.C., 2000).

The animals received fresh water daily on an *ad-lib* basis and the remaining water was measured back and recorded before feeding commenced the next morning. Water consumption of individual animals was recorded daily for each collection period and it was corrected for evaporation losses. Maximum and minimum temperature fluctuations during each period of data collection were also recorded daily.

Rumen contents, of liquid and dry matter, were estimated from ingesta manually removed at 11:00, 21:00 and 01:00 on three consecutive days while the animals had free access to feed and water. The rumen content was removed according to the method described by

Robinson *et al*. (1987). All rumen contents that could be removed by hand were emptied into a 50 l insulated cooler container with a lid and continuously flushed with CO_2. This material, referred to as mat, was weighed and sub-sampled (about 500g regardless of total mass). Two sub-samples were taken at each sampling time. Material not removed by hand was bailed into a similar 50 l insulated container, covered with a lid into which a large funnel had been fitted. This container was also continuously flushed with CO_2. This material, referred to as bailable liquid, was weighed and sub-sampled roughly in proportion to volume (about 500 ml). The liquid was then returned to the rumen followed by mat. All samples were frozen and rumen dry matter and non-dry matter was calculated for each time of incubation (Robinson *et al*., 1987).

The first two samples taken at each sampling time were freeze-dried and analysed for DM, N and NDF. The second was washed through a sieve with a pore size of 2 mm under cold tap water until the water that washed out was clear. The residue that remained on the sieve was freeze-dried and analysed for DM, N and NDF. Total rumen pool and pool of particles larger than 2 mm (DM, NDF and N) were measured, while the pool of particles smaller than 2 mm was calculated by subtraction. The rate of clearance of DM, N and NDF of both the total rumen pool and pool of particles larger than 2 mm was calculated as - rate of clearance (K_c)= ((feed intake, kg/d)/ (average rumen pool, kg/d))/ 24. The rate of clearance for DM, N and NDF were all calculated using the same equation (De Visser *et al.,* 1992).

5.3 Statistical analysis

An analysis of variance with the Proc GLM model (SAS, 1994) was used to determine the significance between treatments and different levels of supplementation. Least square means and standard deviations (SD) were determined. Significance of difference (5%) between least square means was determined by using Bonferroni's test (Samuels, 1989).

5.4 Results and discussion

The inclusion of concentrates in diets changes the total dry matter intake, particle size distribution of rumen content and often the chemical composition of the diet (De Visser *et al*., 1992). Changes in the chemical composition of the diet are reflected in the degradability of the organic matter, fermentation pattern and kinetics of particle size digestion and passage in the rumen (Robinson *et al*., 1987). De Visser *et al*. (1992) reported a reduction in feed intake when feeding an increased proportion of rapid fermentable carbohydrates to animals. These authors also reported a shift in rumen fermentation when feeding diets high in rapid fermentable carbohydrates toward propionic acid.

When forage quantity and quality limits animal production and performance, supplementation with either protein or energy may be required to increase or maintain a certain level of animal production (Bodine *et al*., 1999). *Atriplex nummularia* has a relatively high crude protein content, which ranges from 10 % to 32 % (Hassan *et al*., 1972; Hassan & Abdel-Aziz, 1979; Correal *et al*., 1990). The main limiting nutrient when feeding *A. nummularia* is energy. Weston (1966) reported metabolizable energy values for *A. nummularia* of 6.1 MJ/ kg DM.

The effect of energy supplementation on the ruminal kinetics of sheep fed *A. nummularia* cv. De Kock is reported in Table 5.1

Table 5.1 The effect of energy supplementation on the dry matter intake, body weight and dry matter pool of sheep fed *A. nummularia* cv. De Kock

	% Rate of Supplementation	Maize	Barley
DM intake/ kg body weight (g)	0	22.96[a] $_1$ (±3.36)	22.69 [a] $_1$ (±3.76)
	15	27.51 [a] $_1$ (±3.35)	26.34 [a] $_1$ (±3.80)
	30	40.21[b] $_1$ (±10.30)	36.99 [a] $_1$ (±15.89)
	45	33.76 [a] $_1$ (±4.20)	29.14 [a] $_1$ (±4.66)
Body weight (kg)	0	55.5	55.5
	15	61	52.8
	30	57.17	41.8
	45	66.77	56.77
DM pool/ kg body weight (g)	0	59.30[a] $_1$ (±33.56)	59.30 [a] $_1$ (±33.56)
	15	64.61 [a] $_1$ (±16.77)	79.32 [a] $_1$ (±7.56)
	30	92.26 [a] $_1$ (±34.67)	93.26 [a] $_1$ (±21.91)
	45	46.69 [a] $_1$ (±12.23)	52.85 [a] $_1$ (±10.14)

[a,b] Column means with common super scripts do not differ significantly

$_{1,2}$ Row means with common subscripts do not differ significantly

Values in brackets designates standard deviations

The supplementation of *A. nummularia* with energy sources tended to increase dry matter intake of sheep with 30% maize being the only significant increase. There were no further significant differences found between the two energy sources used in the present trial, although maize tended to result in higher dry matter intakes per kg body weight, for each level of supplementation. This slightly higher intake found with maize supplementation could possibly be due to the fact that maize has a higher percentage of rumen resistant starch than barley. De Visser *et al.* (1992) reported *in vitro* and *in vivo* values for rumen resistant starch of maize and barley. The *in vitro* values were 13% and 45% for barley and maize respectively while the *in vivo* values were 26 % and 34 % for barley and maize respectively. Nocek & Tammanga (1991) reported that the rate of degradation of the potentially degradable starch fraction of barley to be 24.2 %/ h

compared to 4.0 %/h of maize. The higher rate of degradation of barley starch and the higher percentage of rumen degradable starch found in barley could lead to a lower ruminal pH that might have a negative effect on DM intake of sheep.
In both energy treatments the DM intake increased from 0 % to 15 % and from 15 % to 30 % supplementation rates, but decreased from the 30 % to 45 % supplementation level. The decrease in dry matter intake could possibly be due to negative associative effects occurring in the rumen. Mould *et al*. (1983) reported a decrease in dry matter intake due to negative associative effects when barley was used as an energy supplement for hay. In the maize treatment there were significant increases in intake from 0 % to 30 % supplementation and from 15 % to 30 % supplementation levels.

The results obtained for DM intake in the present study are supported by McCarthy *et al.* (1989) and Overton *et al.* (1995), who reported a tendency for higher DM intakes of animals receiving maize based diets when compared to barley based diets. These authors also reported no significant differences between the intakes of animals receiving diets based on maize and barley.

Increasing the amount of carbohydrate in diets will result in an increase in organic matter intake (Overton *et al*., 1995). Data from the present study supports this statement. Dry matter intake increased from 22.96 to 27.51 g/ kg body weight and 22.69 to 26.34 g/ kg body weight per day for maize and barley respectively. The increase in dry matter intake when compared to the control diets may be due to the higher digestibilities of the carbohydrate sources.

The ruminal dry matter pool per kg body weight followed the same pattern as DM intake/ kg body weight. The fill or pool size of a component varies with its potential digestibility (Galyean & Owens, 1988). The dry matter pool/ kg body weight increased as the rate of supplementation increased and the digestibility of the diets increased except for the 45 % supplementation rate where the dry matter pool was lower than the control diets. This may be due to negative associative effects that decreased the apparent digestibility of the 45 % supplementation diets. There were no significant differences in DM pools between

the maize and barley treatments and between the different levels of supplementation within the treatments. The DM pool decreased with 49 % and 43 % respectively for maize and barley treatments from the 30 % to the 45 % supplementation levels from 92.26 to 46.69 and 93.26 to 52.85 g/ kg body weight (compared to a decrease in DM intake of 16 % and 21 % for maize and barley respectively) for the 30 and 45 % levels of supplementation respectively.

Table 5.2 The effect of different carbohydrate sources and levels on the total rumen bailable liquid volumes, rumen mat, total digesta and percentage dry matter of sheep fed *A. nummularia* cv. De Kock

Total Rumen contents			
	% Rate of Supplementation	**Maize**	**Barley**
Bailable liquid/ kg body weight (g)	0	78.52$^{a}{}_{1}$(±12.49)	78.52$^{a}{}_{1}$ (±12.49)
	15	100.0$^{a}{}_{1}$ (±54.78)	119.69$^{a}{}_{1}$ (±8.48)
	30	75.98$^{a}{}_{1}$ (±3.5)	58.39$^{a}{}_{1}$ (±13.14)
	45	88.46$^{a}{}_{1}$ (±48.46)	60.47$^{a}{}_{1}$ (±24.58)
Mat material/ kg body weight (g)	0	64.25$^{a}{}_{1}$ (±30.01)	64.25$^{a}{}_{1}$ (±36.01)
	15	70.41$^{a}{}_{1}$ (±18.68)	86.24$^{a}{}_{1}$ (±7.14)
	30	101.53$^{a}{}_{1}$(±37.09)	101.40$^{a}{}_{1}$ (±23.07)
	45	50.98$^{a}{}_{1}$ (±13.62)	57.73$^{a}{}_{1}$ (±10.81)
Total ingesta/ kg body weight (g)	0	137.82$^{a}{}_{1}$(±41.33)	137.82$^{a}{}_{1}$(±41.33)
	15	164.61$^{a}{}_{1}$(±38.46)	199.01$^{a}{}_{1}$(±16.01)
	30	168.24$^{a}{}_{1}$(±31.18)	151.66$^{a}{}_{1}$(±32.71)
	45	135.15$^{a}{}_{1}$(±38.56)	113.33$^{a}{}_{1}$(±34.67)
Ruminal DM (%)	0	41.13$^{a}{}_{1}$ (±12.18)	38.13$^{a}{}_{1}$ (±8.73)
	15	42.02$^{a}{}_{1}$ (±17.84)	39.83$^{a}{}_{1}$ (±0.60)
	30	53.65$^{a}{}_{1}$ (±9.67)	61.47$^{a}{}_{1}$ (±3.85)
	45	37.35$^{a}{}_{1}$ (±16.08)	47.81$^{a}{}_{1}$ (±5.76)

^{a,b} Column means with common super scripts do not differ significantly

$_{1,2}$ Row means with common subscripts do not differ significantly

Values in brackets designates standard deviations

Ruminal bailable liquid, mat material, total ingesta and dry matter percentage did not differ significantly between the different energy sources or supplementation rates. Ruminal liquid (Table 5.2) reached a peak at the 15 % supplementation rate of both the carbohydrate sources. Animals receiving the maize treatment had slightly higher rumen liquid volumes in g/kg body weight, except at the 15 % supplementation rate where the barley treatment registered higher volumes. These results differ from those reported by De Visser *et al*. (1992), who found significantly higher rumen liquid volumes in animals receiving barley based diets than in those receiving maize based diets. The decrease in rumen liquid volumes in both energy treatments could partially be explained by the smaller component of *Atriplex* in the diet as the rate of supplementation was increased. The results for bailable liquid followed the same pattern as the results for water intake per kg body weight found in an earlier trial (Chapter 4).

Rumen mat material is defined as all solid material that could be removed from the rumen by hand during the sampling period. The results show that, as the level of supplementation increased from 0 % to 30 %, the amount of mat material (Table 5.2) increased from 64.25 g/kg body weight, in the control groups, to 101.53 and 101.40 g/kg body weight, for maize and barley groups respectively. When supplementation increased from 30 % to 45 % in both the energy sources, however, there was a sharp decline in mat material. This decline was also evident in the DM intake results, that were discussed earlier, and could possibly be as a result of negative associative effects that affected the intake. There were no significant differences between the two energy sources, although barley tended to have higher rumen mat material weights than maize. These results are supported by De Visser *et al.* (1992) who also found higher mat material weights in animals receiving barley based diets when compared to maize based diets. Although the animals in the barley treatment had higher weights for rumen mat material, these animals had lower intakes per kg body weight when compared to the maize treatment. The lower intakes and higher rumen mat material values suggests a slower breakdown of fibre in the animals receiving the barley treatment when compared to animals receiving the maize treatment. The results for total ruminal ingesta (Table 5.2) followed the same pattern as

the results for bailable liquid and rumen mat material. There were no significant differences found between the two energy sources for total ingesta although the animals receiving maize as energy supplement tended to have a higher total ingesta, except for the 15 % supplementation level where the barley treatment had higher total ingesta values. There were also no significant differences between different levels of supplementation within each energy treatment.

The data for ruminal dry matter percentage is also presented in Table 5.2. Although there were no significant differences between the two energy sources for rumen DM percentage, animals receiving maize as supplement tended to have a slightly higher ruminal DM percentage than the barley supplemented animals at the 0 % and 15 % supplementation rates. This corresponds well with the higher DM intakes of the animals receiving the maize supplement at the same levels of supplementation. In contrast De Visser *et al*. (1992) found that animals receiving barley based diets had higher ruminal DM percentages than animals receiving maize based diets (not significant). As the rates of supplementation increased to 30 % and 45 % the barley supplemented animals had higher rumen DM percentages. The higher rumen DM percentages found at the 30 % and 45 % supplementation rates correspond with the higher rumen mat material for animals receiving the barley supplements and the lower intakes of animals receiving barley. The higher percentage of rumen DM found with the barley supplement suggests a slower rate of degradation of fibre material which will result in lower intakes, as can be seen with the DM intake per kg body weight data shown in Table 5.1.

Table 5.3 The effect of different carbohydrate sources and levels on the total rumen pools of dry matter, neutral detergent fibre and nitrogen of sheep fed *A. nummularia* cv. De Kock

Rumen pool sizes			
	% Rate of Supplementation	**Maize**	**Barley**
DM/ kg body weight (g)	0	59.30 $^{a}{}_{1}$ (±33.56)	59.30 $^{a}{}_{1}$ (±33.56)
	15	64.61 $^{a}{}_{1}$ (±16.77)	79.32 $^{a}{}_{1}$ (±7.56)
	30	92.26 $^{a}{}_{1}$ (±34.67)	93.26 $^{a}{}_{1}$ (±21.91)
	45	46.69 $^{a}{}_{1}$ (±12.23)	52.85 $^{a}{}_{1}$ (±10.14)
NDF/ kg body weight (g)	0	49.79 $^{a}{}_{1}$ (±28.11)	49.88 $^{a}{}_{1}$ (±28.10)
	15	51.78 $^{a}{}_{1}$ (±14.82)	62.41 $^{a}{}_{1}$ (±7.77)
	30	69.28 $^{a}{}_{1}$ (±27.66)	68.77 $^{a}{}_{1}$ (±11.73)
	45	31.51 $^{a}{}_{1}$ (±7.50)	35.30 $^{a}{}_{1}$ (±7.93)
N/ kg body weight (g)	0	0.65 $^{a}{}_{1}$ (±0.36)	0.65 $^{a}{}_{1}$ (±0.36)
	15	1.17 $^{a}{}_{1}$ (±0.24)	1.46 $^{a}{}_{1}$ (±0.14)
	30	1.39 $^{a}{}_{1}$ (±0.64)	1.33 $^{a}{}_{1}$ (±0.36)
	45	0.92 $^{a}{}_{1}$ (±0.23)	1.04 $^{a}{}_{1}$ (±0.20)

^{a,b} Column means with common super scripts do not differ significantly

$_{1,2}$ Row means with common subscripts do not differ significantly

Values in brackets designates standard deviations

The total dry matter pool in the rumen tended to be slightly higher in animals receiving the barley supplemented diet (not significant). There were also no significant differences in the total rumen pools of dry matter, neutral detergent fibre and total nitrogen between the two different carbohydrate sources. The NDF pool increased as the level of supplementation increased in both the carbohydrate sources from 0 % to 30 %. At the 45 % supplementation there was a sharp drop in the NDF pool from the 30% level, of between 49 % and 55 % for maize and barley respectively. No significant differences were, however, found between the different levels of supplementation of either of the

carbohydrate sources. The decrease in the size of the NDF pool at the 45 % supplementation rate could be due to the decrease in intake noted when the 45 % supplemented diets were fed to the animals in both energy treatments. At the 45 % supplementation rate the animals received the least amount of *A. nummularia* in their diets. It can, therefore, be expected that the 45 % supplementation diets contain the least amount of NDF. The lower percentage of NDF in the diet and the lower intakes, found when the animals received the 45 % supplemented diets, could therefore, be the reason for the large difference in total NDF pools between the 30 % and 45 % supplementation rates.

The total nitrogen pools followed the same pattern as the DM and NDF pools with an increase in pool size from 0 % to 30 % supplementation, for both the carbohydrate sources, and then a decrease as the level of supplementation increased to 45 %. There were also no significant differences found for the total nitrogen pool between the two different carbohydrate sources and between the different levels of supplementation. Animals receiving the barley treatment tended to have a larger total nitrogen pool when compared to the maize treatment, except at the 30 % level of supplementation, where the total nitrogen pool for the maize treatment was 0.06 g/kg body weight larger.

The results for the rumen pool of large (>2mm) particles is reported in Table 5.4. These values are higher than expected when compared with values cited in the literature (De Visser *et al.,* 1992). In a previous trial these authors made use of wilted grass silage and maize silage as sources of roughage. The values in the present trial may be higher because of a higher fibre content and a slower rate of degradation of the diets due to the *A. nummularia* content when compared to that of De Visser *et al.* (1992). The size of particles leaving the rumen decreased when the proportion of concentrate in the diet was increased, due to a bigger pool of small particles probably caused by a smaller initial size of the concentrate diets (Bosch *et al.,* 1992). The pool of small particles (<2mm) in the present trial is smaller than what was expected. This could be because of separation of the carbohydrate and roughage components in the feed troughs, and animals consuming mainly roughage even at the higher levels of supplementation. Individual animal

selection for the roughage component could also play a part in the size of the small particle pool. Small particles can get trapped in the floating material and their gas content will influence their fundamental specific gravity (Bosch *et al.,* 1992).

Table 5.4 The effect of different carbohydrate sources and levels on the large particle pool of dry matter, neutral detergent fibre and nitrogen of sheep fed *A. nummularia* cv. De Kock

Rumen pool sizes of large particles (>2mm)			
	% Rate of Supplementation	**Maize**	**Barley**
DM/ kg body weight (g)	0	57.69 $^{a}{}_{1}$ (±32.99)	57.69 $^{a}{}_{1}$ (±32.99)
	15	63.60 $^{a}{}_{1}$ (±16.90)	73.67 $^{a}{}_{1}$ (±3.16)
	30	90.55 $^{a}{}_{1}$ (±34.21)	91.63 $^{a}{}_{1}$ (±20.77)
	45	46.18 $^{a}{}_{1}$ (±11.86)	51.90 $^{a}{}_{1}$ (±9.69)
NDF/ kg body weight (g)	0	45.27 $^{a}{}_{1}$ (±26.01)	45.27 $^{a}{}_{1}$ (±23.73)
	15	44.94 $^{a}{}_{1}$ (±11.55)	51.82 $^{a}{}_{1}$ (±47.18)
	30	56.08 $^{a}{}_{1}$ (±16.32)	57.46 $^{a}{}_{1}$ (±47.85)
	45	25.82 $^{a}{}_{1}$ (±9.14)	32.51 $^{a}{}_{1}$ (±25.97)
N/ kg body weight (g)	0	0.47 $^{a}{}_{1}$ (±0.26)	0.47 $^{a}{}_{1}$ (±0.26)
	15	0.86 $^{a}{}_{1}$ (±0.17)	1.07 $^{a}{}_{1}$ (±0.22)
	30	0.96 $^{a}{}_{1}$ (±0.59)	1.03 $^{a}{}_{1}$ (±0.29)
	45	0.71 $^{a}{}_{1}$ (±0.16)	0.78 $^{a}{}_{1}$ (±0.09)

^{a,b} Column means with common super scripts do not differ significantly

$_{1,2}$ Row means with common subscripts do not differ significantly

Values in brackets designates standard deviations

There were no significant differences between the different carbohydrate sources and between the different levels of supplementation within each energy treatment for the pool of large (>2mm) particles.

The rumen pool of small particles (<2mm) was calculated as the difference between total rumen pool size and large particle pool size (>2mm). As the level of supplementation increased from 0 % to 30 % the small particle (< 2mm) pool for NDF increased by 10 % and 7 % for maize and barley respectively, from 4.52 to 13.2 g/kg body weight and 4.61 to 11.31 g/kg body weight. The increase in the small particle pool may be due to an increased rate of degradation as the rate of supplementation increased to 30 %. As the rate of supplementation was increased so did the proportion of small particles in the diet. As the level of supplementation increased from 30 % to 45 %, for both carbohydrate sources, the size of the small particle pool decreased from 13.2 to 5.69 g/kg body weight and 11.31 to 2.79 g/kg body weight for maize and barley respectively. This is possibly due to a decrease in intake of small particles and a decrease in the rate of degradation due to negative associative effects occurring in the rumen. Gazza *et al*. (1991) reported that higher levels of concentrate supplementation reduced the fractional rate of digestion of fibre and tended to reduce that of NDF. These authors found that lag time to be longer with a larger amount of concentrate in the diet.

The nitrogen pool of large particles (> 2mm) presented in Table 5.4 increased as the rate of supplementation of both carbohydrate sources increased, although this was not significant. The nitrogen pool peaked at the 30 % supplementation level for maize with 0.96 g/kg body weight and at the 15 % supplementation level for barley with 1.07 g/kg body weight. As the level of supplementation increased from 30 % to 45 %, for both the carbohydrates sources, the size of the nitrogen pool (> 2mm) decreased markedly, but it was still larger than that of the control diets. The size of the large nitrogen pool stayed relatively constant as the levels of supplementation increased from 0 % to 45 % for both the carbohydrate sources. The large particle pool for nitrogen varied from 72.3% of the total nitrogen pool, in the control group, to 77.2% and 75% for maize and barley respectively at the 45 % supplementation rate. The small particle pool for nitrogen was smaller that expected.

This suggests that the protein and non-protein nitrogen of *A. nummularia* is rapidly degraded in the rumen and that the values for nitrogen reported in Table 5.3 and Table 5.4 is for nitrogen of dietary origins and not that of ammonia and microbial protein.

5.5 Ruminal clearance rates for DM, NDF and N

Nutrient supply and, as a result, production in animals is influenced by the capacity of the rumen to clear the ingested feed (rumen content × clearance rate). The ingested feed can be cleared from the rumen either by microbial degradation or by passage to the lower gut. The clearance rate of DM from the rumen is the sum of disappearance due to passage and due to degradation. The rate of clearance of rumen contents has been shown to depend on the chemical and physical composition of the feedstuff, the rate of microbial degradation, particle size and pH in the rumen (Bosch & Bruining, 1995).

The results for the clearance rate of total rumen dry matter, neutral detergent fibre and nitrogen are presented in Table 5.5.

Table 5.5 The effect of different carbohydrate sources and levels of supplementation on the ruminal clearance rate (K_c, g/kg.h^{-1}) of DM, NDF and N of sheep fed *A. nummularia* cv. De Kock

		Energy Sources	
Ruminal clearance rates	**Rate of supplementation**	**Maize**	**Barley**
DM	0	0.0187$^{a}{}_{1}$(±0.007)	0.0187 $^{a}{}_{1}$ (±0.007)
	15	0.0190 $^{a}{}_{1}$ (±0.008)	0.137 $^{a}{}_{1}$ (±0.003)
	30	0.180 $^{a}{}_{1}$ (±0.002)	0.0170 $^{a}{}_{1}$ (±0.003)
	45	0.0317 $^{a}{}_{1}$ (±0.010)	0.024 $^{a}{}_{1}$ (±0.006)
NDF	0	0.0370 $^{a}{}_{1}$ (±0.007)	0.030 $^{a}{}_{1}$ (±0.005)
	15	0.127 $^{a}{}_{1}$ (±0.006)	0.009 $^{a}{}_{1}$ (±0.002)
	30	0.0120 $^{a}{}_{1}$ (±0.001)	0.011 $^{a}{}_{1}$ (±0.001)
	45	0.0170 $^{a}{}_{1}$ (±0.005)	0.013 $^{a}{}_{1}$ (±0.004)
N	0	0.027 $^{a}{}_{1}$ (±0.010)	0.027 $^{a}{}_{1}$ (±0.010)
	15	0.016 $^{a}{}_{1}$ (±0.004)	0.012 $^{a}{}_{1}$ (±0.002)
	30	0.026 $^{a}{}_{1}$ (±0.003)	0.026 $^{a}{}_{1}$ (±0.005)
	45	0.027 $^{a}{}_{1}$ (±0.009)	0.021 $^{a}{}_{1}$ (±0.005)

^{a,b} Column means with common super scripts do not differ significantly

$_{1,2}$ Row means with common subscripts do not differ significantly

Values in brackets designates standard deviations

In the present trial animals receiving the maize treatment tended to have slightly higher DM clearance rates, when compared to the barley treatment, although there were no significant differences found between the two different carbohydrate sources. Bosch & Bruining (1995) defined the rate of clearance as the sum of disappearance due to passage and degradation. These authors found that as the fibre content in a diet was increased the rate of clearance from the rumen decreased and reported clearance rates of 0.0422 and 0.0442 for first cut and second cut grass silage from the same stand (first cut silage being

higher in fibre than second cut). The result in the present study supports the result reported by Bosch & Bruining (1995). The results in the present study differ, however, from results reported by De Visser *et al.* (1992), where the latter authors reported higher clearance rate for barley based diets, when compared to maize based diets (not significantly). The higher clearance rates for maize supplemented animals could be explained by the lower ruminal degradability of maize when compared to barley (De Visser, 1990). The lower ruminal degradability of maize will result in a more stable rumen pH, which will improve fibre degradability in the rumen. In order for particles to pass out of the rumen, particles must be transferred to the floor of the reticulum (Ehrlein, 1979, as cited by Gazza *et al.,* 1991). The density of particles must be sufficiently high to allow them to settle. Newly ingested feed particles generally have a low functional density, due to chemical composition, trapped air and gas formed by microbial fermentation. It could be that maize particles had a higher functional density than barley particles. The large particle mat in the rumen where smaller particles get trapped, will also influence the probability of smaller particles leaving the rumen.

The clearance rates for neutral detergent fibre decreased for both energy treatments as the level of supplementation increased from 0 % to 45 %. The smaller proportion of *A. nummularia* in the diets as supplementation increased could partially explain this. There were no significant differences in the NDF clearance rates between the two different carbohydrate sources, although the maize treatment tended to have a higher NDF clearance rate. In the present study the NDF clearance rate decreased (not significantly) from 0.037 to 0.017 and 0.030 to 0.013 for maize and barley respectively, as the level of supplementation was increased from 0 % to 45 %. Gazza *et al.* (1991) reported that higher levels of concentrate supplementation reduced the fractional rate of digestion of fibre and tended to reduce that of NDF. These authors found that lag time, defined by Givens *et al.* (2000) as the initial lag phase due to the inability of the rumen microbial population and its enzymes to degrade a substrate at a significant rate until microbial growth is sufficient for enzyme production to increase and ultimately saturate a substrate, tended to be longer with a larger amount of concentrate in the diet although this was not significant. The increase in lag time as the level of concentrate in the diet is increased

could also partially explain the decrease in the NDF clearance rates found in this trial. There were no significant differences found between the different carbohydrate sources and between the different levels of supplementation, within each treatment, for nitrogen clearance rates.

5.6 Conclusion

The supplementation of *A. nummularia* cv. De Kock with an energy source tended to increase dry matter intake and rumen fill. Rumen pools of dry matter, neutral detergent fibre and total nitrogen tended to increase as supplementation was increased. The tendency of energy supplementation to increase the different pools diminished as the supplementation level increased from 30 % to 45 % for both the energy sources. These results indicated that the ruminal environment was more favourable for fibre degradation in maize supplemented animals.

Chapter 6

6.1 General discussion

The work in this dissertation indicates that the digestion of *A. nummularia* cv. De Kock is influenced by the supplementation of different carbohydrate sources at different levels of supplementation.

The basal diet of *A. nummularia* used in this experiment was harvested from mature plants, which had been heavily grazed by sheep prior to collection. It is feasible that more fibrous and less palatable material was harvested for use in this trial, as sheep are selective grazers and would have selected the most palatable and nutritious material.

The results in Chapter 4 indicate that supplementation with both energy sources tended to increase the dry matter intake of sheep (not significant), when compared to the control diets. There were significant differences found at the 45 % maize supplementation and the 30 % barley supplementation levels compared to the lower levels of supplementation. The higher intake of supplemented diets could have been as a result of the higher palatability and digestibility of the energy sources.

The poor quality of *Atriplex* material is reflected in the low dry matter digestibility when compared to data from the literature. There was a significant increase in the dry matter digestibility from the control diet to the 15 %, 30 % and 45 % supplemented diets for both energy sources. The increase in digestibility, when diets are supplemented with an energy source, can partially be explained by the higher content of digestible organic matter in the energy sources. It was also reported that as the level of supplementation was increased the incremental increases in dry matter digestibility decreased. This could possibly be due to negative associative effects occurring in the rumen. Barley supplemented diets tended to have higher dry matter digestibilities. De Visser (1990) stated that the use of concentrates high in ruminal degradable starch negatively influences rumen fermentation conditions, which may in turn reduce neutral detergent fibre

degradation. This was apparent in the present investigation at supplementation levels of 30 % and 45 % of both the energy sources. The decrease in neutral detergent fibre digestibilities found in the present trial was most probably due to negative associative effects in the rumen.

The inclusion of concentrates in diets changes total dry matter intake, particle size distribution of rumen content and often the chemical composition of the diet (Bosch *et al*., 1992). Changes in the chemical composition of the diet are reflected in the degradability of the organic matter, rumen fermentation pattern and kinetics of particle digestion and passage in the rumen (Robinson *et al*., 1986; 1987). It was also reported that the ruminal DM pool/ kg live weight increased as the level of supplementation increased to 30 % for both the energy sources. Negative associative effects in the rumen could possibly explain the decrease in DM pool when the level of supplementation was increased from 30 % to 45 % and it is reflected in the decrease in dry matter intake at the same levels of supplementation. During this trial there were no significant differences found in the total rumen pool of dry matter, neutral detergent fibre and nitrogen between the two carbohydrate sources. The neutral detergent fibre and nitrogen pools increased as the level of supplementation increased up to 30 %. At the 45 % level of supplementation of both the energy sources there was a decrease in all the rumen pools.

The supplementation of *A. nummularia* with an energy source also had an effect on ruminal clearance rates, which are defined as the sum of disappearance of material due to passage and degradation. The results reported indicate that supplementation with energy increased the clearance rate of dry matter from the rumen. Animals receiving the maize supplemented diets tended to have higher clearance rates for dry matter and this could be due to the lower rumen degradability of maize starch compared to that of barley (De Visser, 1990). Neutral detergent fibre clearance rates had an inverse relationship with energy supplementation. This could be due to the smaller proportion of neutral detergent fibre in the diets as the level of supplementation increased. Gazza *et al*. (1991) also reported that an increase in concentrates in a diet reduced the fractional rate of digestion of fibre and reduced that of neutral detergent fibre.

Extensive ruminal fermentation results in the absorption of large amounts of the end products of fermentation from the rumen and an increased rate of passage of undegraded dietary residue and ruminal microbes to the small intestine for digestion and absorption. Energy and amino acids form a combination of dietary and microbial sources that are absorbed and utilized by animals. Overton *et al*. (1995) reported an increase in passage of amino acids to the duodenum as the percentage of maize and barley in diets increased because of the influence of dry matter intake and microbial protein synthesis. Protein in *A. nummularia* is extensively degraded to ammonia in the rumen. Weston *et al*. (1970) reported high losses of N in the urine of sheep receiving *Atriplex* diets. This may have been due to inefficient utilization of ammonia by rumen microbes. The results suggest that energy supplementation at a level of 30 % should be sufficient to optimize rumen ammonia nitrogen utilization.

Increasing the level of supplementation from 0 % to 45 % in both the energy sources decreased ruminal pH. There was a significant decrease in rumen pH when the level of supplementation was increased from 0 % to 30 % in the maize treatment and in the barley treatment when the level of supplementation was increased from 0 % to 30 % and from 30 % to 45 %. The decrease in rumen pH corresponds with the increase in rumen volatile fatty acid production reported earlier.

It can be concluded from the results reported in this dissertation that it is not necessarily the type of carbohydrate supplementation but the level at which the energy source is supplemented that will have an effect on the digestibility and rumen fermentability of *A. nummularia* cv. De Kock when it is fed to sheep.

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