NUTRITIONAL EVALUATION AND UTILISATION OF AN AQUATIC PLANT, *POSIDONIA AUSTRALIS* (SEAGRASS) IN SHEEP

by

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DECLARATION

I hereby declare that this thesis contains no work which has been accepted for the award of any other degree or diploma in any university and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the university library, being available for loan and photocopying.

Nourmohammad Torbatinejad

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ABBREVIATIONS

ADF	acid detergent fibre
ADL	acid detergent lignin
BA	Butyric acid
CF	Crude fibre
$CO(NH_2)2$	Urea
CP	Crude protein
DCP	Digestible crude protein
DE	Digestible energy
DMD	Dry matter digestibility
EDTA	Ethylenediaminetetraacetic acid
FD	Fibre diameter
M	Molar
ME	Metabolisable energy
MFS	Methylegreen Formalin-sa
mV	Millivolt (s)
N/Ktex	Newton/Kilotex
NDF	Neutral detergent fibre
NFE	Nitrogen free extract
OMD	Organic matter digestibility
OMf	Organic matter fraction
PA	Propionic acid
RCBD	Randomized completly bolock design
rpm	Revolutions per minutes
TDN	Total digestible nutrients
TFA	Triforoacetic acid
tVFA	Total volatile fatty acids
UV	Ultraviolet
V	Volts
VFA (s)	Volatile fatty acid (s)

ABSTRACT

This thesis describes research into the nutritional value of aquatic plants as novel or non-conventional feedstuffs for ruminants in general and for sheep in particular, with especial reference to those which are available in high amount in southern Australia, such as the seagrass, *Posidonia australis*. An understanding of the nutritional characteristics of *Posidonia australis* and of the potential for its improvement, together with an estimation of its possible role in lot-feeding, were the most important practical objectives of this study.

The thesis contains a detailed discussion of the results obtained and suggestions for future work. After an introduction (Chapter 1) the associated literature (Chapter 2) survey covers distribution, productivity, nutritive value and uses of aquatic plants, concentrating on seaweeds and seagrasses; evaluation and characteristics of lignocellulosic materials; possibility of improving nutritive value of low quality feedstuffs; rumen ecosystem in response to fibrous material ; and finally, lot-feeding systems in the animal industries.

The experimental component of the thesis involves five broad parts, as follows:

(i) Selection of seagrass, *Posidonia australis*, from among other aquatic plants by chemical estimation of their nutritive value (Chapter 3);

(ii) Nutritive evaluation *in vivo* of both fresh and partially decomposed *Posidonia australis* both before and after its use as litter for broiler chickens, i.e. one potential way to improve nutritional quality (Chapter 4);

(iii) Nutritional studies *in vitro* and *in sacco* following pre-treatment of *Posidonia australis* with alkalis and fungi (Chapter 5);

(iv) Study of ruminal parameters in sheep fed untreated and treated seagrass, *Posidonia australis* (Chapter 6),

(v) Estimation of the potential of treated and/or supplemented *Posidonia australis* for sheep maintenance and production in lot-feeding (Chapter 7).

Chapter 3: First, in order to screen appropriate species of aquatic plants from amongst the hundreds of species available in South Australia, the beach at Kingston was sampled, based on information from the Department of Botany, the University of Adelaide and local knowledge. Thirteen species of plentiful and readily-available aquatic plants (12 species of seaweed, 1 species of seagrass) were collected and identified. Chemical analysis and digestibility *in vitro*, as simple and fast methods, were applied to estimate the possible nutritive value of the collected species, as compared with a normal feedstuff, lucerne hay. From among the marine plants readily available in South Australia the seagrass, *Posidonia australis*, was regarded as a potential alternative animal feedstuff.

Samples of four different physical forms of this plant were collected, from the water (green and fresh) and from on the beach (washed and un-washed), and were examined and compared for chemical composition using advanced methods, including non-starch polysaccharides (NSP) , uronic acids, lignin, tannin, amino acids, soluble and insoluble ash and mineralss. The result of this second experiment lead to the selection of the dry unwashed seagrass, which is particularly plentiful and is possibly important in commercial terms. The selected seagrass, with its high content of crude fibre and lignin and being low in both digestibility and crude protein, can be regarded a as low-quality, lignocellulosic roughage for ruminants.

Chapter 4: Experiments were then carried out based on two main objectives; namely determination of voluntary intake and digestibility *in vivo* of the selected seagrass and study of the possibility of improving its nutritive value by physical, biological and supplementation methods, including: a) decomposition, b) using both fresh and decomposed material as chicken litter, and c) supplementation with molasses. Five experiments were carried out separately, each with 20 Merino wethers.

One important result from this chapter was that clearly the voluntary intake of *Posidonia* and decomposed *Posidonia* is so low that these materials on their own cannot meet sheep requirements for a whole diet.

It is evident also that the dry-matter digestibility (DMD) of pure *Posidonia* is very low, so that it can be classified only as a poor-quality roughage. The results show, however, that decomposition and supplementation with a protein source or a more palatable forage, such as chicken manure and lucerne, improved digestibility of *Posidonia* .

Chapter 5: Experiments *in vitro* and *in sacco* were then undertaken to estimate the nutritive value of *Posidonia* following pre-treatment with NaOH, Ca(OH)2, ammonification and fungi (with various concentrations and interaction times), concentrating on possible improvement to digestibility and rumen degradability of dry matter, organic matter and cell-wall structural carbohydrates (NDF, ADF, ADL, cellulose and hemicellulose). Substantial ruminal disappearance of the DM and OM of untreated *Posidonia* occurred in 72 hours incubation time, but all treatments increased this. Of the various treatments applied in this experiment, alkali treatment was more effective than fungal treatment and amongst the alkalis NaOH had the greatest effect on rumen disappearance of *Posidonia*.

The effect of alkalis on the degradability of the NDF and ADF was similar to that on dry-matter degradability, but between fungi only *P. gigantea* treatment was able to increase NDF and ADF degradability, and then only as long as the *Posidonia* was first ground.

The results of these experiments lead to estimates of the optimum condition of treatment in terms of possible commercial practice.

Chapter 6: The objectives of the experiments described in this chapter were two fold - first to measure voluntary intake and digestibility (DM, OM, cell-wall constituents) *in vivo* of treated *Posidonia* and to compare this with the previous results *in vitro*, and then to determine the pattern of ruminal parameters in sheep fed treated seagrass (NaOH, ammonia, molasses and ammonia+molasses) and untreated, in comparison with similar treatment of more conventional lignocellulosic feedstuffs. This latter objective was undertaken to test the hypothesis that the apparent improvements in dry matter and organic matter digestibility and in cell wall degradability observed in seagrass following chemical treatments are reflected in appropriate changes in rumen metabolism which could be seen as a advantageous i.e. to provide more useful products and metabolites for the sheep's nutritional requirements.

Nitrogen balance, the levels of total and specific volatile fatty acids and ammonia, rumen pH and the number of total, viable, cellulolytic and proteolytic bacteria and of protozoa in the rumen before and after feeding were determined.

In this study the pH of rumen digesta was higher when sheep were fed the alkali treated *Posidonia* diet than when fed the untreated material. Considerable diurnal variation in ruminal NH_3 concentration was observed in sheep fed both untreated and treated seagrass. As expected NH_3 and NH_3/molasses treated seagrass increased the average rumenal concentration of NH_3, although NaOH and molasses alone did not. The results of N-balance studies in this thesis indicate that the feeding of chemically-treated seagrass significantly improves the proportion of nitrogen retained by sheep. Data also showed that the average ruminal total VFA concentration in sheep fed untreated seagrass was 86.6 mM, which is comparable to that reported for sheep fed untreated rice straw, namely 101.6 mM. Among the experimental diets all treated seagrasses resulted in lower levels of mean total VFA, in spite of increased digestibility of cell wall contents such as cellulose. The number of all microorganisms including total, viable, cellulolytic and proteolytic bacteria and protozoa of rumen of sheep fed with treated seagrass increased, both after 12 hours feeding and on average in all groups of sheep.

Chapter 7: The final experimental chapter considers an experiment related to lot-feeding of Merino wethers with *Posidonia*, using the results of all previous experiments. Five groups of ten Merino wethers were handfed with differing treated-seagrass-based rations (treatments) and treated wheat straw (control), while one further group was released to green pasture (additional control).

The specific objectives here were first, to compare the effect of feeding seagrass as a non-conventional feedstuff, with cereal straw as a conventional feedstuff, for sheep maintenance and to compare seagrass/chicken litter with lucerne hay or with pasture grazing for sheep production; and second to provide some basis for a financial or economic analysis of the possible use of the seagrass *Posidonia australis* as a ruminant, particularly sheep, feed (in a lot-feeding system) in drought and during summer food shortage.

The major measurements of this experiment included feed intake, body weight gain, feed conversion ratio, feed cost and wool production characteristics, e.g. clean fleece weight, staple length, fibre diameter, length:fibre ratio and staple strength.

The results of this experiment showed that ammonia/molasses treated *Posidonia* can be fed to sheep in considerable amounts (50% of the required dry matter), with wheat straw, mainly at maintenance or drought-feeding levels. Additionally, supplementation of treated *Posidonia*-based diets with the inclusion of around 25% lucerne hay can meet body-weight gain requirements of about 80 g/day. A very useful and commercially-recommendable result from the studies of this chapter was that when *Posidonia* is used as a bedding material for broiler chickens for 6 weeks then the resultant litter, with amounts of 25% in the ration, could meet body gain requirement of 100 g/day. This result in terms of feed efficiency and cost, statistically, was equal to the value of lucerne hay at the same level in the ration.

In general, in groups of sheep fed with *Posidonia*-based diets the rate of wool growth was in the range found with other, more traditional, diets. Also wool growth and its characteristics in experimental sheep in this lot-feeding were comparable with those from sheep grazed in green pasture.

In summary, from the results presented in this chapter it can be recommended that lot-feeding of sheep with treated *Posidonia* and *Posidonia* litter-based diet during the dry period of any region can be successfully established with simple and inexpensive facilities at the farm level, for both maintenance and production.

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CHAPTER 1

General introduction

Christianson and Allender (1988)

"The saline waters which cover about 71% of our planet's surface support many different kinds of plants which can be regarded as unconventional feedstuffs for ruminants."

CHAPTER 1

General intruduction

1.1 World population and animal protein requirements

The world population is increasing rapidly. The United Nations has predicted that by the year 2050 the world population will reach 10,000 million people (Langer and Hill 1991). If the present trends continue, there will be only 0.3 hectare arable land per capita by the year 2000. In order to nourish adequately the world population yield per hectare needs to be three times higher than at present (Boda 1990). Many third-world countries do have increased food production, but the increase is not keeping place with rapid population growth. It is estimated that over 1 billion people now suffer from chronic malnutrition (Minson 1990).

Protein sources are particularly important among earth's food. Although some protein is formed in plants, especially as reserve food in their seeds, the bodies and milks of grazing mammals have long been regarded as a more important source of protein (Hungate 1988). According to many scientists there is a need to double animal protein production in the next 20 to 25 years in order to improve the protein intake status of the world's rapidly growing human population. The developing countries have about 60 percent of the world's animals but produce only 20 percent of the world's meat, milk and eggs. Better feeding and nutrition programs in particular would increase their production of animal food for human consumption. Since the total population of the most important ruminants (sheep, cattle and goats) that can make some contribution to food for humans approaches 2.5 billion head. It is clear that ruminant animals are especially important in this context (Minson 1990).

1.2 Animal production systems

A number of different animal production systems are used world wide. The cheapest and most common, however, and the one which predominates in Australia, is that involving grazing lands (Jarrige 1980; Morley 1981). In fact nearly all of the agriculturally-important animals in Australia and worldwide, such as sheep, cattle, horse, goat and deer, are

herbivorous. They supply the majority of meat and milk consumed by humans, as well as large quantities of textile and leather goods, and provide much of the draught power in many areas of the world (Church 1979; Jarrige 1980; Morley 1981; Orskov 1982).

In the agricultural sector the sheep industry is one of the major and largest enterprises. Australia and the sheep industry have been synonymous for more than 150 years (Bryant 1983). Australia has the largest sheep industry in the world. Sheep numbers reached a peak of 180 million in 1970. However, due to the effects of poor international markets the number declined to 148 million in 1992 (ABARE 1992). The most important product in this sector has always been wool, although in recent decades sheep meat has increased in importance. Wool, however, is still Australia's dominant single agricultural product. Australia produces nearly 30 percent of the world's wool and it occupies first place in the world export trade in this product. According to figures estimated by ABARE (1992) Australia supplies over 50 percent of the world wool export among the main producing countries. This leading position has increased over the years. Wool export is therefore a major source of foreign exchange income for both the farmers and the Australian economy as a whole.

Maximum production of meat, milk or wool can be achieved only if animals are supplied with sufficient quantities of the raw materials required for the synthesis of those products. This can readily occur when housed ruminants are fed grain-based diets supplemented with proteins, minerals, and vitamins, but when forage is the sole source of nutrients production is invariably much lower than the genetic potential of the animal. Approximately 90 percent of the feed available to ruminants throughout the world consists of forage - grass, browse, legumes, hay, and straw (Minson 1990).

In Australia the forage base of feed to provide the needs of ruminants, especially sheep, is supplied in three main ecological zones : (a) the pastoral zone; (b) the cereal-sheep zone and (c) the high-rainfall zone (Pratley 1991).

The pastoral zone is characterised by marginal cropping and extensive grazing of native pastures with low stocking rates. About 10-20% of the total sheep numbers are found in

this zone (AWC 1989). In the cereal-sheep zone regular cropping is practised in addition to the grazing of mainly sheep on a more intensive basis than in the pastoral zone. Although this zone occupies about 11 percent of Australian land, because of its favorable conditions, such as a longer growing season, it supports nearly 45-55% of the total sheep population (BAE 1983). The third zone includes the coastal mainland with higher rainfall and hillier topography. This zone is generally more suitable for intensive grazing rather than for grain production. About 20-30% of the nation's (Merino) sheep are maintained in this zone.

The three different agricultural zones mentioned above are all found in the state of South Australia (Jefferies and Nash 1989). The cereal zone accounts for more than 65% of the annual gross value of agricultural production in the state. The cereal zone production is based on a cereal-livestock ley farming system. Overall most of the sheep population is grazed on annual legume-based pastures (South Aust. Dept. Agric. 1991).

A major characteristic of sheep husbandry in Australia is the dependence on pasture. Pastures are the main source of nutrients for the nation's sheep (Squires 1981). By world standards grazing practice in Australia can be defined as extensive, a system of animal husbandry that is very different from that practised in many other countries with similar vegetation types. The major characteristic of Australia's livestock production system is its great dependence on year-round, low-input and continuous grazing on large properties (Wheeler and Freer 1986; Squires 1991). Pastures are therefore a valuable national resource contributing over 60% of the value of all agricultural products to the nation's economy (Archer *et al.*. 1993).

In southern Australia it has been well documented that annual pastures have declined in productivity and quality in recent years, due primarily to loss of legumes (Carter 1982; Gillespie 1983; Dear and Loveland 1985). In pasture/cereal rotation systems the key requirement for persistence of the annual pasture legumes is that appropriate amount of seeds must be produced and survive both under grazing and through the year of the cereal crop (ICARDA 1984; Jones and Carter 1989). In the mediterranean-type environment of southern Australia the pastures available to the grazing animals during the summer months are the dry residues from the previous growing season. Livestock production is often

limited by the quality and quantity of this dry feed on offer (Brown 1976). Research carried out by Carter and his group at the University of Adelaide over the past 20 years has highlighted that generally about 80-90% of farms have insufficient legume seed reserves following the cropping sequence to regenerate a reasonable legume-based pasture. It has been demonstrated that one of the major causes of pasture legume deterioration in the cereal belt is excessive consumption of legume seed by sheep during the dry and hot period of late summer and early autumn (Carter 1981; de Koning and Carter 1989; Squella 1992).

To reduce the dramatic effects of over grazing during the dry season on pasture deterioration and soil erosion, declining sheep body weight and wool production and high death rates one of the most effective management methods for sheep in the Mediterranean-type environment of southern Australia can be lot-feeding (Carter *et al.*. 1993). This practice can be used for a number of different purposes, e.g. lot-feeding of sheep for maintenance during a period of feed shortage or where there is the erosion risk in running sheep in paddocks, lot-feeding for production or finishing and lot-feeding after the break of the season in order to conserve the newly emerged annual legume seeds in annual pasture (Rehn 1988; Ashton 1989).

In Australia lot-feeding is a rapidly-expanding sector of both the beef and dairy industries. The number of cattle in feedlots increased from around 25,000 in the early 1980s to around 75,000 in the later years of the decade. This is coupled with an even greater increase in feedlot capacity since 1990, so that Australia now has the potential to a accommodate some 400,000 head. Currently about 95% of the cattle on feed are in commercial feedlots, with the remainder in opportunity feedlots that are used mainly during droughts. Such increases in industry growth and expectations are due to a combination of factors, one of which is "the integration of lot-feeding as a drought mitigation strategy" (Howard and Plasto 1991).

In lot-feeding of sheep and cattle nutrition represents the most important factor in their performance and profitability. Lot-fed sheep should be handfed with a mixture of feedstuffs such as grain, forage (hay, crop residues) and agro-industrial by-products. If this management practice is to be both acceptable and profitable, then crop residues must be used (Burns 1981).

The most dominant crop residue in the world, especially in Australia, is cereal residue. Cereals are grown on 2/3 of the world's arable land and arable soil covers 10% of the earth's surface. But, as previously mentioned, the world's population is rapidly increasing and as a result the area of cereal arable land per capita and per head of animal is decreasing. The possibility of gaining new areas for cultivation is unlikely (Boda 1990). The situation could theoretically be relieved by several solutions including: breeding more efficient animals, breeding plants which are able to fix more than the current maximum of 3% of solar energy and finding new protein and energy resources. Among these solutions the use of many non-traditional resources should examined for both animal and human nutrition.

1.3 Aquatic plants

One of the most important non-conventional resources that could be considered seriously throughout the world and, especially in Australia for animal nutrition, is aquatic plant life.

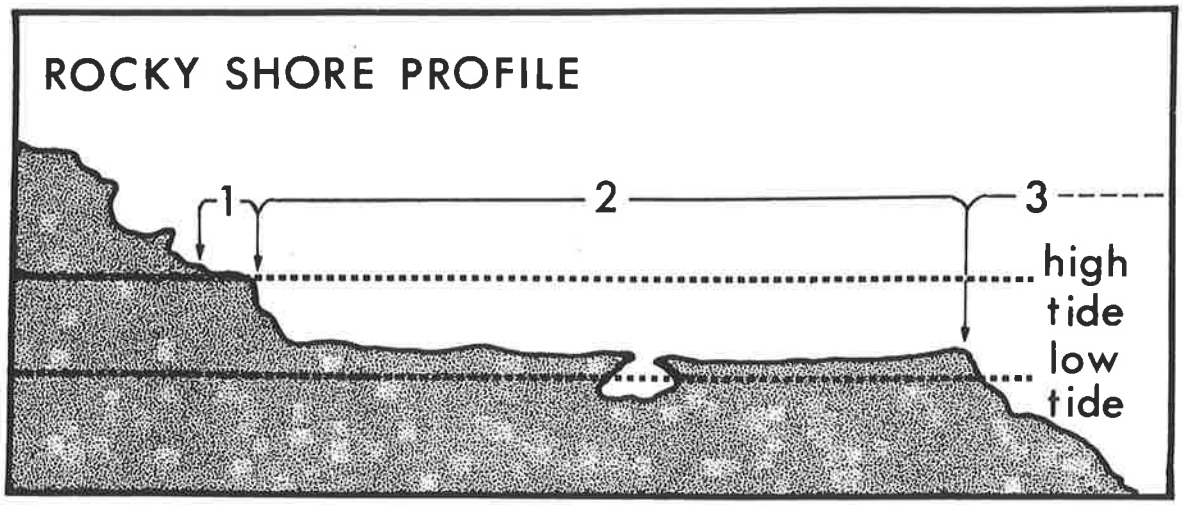
The saline waters which cover about 71% of our planet's surface support many different kinds of plants. These include the various types of larger algae, popularly known as seaweeds, which grow freely in shallow waters throughout the world. Also conspicuous on many coasts are the marine angiosperms, comprising seagrasses and saltmarsh plants, and to a lesser extent the marine lichens. These plants typically grow fixed to the sea bottom or other substrates and are described as benethic. Other plants lead a free floating, or planktonic, existence in the seawater itself. For example, many different types of planktonic algae invariably occur in the surface layers of seas and oceans. In most situations they are invisible to the naked eye, because of their microscopic size, and thus their abundance and great ecological importance is not generally appreciated. It is noteworthy also that certain plant types which are widespread on land, such as the gymnosperms, pteridophytes and bryophytes, are rarely encountered under marine conditions (Price 1980).

Throughout the world, including Australia, marine flora is dominated by algae and seagrasses, although in estuaries and other coastal waters flowering plants may be locally abundant (King 1980).

The term "seaweed" refers to the majority of the macroscopic plants which inhabit the intertidal region of the seashore and the permanently submersed shallow sunlit regions of the continental shelf. Seaweeds are alga, structurally simple plants without roots, stem, or leaves, and having primitive methods of reproduction. Especially on rocky shores most species of seaweeds and marine animals have relatively restricted vertical distributions, occurring in horizental bands or zones which are related primarily to tidal fluctuations. Three zones have been recognised on coasts throughout the world, and although both their vertical and horizontal extents may vary they are always characterised by particular kinds of organisms. The majority of seaweeds occur around low tide level or subtidally. Figure 1.1 and plate 1.1 (upper) show a typical rocky shore profile and some coastal seaweeds in south-east Australia, respectively (Christianson *et al.* 1988).

In many bays, estuaries and other shallow, relatively sheltered parts of the sea the flowering plants known as seagrasses (Plate 1.1 lower) are an important constituent of the marine vegetation. They form large underwater meadows which are breeding grounds for fish and invertebrates and also stabilise the sand or mud surfaces. Seagrass are not related to the algae. They have true stems and leaves, develop inconspicuous flowers and form fruits and set seeds like flowering plants on land. They are, however, quite distinct from the land grasses. Great masses of seagrasses often pile up on sandy shores after storms (Plate 1.1 lower) (Christianson *et al.* 1988).

Figure 1.1: Rocky shore profile and the three major coastal zones (Christianson *et al.* 1988)



Three major zones	Characteristic organisms	Approximate tide levels
1 Littoral fringe	Periwinkles; black lichens and blue-green algae	Splash (above high tide)
2 Eulittoral zone	Barnacles and limpets	Intertidal (between low and high tide)
3 Sublittoral zone	Kelps (corals)	Subtidal

(In the notes accompanying the photographs in this book, the terms 'Intertidal' and 'Subtidal' are used to describe seaweed habitats.)

Seagrass is the one group of angiosperms which is restricted to the marine environment. None of the seagrass are true grasses (family *Gramineae*) but all are monocotyledons in the single subclass Helobiae (*Alismatidae*). Within this subclass den Hartog (1970) placed all seagrasses in two families: the *Hydrocharitaceae* and the *Potamogetonaceae*. The family *Potamogetonaceae* in this sense is very heterogeneous and it is variously divided by other into five or more separate families. Seagrass leaves are often long and narrow, but maybe broad or rounded, and they are simpler anatomically than those of typical land plants. The flowers are considerably reduced and often inconspicuous. In most cases, pollination takes place under water. Seagrass vegetation occurs in tropical, temperate and even polar regions, but attains its greatest diversity in tropical areas (Clayton 1975).

Plate 1.1: Upper: Brown seaweeds as the dominant plant of the lower intertidal zone. Lower: Seagrass along the South-eastern coast of Australia (Christianson *et al.* 1988)

1.4. Objectives of thesis

This thesis describes research into the nutritional value of aquatic plants, especially seaweeds and seagrasses, as novel or non-conventional feedstuffs for ruminants in general and for sheep in particular, with especial reference to those which are available in high amount in southern Australia, such as the seagrass *Possidonia australis* from Kingston, South Australia. Understanding the nutritional characteristics of *Posidonia australis*, it's nutritive value and the potential for improvement in this and finding a possible role for this plant in lot-feeding were the most important objectives in this context.

The experimental component of the thesis involves five broad parts, as follows:

(I) In order to screen appropriate species of aquatic plant in terms of both nutritive and possible commercial value, the general nutritive quality of 13 species of aquatic plants (12 species seaweed, 1 species seagrass) which are readily available in the South Australia was evaluated by chemical analysis and digestibility *in vitro* determination and compared with lucerne chaff as a standard feedstuff.

(II) The nutritive value of both fresh and partially decomposed *P. australis* and these two types after their use as litter for broiler chickens, was determined *in vivo*, using sheep.

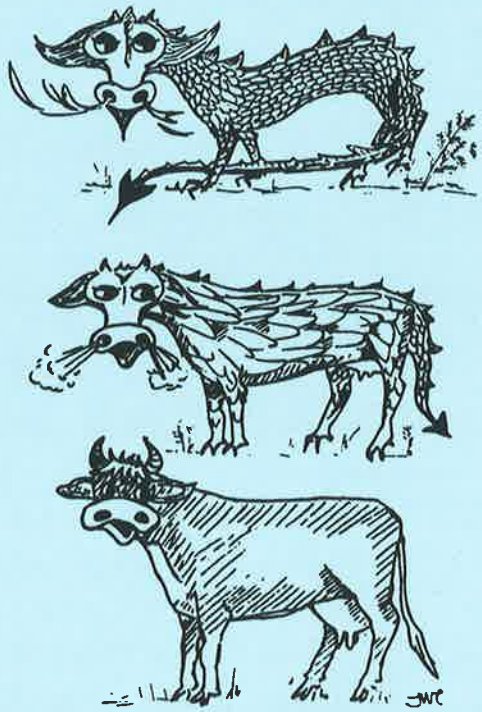
(III) The possibility of improving the nutritive value of *P. australis* by both chemical and biological treatments, concentrating on modification of structural cell-wall components, was studied both *in vitro* and i*n sacco* .

(IV) Diurnal changes in rumen parameters of sheep fed treated and untreated *P. australis* were studied.

(V) This section, as a final experimental part of the thesis, describes the lot-feeding of Merino sheep with a low-cost, balanced ration that included treated *P. australis*, at both maintenance and production levels and in comparison with wheaten straw as a conventional lignocellulosic feedstuff.

CHAPTER 2

Review of literature



Czerkawski (1986)

"Sans les animaux, la nature de l'homme serait encore plus incompre'hensible"

"Without animals, human nature would be even more incomprehensible" (Georges Louis Leclerc, Comte de Buffon: Historie Naturelle 1779).

CHAPTER 2

Review of literature

2.1 Introduction

This review has been planned to cover all broad areas of the experimental components of the thesis, all of which are related to the nutritional study of aquatic plants in sheep.

(i) The review will start by describing characteristics of aquatic plants , concentrating on seaweeds and seagrasses with respect to distribution, productivity, nutritive value and uses.

(ii) next, nutritional evaluation of feedstuffs is considered, concentrating on the methods which were applied in this thesis.

(iii) Because of subsequent experimental emphasis on *Posidonia australis* the characteristics and utilisation of lignocellulosic feedstuffs were reviewed.

(iv) In this section the possibility of improving nutritive value of lignocellulosic feedstuffs in general are reviewed.

(v) The response of experimental animals to lignocellulosic materials, with respect particularly to the rumen ecosystem is then covered.

(vi) Finally In order to cover the last experimental chapter of the thesis lot-feeding in the sheep industry is reviewed.

2.2 Aquatic plants

2.2.1 Seaweeds

2.2.1.1 Seaweed distribution and productivity

There are about 8000 known species of seaweed along the world's coast lines, and they may extend out to water as deep as 270 meters (Womersley 1980). These are important contributors to food webs in coastal waters . Data on the benthic algal flora of various parts

of the world are presented in Table 2.1. The present distribution of benthic marine species is the result both of their migration and of the displacement of coastlines in earlier geological periods (Womersley 1980).

Several distinct biogeographical provinces (coastal regions with homogeneous flora) have been identified around Australia (Clayton and King 1975)). In terms of algae the most important province is southern Australia and there has been considerable improvement recently in data for this region, especially with regards to detailed monographs on various families and genera, especially of the Rhodophyta (Womersley 1959). This southern Australian region extends from the south-west corner of Western Australia to about the Victoria-New South Wales border, and includes Tasmania (Womersley 1959). The results of an analysis of 329 genera and 1010 species in southern Australia are shown in Table 2.2.

According to Mann (1972) and Luning (1990) the maximum algal biomass on rocky substratum in the sublittoral zone may be as much as 16 Kg of fresh weight per square meter, and perhaps half this value in the mideulittoral zone. The annual primary productivity (carbon per square metre per year) may be several times higher than the standing biomass since, for example, part of the annually-fixed carbon is continuously lost in the form of eroding tissue.

In the world's oceans phytoplankton produce up to an estimated 30×10^9 tonnes of carbon per year, while terrestrial plants contribute up to 50×10^9 tonns of carbon per year (Bunt 1975). Marine-benthic plants (macro algae, seagrasses, and micro algae) produce probably about 3% of the organic carbon produced by phytoplankton. This approximation is arrived at by assuming that the mean rate of production is 1000 g Carbon m^{-2} yr^{-1} in a 2 Km wide strip along a global coastline of 400000 Km (Schopf et al. 1978). The annual global seaweed harvest amounts to about 3×10^6 tonnes of algal fresh weight (Blunden et al 1975). Table 2.3 shows harvested-production of seaweeds and aquatic plants in different continents in 1960, 1967 and 1973.

Table 2.1: Approximate numbers of genera and species of benthic algae and length of coast lines in various parts of the world (Womersley 1959).

Region	Length of coast (Km)	Genera	Species	Nature of Region
Britain	5000	261	604	Cold temperate
North, Eastern North America	8000	170	392	Arctic to warm temperate
Tropical Eastern America	16000	237	752	Tropical
Pacific North America	12000	366	1254	Arctic to tropical
California	1400	282	666	Cold temperate
Malaysia Indonesia	-	189	629	Tropical
Japan	6500	411	452	Subarctic to subtropical
South Africa	2500	254	539	Cold temperate to subtropical
Southern Australia	5500	400	1100	Cold temperate to warm temperate
New Zealand	4500	226	649	Sub-antarctic to warm temperate

Table 2.2: Number and percentage of endemic genera and species of seaweeds in southern Australia (Womersley 1959).

Family	Total genera	Number and % endemic		Total species	Number and % endemic	
Chlorophyta	27	3	11%	94	43	46%
Phaeophyta	63	12	19%	191	134	70%
Rhodophyta	239	72	30%	725	538	75%
Total	329	87	26%	1010	715	71%

2.2.1.2 Uses of seaweeds

In some countries animals regularly feed upon fresh seaweed or are given a prepared seaweed feed. In Iceland fresh seaweed is commonly employed as a feed for sheep, cattle and horses; the animals are encouraged to stay browsing on the shore during the whole of the winter and in some places during the summer as well (Chapmanan and Chapman 1980).

The Icelanders lay in a store of seaweed for a winter supply by washing the plants and then packing them in trenches where they are compressed with heavy oak planks and stones. The compressed mass is broken as required and fed to the animals without the smell or taste of milk from cows being affected. Sometimes some species (e.g. *Alaria*), after washing, are air-dried and then stored in layers in barns, each layer alternating with a layer of hay. Since 1960 some seaweed meal factories have existed in Iceland and some seaweeds are extracted to provide a liquid plant nutrient (Hallson 1964). In Finland both *Laminaria* and *Alaria* are used as fodder for cattle.

In certain coastal area of Norway sheep are fed regularly on seaweed and it has been found that after several generations coastal sheep digest it far better than inland animals (Sheehy *et al.* 1942).

In Scotland sheep and cattle wander down on to the foreshore and eat various algae. In the small, most northerly island of the Orkneys, North Ronaldsay, there is a local race of small black sheep which feed entirely on seaweed. The whole island is surrounded by a wall which keeps the animals out on the shore. Several hundred sheep browse on the seaweeds and are allowed to enter a pasture only when in lamb or just before slaughter. They suffer from very few diseases (Stephenson 1974). Opinion differs as to whether the meat tastes fishy or not, but the wool is regarded as being of a superior quality. On the west coast of Scotland, around Loch Feochan, *Pelvetia* is fed to pigs when they are being fattened for market, the weed being given raw or boiled up and mixed with oatmeal, in which form it is also fed to calves.

In the Commander Islands in the Behring sea, polar foxes are fed seaweed as part of their normal diet and *Alarialosa fistu, Laminaria bongardiana, Fucus elliplica* and *Fucus evanescens* are collected, mixed with meal and fed to pigs (Kirby 1953). In Cuba experiments have been carried out with *Ulva spp* as an additive to poultry meal, with 10% addition giving optimal results (Chapman and Chapman 1980).

The only reference to seaweed used for animal feed in the tropics comes from Hong Kong, where species of *Sargassum* are dried and used as pig feed (Kirby 1953).

Seaweed as an item in the human diet has been used in Japan and China for a very long time. To a lesser extent various species have been employed in this fashion in Europe and North America.

On the continents and larger islands, where normal agriculture can be supported, there is generally no great demand for seaweed as food. There are, however, many islands where conventional agriculture cannot meet local demands and it is here that people have, of necessity, relied on the sea as a major source of food (Chapman and Chapman 1980).

Many publications have described how seaweeds play a considerable part in the economic life of some nations, and that in certain circumstances, e.g. stress of war or famine, their use increases and may extend even to countries that normally do not employ them. An initial indication of important areas of actual or potential seaweed usage was provided in 1975 by

FAO (Michanek 1975). Due to the continued growth of the world's population and resulting increasing pressure for food and energy seaweeds, which form an annually renewable resource, are likely to become increasingly important.

In recent years dried seaweed meal and liquid extracts have been increasingly employed by horticulturists, market gardeners, farmers and orchardists. With the gradual exhaustion of presently-known mineral fertiliser supplies it is again likely that in the future even more use will be made of the annually renewable source in seaweeds (Chapman and Chapman 1980).

Finally, seaweed can also be converted to methanol and under some circumstances used economically in place of gasoline (Wise and Silvestri 1976).

2.2.1.3 Composition and nutritive value of Seaweeds

The fresh weight of seaweed consists of 75-90% water. Of the remaining dry weight, about 75% is organic matter and 25% mineral ash, consisting mainly of potassium, sodium, magnesium and calcium salts. About half of the organic dry weight of algae consists of carbon (Morgan *et al.* 1980). Generally some 10% of the organic dry weight is protein, but this value may increase to as much as 20-40% in genera such as *Ulva, Hypnea Porphyra* and *Palmaria*. (Durako and Dawes 1980). Black (1955) reported that the proteins of seaweeds, in common with those of most land plants, are less assimilable than animal proteins. Presence of all the essential amino-acids in seaweed has been reported, but the crude protein content rarely exceeds 15 percent (5 - 15 percent on dry weight basis) and seaweed can not therefore be regarded as a major source of proteins (Black 1955). The remaining organic dry weight is mainly low-molecular-weight carbohydrates and polysaccharides. In *Laminaria* species the content of lipophilic substances may amount to 1% and the iodine content to 4% of the dry weight (Haug and Jenson 1954).

The brown seaweeds contain amounts of fat varying from less than 1 percent to 8 or 9 percent, and there seems to be very little difference between the compounds present in seaweeds and those in land plants (Black 1955).

In the absence of free sugars seaweeds contain the hexahydric alcohol mannitol, which varies from 5 to as much as 25 percent of the dry matter. D-mannitol, or manna sugar, is a colourless, odourless, crystalline powder with half the sweetness of sucrose. In place of the starch of land plants a glucose polymer called " laminarin" is present in the brown seaweeds, and in autumn it may make up to 25 percent of the dry matter of the plant (Black 1955).

Primary photosynthetic products, as indicated by ^{14}C tracer studies, are besides the usual amino and organic acids, sucrose in green algae, mannitol in brown algae, and the glycosides (substances with both sugar and non-sugar parts) floridoside and isofloridoside in the *Bangiophycidae*, and digeneaside in the *Ceramiales*. Reserve polysaccharides, including starch, are synthesized in green algae and floridean starch in red algae. The structural polysaccharides represent important substances for industry, e.g."phycocolloids", "algal mucilages", such as the sulfated galactan agar, whose structural unit is agarose, and carrageenan in the red algae, and alginic acid and its salts, the alginates, in the brown alga (Haug *et al.* 1974, Cheshire and Hallam 1985).

There are diverse types of cell coverings in the alga. It should be noted that many alga form cell walls external to their plasma lemma. These are generally composed of chemically distinct fibrillar and amorphous components. The most familiar and widespread fibrillar component is the polysaccharide cellulose, although mannan, xylene and alginic acid are also important in some algal groups. The mucilaginous texture of many algae is due to the presence in the amorphous matrix of the cell wall of characteristic sulphated polysaccharides, such as fucoidan in the *Phaeophatea* and agar in the *Rhodophyta*. Small quantities of lipid and protein may also occur in the cell wall. In addition to the organic compounds, deposits of inorganic substances such as calcium and magnesium carbonates, or silica, are associated with the cell wall or other skeletal structures in particular algae (Ducker 1978).

Seaweeds have a low crude fibre content (2 - 10%), the place of cellulose in the cell wall structure being largely taken by alginic acid (15 - 25% of the dry matter). Nelson and Lemon (1942) have investigated this substance and have concluded that it could have considerable nutritive value.

Seaweeds can be considered a valuable source of vitamins. Although they do not contain vitamin A, they do posses its precursor, carotene, as well as fucoxanthin, a pigment which may also give rise to vitamin A. They also contain vitamins B1 and B2, while work by Ericson (1952) has shown the presence of vitamin B12 in amounts comparable of those in liver. Vitamin C can occur in appreciable quantities and there is evidence of the presence of vitamins D and E. Stephenson (1974) reported that seaweed use can increase the fertility and birth-rate of animals due to the presence of tocopherol, the anti-sterility factor of vitamin E. The use of seaweed meals improves both the yolk colour of eggs and their iodine content, primarily because of the fucoxanthin present (Jensen 1966). In addition to these vitamins, seaweeds contain other growth-promoting substances (Black 1955). In other studies, however it appears that with well-balanced rations the addition of seaweed meal has little or no effect on chicks or hens and the use of the weed is beneficial only if the ordinary ration is deficient in vitamins A or B_2 (Chapman and Chapman 1980).

A characteristic feature of seaweeds is their high mineral content (up to 35 percent of the dry matter), and it can be said that they contain all the elements which have so far been shown to play an important part in the physiological processes of animals. For a well-balanced diet, therefore, seaweed would seen to be an excellent mineral supplement. (Black 1955). The occurrence of iodine is also a point of major importance. Depending on the species and season of the year, the brown seaweeds contain 0.03 - 1.5 percent iodine (dry basis) in a form more valuable than in iodine salts, being partly present as the precursor of thyroxine (Black 1955). It also seems clear that one can add up to 7% meal for chicks and up to 15% for laying hens without apparent harm. Higher quantities, though, are unfavourable (Black 1955; Hoie and Sann 1960). In one US experiment 6000 hens were fed 1.25% meal in their ration and this reduced the proportion of thin-shelled eggs (Chapman and Chapman 1980). A further advantage is possibly that the trace elements are present in organic form which makes them more readily assimilated. Seaweed principle value is regarded as being in its iodine and other mineral content, essentially because the various elements are naturally dispersed. In practice amounts used rarely if ever approach values that could be dangerous.

It is particulary important to remember that analyses of raw plants and manufactured products vary extensively from locality to locality and are also dependent upon both the season of the year and the relative proportions of the different parts of the plants in the samples (Champan Chapman 1980). It is known, for example, that the carbohydrate content of the algae is highest in the autumn. In addition the composition of any commercial product depends heavily upon its method of preparation (Chapman and Chapman).

Experiments have been carried out on the digestion of the various component in seaweed meal. In one set of experiments the digestibility coefficient of *Ascophyllum* meal dry matter was found to be 29.7% for sheep and 26.2 for pigs, though much of the nitrogenous material was undigested (Beckmann 1977). The most important digestible component would appear to be laminarin, which forms 3 - 6% of *Ascophyllum* meal. Healthy animals appear to digest about 20%, whereas those in poor condition will take up to only 5%. Experiments in Ireland using pigs indicated that the chief value of adding seaweed meal was to improve the amount of basic ration that could be digested (Sheehy *et al.* 1942). After two years of trials Sater and Jensen (1957) found that the addition of seaweed meal had no effect on breeding or fertility of sheep. All breeds, however, did show an increased winter wool production. The effects of the meal were more pronounced after a dry summer, when hay quality would have been reduced. Average good quality Norwegian meal prepared from *Ascophyllum* has a composition equal to that of good hay and oats (Chapman and Chapman 1980). Figures also showed that *Ascophyllum* and potato tops have comparable protein (nitrogen) contents, though less than that of hay. Considerable differences are to be found in the carbohydrate component (Chapman and Chapman 1980).

In carrying out digestibility trials with seaweed one of the main difficulties of course is the great variation found in its composition. This, of course, is also a marked feature of many feedstuffs, especially grassland products. In seaweed feed preparations the composition depends greatly on the ratio of frond (leaf) to stipe (stalk) (Black 1955).

Table 2.3: Harvested production of seaweeds and aquatic plants (Naylor 1976)

	Metric tons x 1000 (wet weight)		
Continent	1960	1967	1973
Africa	17.5	63.0	37.4
North and Central America	119.7	75.3	77.9
South America	8.1	66.3	154.9
Asia	667.4	627.5	1603.3
Europe	183.9	277.9	381.8
Oceania	0.4	7.5	0.6
World Total	1171	1886	2402

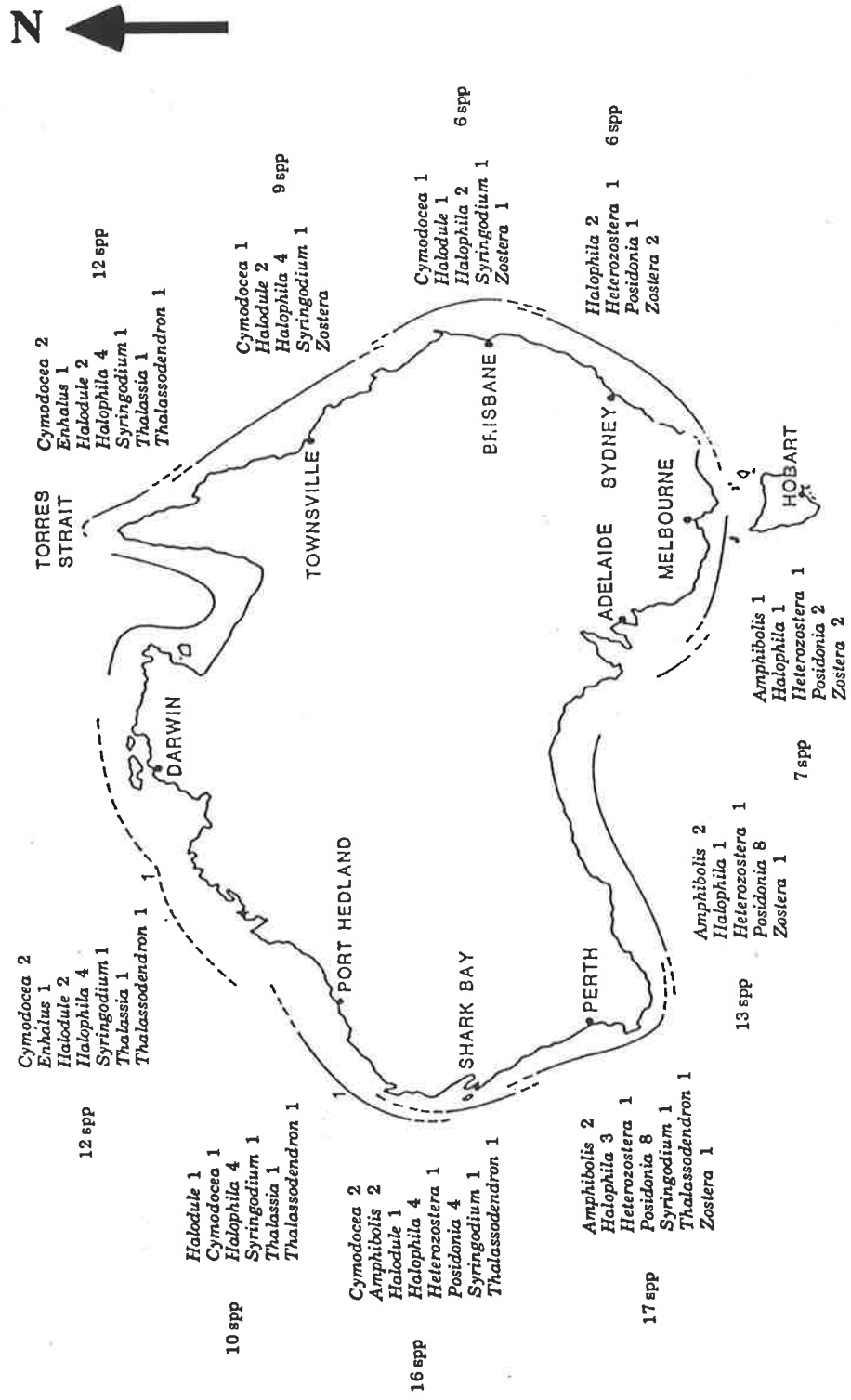
2.2.2 Seagrass

2.2.2.1 Distribution and productivity

Of the 12 seagrass genera recognised at present 7 are characteristic of the tropics (*Haodule, Cymodocea, Syringodium, Thalassodendron, Enhalus, Thalassia, Halophila*) while 5 are confined to temperate waters (*Zostera, Phyllospadix, Heterozostera, Posidonia, Amphibibolis*). The distribution boundaries are not always clear cut, however, especially where there are north or south flowing currents of warm or cold water (den Hartog 1970).

Australia presents a special case in terms of seagrass distribution. It has both the highest number of species of any continent in the world and communities with very nearly the highest seagrass species diversity in the world . It is a country that was once part of Gondwana and has drifted North, encountering the Asian continent in the Pliocene period and having experienced extensive interchange of biota during the Pliocence and Pleistocene, when lower sea levels occurred. By contrast New Zealand has only two seagrass species. These facts would seem to demand special attention and an explanation in terms of seagrass biogeography (Johnstone 1982; Walker and Prince 1987). The distribution of Australian seagrasses is shown in Figure 2.1.

Figure 2.1: Distribution of Australian seagrasses (Larkum and den Hartog 1989)



Aquatic angiosperms are without doubt the most productive plants on the earth today (Westlake 1963). The importance of seagrasses (marine angiosperms) as primary producers, however, has only been fully recognised recently. For example *Thalassia testudinum* (tropical seagrass) has an estimated primary productivity of 0.8-1.9g cm^{-2} day^{-1} or 9-18 tonne ha^{-1} yr^{-1} (Zieman 1975) and *Zostera marina* (temperate seagrass) 0.9 g cm^{-2} day^{-1} or 8.6 tonnes ha^{-1} yr^{-1} (Sand-Jensen 1975). The primary productivity of seagrass may be considerably higher than pasture growth rate e.g. some 5 tonnes ha^{-1} yr^{-1} in the south east of Australia with 430 mm annual rainfall (Ransom 1991).

Few measurements are available of the productivity of seagrasses in Australia . *Possidonia australis* from New South Wales and South Australia has a leaf productivity of between 0.2 and 1.7 g cm^{-2} day^{-1} (West and Larkum 1979). *Zostera capricornia* in Sydney waters has a mean annual above-ground productivity of 1.4 g cm^{-2} day^{-1}. Because of its abundance and wide distribution *Possidonia australis* must make a large contribution to the total primary productivity of sheltered waters of NSW, South Australia and Western Australia. *Zostera capricornia* and other temperate seagrasses form less dense communities but nevertheless can contribute significantly to overall production in shallow areas, such as Victoria's Westernport Bay (West and Larkum 1979).

The productivity of tropical Australian seagrasses has yet to be measured and may be even higher than that of the temperate species.

2.2.2.2 Uses of seagrasses

There are numbers of organisms that use seagrass directly as a food source. Most of the plant material apparently enters the food web through detrital food chains (Fenchel 1977). McRoy and Helffrich (1980) assembled a list of 154 species, including invertebrates and fish, which consume living seagrass in an attempt to dispel the popular notion that few animals eat seagrass. Over half the species listed have diets in which more than 10% is seagrass tissue and this food type even predominated in the diets of some of the consumers listed. The intensity of grazing varies with locality. Grazing on live seagrass appears more common in tropical waters, such as the Caribbean (Randall 1965, 1967; Ogder 1976, 1980;

Weinstein and Heck 1979; Thayer *et al.* 1984). Dawes *et al.* (1979) measured energy levels available to herbivores in marine plants from Caribbean waters and showed that seagrasses were similar to algae. It is therefore clear that seagrasses could offer potential nourishment to herbivores, including both ruminants and non-ruminants.

2.2.2.3 Structure and composition of seagrasses

The roots of seagrasses are adventitious, as in all monocotyledons, and arise from the lower surface of the rhizomes, generally from the nodes. They show many specialised features, however, that are thought to be adaptations to an aquatic environment (Arber 1920; Sculthorpe 1967).

The roots of all seagrasses have distinct root caps. The mature root has an epidermis, which may bear root hairs, overlying cortical parenchymatous tissue that encloses air lacunae and a central stele. The epidermal cells usually have thin, unlignified walls and a peripheral cytoplasm, and below the epidermis there is a distinct exodermis one or more cells thick, each of which has thickened but unlignified walls containing suberin lamellae (Kuo and Cambridge 1978). The root epidermis of *Posidonia* is lignified but the exodermis is not, and in hard roots, such as those of *Thalass odendron* and *Amphibolis*, the walls of the epidermal cells and three or more layers of exodermal cells are thickened and lignified (Kuo 1983). The rhizosphere of many seagrasses has been found to support a diversity of microorganism, especially bacteria (Kuo *et al.* 1981).

According to Baydoum and Brett (1985) cell walls from rhizomes of *Halophila ovalis*, *H. stipulacea* and *Halodule uninervis* have non-cellulosic polysaccharides containing mainly glucose and arabinose, with very small amounts of pectin. The lignin consists mostly of nonconjugated phenols.

There are leaf sheaths in most seagrasses and these are clearly differentiated from leaf blades and enclose the young, developing leaves. The fibre bundles of the sheath are lignified, and because of this they persist on the rhizomes long after the other tissues of the leaf sheath have rotted away, as for example in *Posidonia* (Kuo 1978). The sheath fibres of *Posidonia* are rolled by wave action to form "marine balls" or "Posidonia balls" of different sizes.

Posidonia fibres had accumulated on the ocean floor in such quantities in the Spencer and St. Vincent Gulfs in South Australia that they were harvested for a time (1905 - 15) to make grain bags, paper and insulation material (Winterbottom 1917; Reid and Smith 1919).

2.2.2.4 Nutritive value of seagrasses

Birch (1975) observed that seagrasses from the Australian tropics had energy and nutrient levels similar to those of poor pastures. Total organic matter is usually in the range 75 - 80% of dry weight, but may be as high as 90% in newly formed leaves. Other nutritional components vary with season, species, age and portion of the plant (Harrison and Mann 1975; Bjorndal 1980; Klumpp and Van der Valk 1984; Pirc 1985). Carbohydrates average 50% of the dry weight of leaves, a high proportion of which is in complex form. The proportion of organic matter as fibre and other structural components is comparatively high in seagrasses, ranging between 30 and 80%, with cellulose as the main fibrous component (50 - 60%) and the remainder as hemicellulose and lignin (Bjorndal 1980; Klumpp and Van der Valk 1984). Whereas hemicellulose and cellulose can be digested and utilised by some consumers, lignin is the most refractory of fibrous components and can also further limit the digestive efficiency of consumers that are otherwise generally capable of utilising fibre (Van Soest 1982).

Most consumers derive their nitrogen requirements entirely from their food. Since animals are considerably richer in nitrogen, and are far less conservative with this element than are plants, the nitrogen composition of food exerts a critical influence upon the feeding rate and food selection of herbivores (Crawley 1983). Nicorti (1980) compared the composition of eighteen different type of marine plant and identified seagrass as the third highest in organic matter and energy, but only thirteenth for nitrogen. New leaves of *Thalassia* were richer in nitrogen than epiphytic algae which colonised older fronds (Odum et al. 1979).

Seagrasses typically contain some 10 to 15% protein, some of which may be inorganic or associated with non-protein amino acids and protein complexes of no known nutritional value. Up to 30% of nitrogen in seagrasses can be in such non-nutritious form (Harrison and Mann 1975; Suberkropp et al. 1976), but this does not appear to be the case in the algae. Hence the ratio of carbon to nitrogen, a widely used index of nutritional quality, can also be

inappropriate when comparing seagrasses and algae. Data obtained by Augier *et al.* (1982) and Pire (1985) showed that protein values are generally 10 to 20%, with the highest level in the new leaves. Interestingly, seagrasses posses higher levels of essential amino acids.

The quantity and quality of soluble organic components in marine plants would presumably influence food selection by herbivores, which obtain feed value by rupturing the plant cells to release their contents (Klumpp and Van der Valk 1984). With algae, this rupturing process can occur by either digestive acid lysis or maceration whereas seagrass tissue, due to the structure of its cell walls, is thought to be unaffected by acidic gut secretions.

Newly formed leaves of *Zostera marina* from Nova Scotia comprise 45% soluble organic matter, which declines significantly as the fronds age (Harrison and Mann 1975). In Australia, *Heterozostera tasmanica* and *Zostera muelleri* have similar proportions of organic matter in a labile form, whereas *Posidonia australis* is, by comparison, fibre rich. More specifically, the "juice" extracted from *H. Tasmonica* formes half the mass, 24% of the energy and 67% of the nitrogen of new fonds. The soluble carbohydrate fraction varies between 17 and 31% of total mass in seagrasses, compared with just 6 to 10% in algae (Walker *et al.* 1985).

2.2.3 Problems with aquatic plants

Aquatic plants have generally been regarded more as problems than as resources. A U.S. National Academy of Sciences report stated that the problem of aquatic plants was reaching alarming proportions in many parts of the world (National Research Council, 1976). The report pointed out the following adverse effects of these plants: blocking canals and pumps in irrigation projects, interfering with hydroelectric production, wasting water by evapotranspiration, hindering boat traffic, increasing waterborne disease, interfering with fish culture and fishing and impeding drainage, which results in flooding. The problems seem to be more severe in tropical areas. Aquatic plants may also foster mosquito borne diseases, because the small sheltered pools formed between floating plants are well adapted for mosquito breeding.

Thus in some regions of the world aquatic plants have become a major ecological and economical problem in recent years. For instance, agencies in Florida spend an estimated $3.5 million per year to control aquatic weeds for navigation improvement, drainage, fishing, water conservation and insect control (Bagnall *et al.* 1977; Lizama *et al.* 1988).

Similarly, on southern Australia beaches, there are hundreds of tonnes of seagrass, *Possidonia australis*, which are massed each year by the action of waves. Local residents consider that this causes a variety of environmental problems.

2.3. Evaluation of specific plants as feedstuffs

Animal feed evaluation is undertaken for different purposes. The main reasons could be summarised as follows (i) to estimate the extent to which one feed can replace another (Oldham and Emmans 1990) or which feed is the most economical to be fed (Flatt 1988) ; (ii) to relate feed attributes to animal requirements (Oldham and Emmans 1990) ; (iii) to direct the performance of farm animals through nutrition (Van der Honing and Steg 1990) ; (iv) to enable livestock feeders to calculate suitable diets for animals and plan for adequate feed supplies (Flatt 1988).

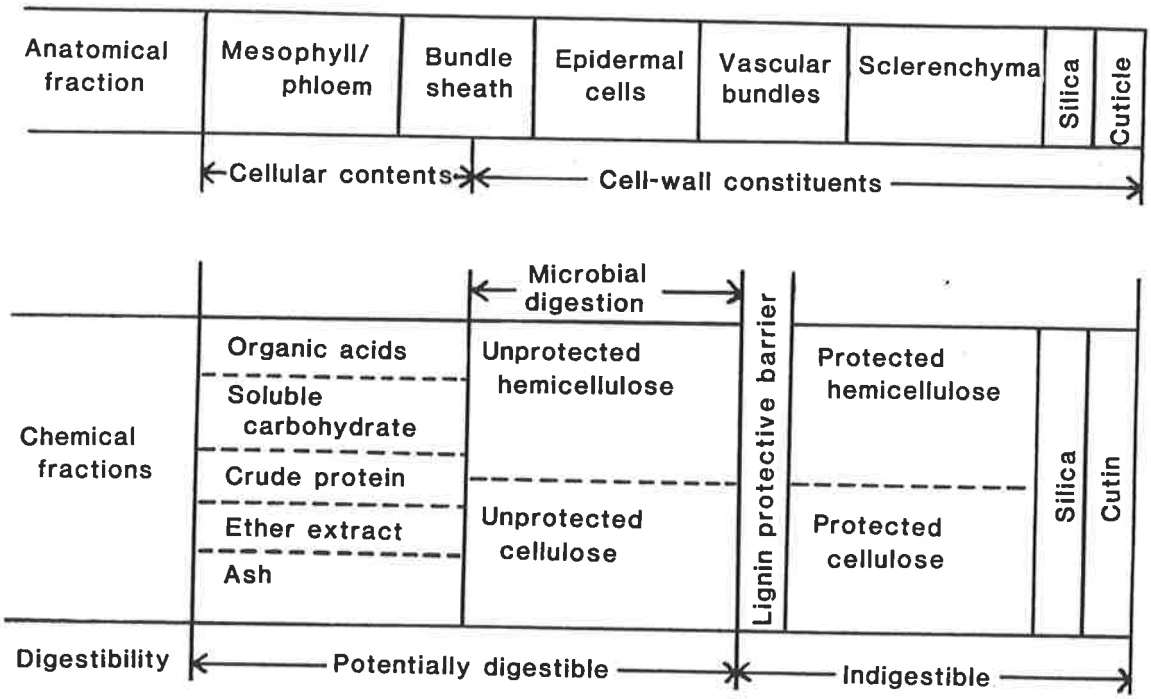
2.3.1 Chemical analysis

Chemical evaluation of plants is essentially aimed at obtaining analytical data that might predict the extent of biological degradation and utilisation under specified conditions. Such assays are usually for compounds that are expedient to handle, are known and or are considered important, and such evaluation is valid only if correct methodological principles are employed (Goering *et al.* 1973).

Forages, in common with all plants, are made up of variously modified cells. These all contain two major components: the cell contents and the membrane or cell-wall constituents (Van Soest 1965). The cell contents fraction contains most of the organic acids, soluble carbohydrates, crude protein, fats, and soluble ash, while the cell-wall fraction includes cellulose, hemicellulose, lignin, cutin, and silica. Figure 2.2) shows a simple model that links plant anatomy to chemical composition and provides a basis for understanding differences in the potential digestibility of the various fractions (Minson 1990).

Chemical analysis of feedstuffs provides a practical means of feed evaluation in the laboratory and helps investigators to assess available feeds and their possible nutritional values. The "proximate analysis" of feeds was originally introduced by the German scientists Henneberg and Stohmann in the 19th century (Van Soest 1969). However, the procedure has since been severely criticised and partially replaced by other analytical procedures e.g. the Van Soest fractionation of carbohydrates, (1967a). Early modified procedures continue to be used, however, because of their simplicity and of difficulties involved in the application of newer procedures.

Figure 2.2: Conceptual model of the relation between plant anatomy and chemical fractions, indicating areas of potentially higher digestibility by ruminants (Minson 1990)



2.3.2 Voluntary intake

Voluntary intake of feedstuffs may be defined as the quantity of dry matter eaten each day by animals when they are offered excess feed (Minson 1990). The amount of feed that an animal consumes in a given time is an important aspect of feed evaluation. The most important factor controlling animal production and the utilisation of roughages by ruminants is the voluntary intake of the material concerned (Hovell *et al.* 1986; Minson 1987; Garnsworthy and Cole 1990). The feed which ruminants voluntarily consume is the first step in the process of conversion of feed into valuable products (Ketelaars and Tolkamp 1992). Our understanding of the mechanisms which control feed intake in ruminants is still poor, because the mechanisms are extremely complex (Grovum 1988). However, it has been suggested that voluntary intake of roughages is determined largely by rumen load, which in turn is influenced by rate of digestion and passage (Balch and Campling 1962; Donefer *et al.* 1963; Montgomery and Baumgardt 1965; Thomas and Chamberlain 1990). Perhaps in diets consisting entirely or mainly of concentrates rather than roughages voluntary intake might be controlled by thermostatic or chemostatic regulatory mechanisms rather than by the rate of passage (Balch and Compling 1962; Weston 1985; Grovum 1988).

2.3.2.1 Factors influencing voluntary intake

Voluntary intake of roughages, concentrates and mixed feeds is influenced by different factors. These factors can be cited in three categories : (a) animal factors (Weston 1966, 1979, 1981; Freer 1981; Dulphy and Demarquilly 1983), (b) dietary factors (Elliott 1967a, 1967b; Weston 1967; Hegarty 1981; Barry and Duncan 1984; Howarth 1988; Pritchard *et al.*1988; SCA 1990; Ketelaars and Tolkamp 1992) and (c) other factors (CAB 1980; Grovum 1988; Ketelaars and Tolkamp 1992).

Animal factors such as species, genotype, age, sex, weight, body composition and physiological status affect the voluntary intake of feedstuffs in ruminants. Blaxter and Wilson (1962) reported that the voluntary intake of roughages by cattle and sheep is similar per unit metabolic body weight ($kgW^{0.75}$). Probably due to this similarity, equations have been proposed for prediction of intake by cattle from measurements made on the standard

sheep (Dulphy and Demarquilly 1983). Domesticated ruminant species are of different sizes (Ketelaars and Tolkamp 1992) and therefore different results with regard to the effect of size on intake will be seen in the literature. Even between ruminants of the same mature size and condition a considerable variability in voluntary intake can be found (Freer 1981). The physiological status of ruminants substantially affects feed intake. Generally, intake is higher if the physiological status is associated with enhanced nutrient demand. Thus, voluntary intake is usually high in young animals, in lactating and pregnant animals and when body tissue stores are depleted (Weston 1979). Ruminants achieve their highest voluntary intake during lactation. During this period animals spend more time both eating and ruminating and the rate of eating is increased (Dulphy *et al.* 1980; Weston 1981). Allden (1979a) reported that in Merino sheep voluntary intake increased progressively until about 30-40% of mature body weight was achieved, after which it remained steady or decreased slightly. Ketelaars and Tolkamp (1992) However, concluded that changes in intake following changes in physiological status (maturity, pregnancy and lactation) are poorly explained by current concepts.

It has been shown that the main characteristics of fibrous feedstuffs that determine intake by ruminants are those which limit the rate at which these can pass through the digestive tract (Balch and Campling 1962; Freer 1981). In ruminants, when a roughage is chopped or ground its voluntary intake increases, due presumably to the faster rate of passage of material from the rumen. On the other hand this physical treatment can result in a slight decrease in digestibility, because the feed is subjected to the microbial digestion for a shorter period of time (Garnsworthy and Cole 1990). Minson (1981) concluded that the quantity and composition of the fibre and the availability of essential nutrients for the microbial population of the rumen are the most important factors that influence intake of pastures. Weston (1979) reported that inadequate levels of essential nutrients depress feed intake. Moir *et al.* (1975) and Freer (1981) stated that generally roughage intake is inversely related to the cell-wall content and its rate of fermentation.

Taste smell and texture of feeds have all been regarded as important palatability aspects that may affect feed intake (Arnold 1981; Grovum 1988). Palatability is clearly a major factor in

feed selection by ruminants when they are grazing or are fed hay of different quality (Doyle and Egan 1987; Minson 1990). Some materials, such as molasses, often make the diet or feed mixtures more acceptable to livestock not only because of their taste but also because of their effect in controlling dust (Grovum 1988). Sometimes, in spite of the rumen being nearly empty, intake of some feeds is very low. This may be attributed to some other roughage characteristics that influence taste and odour (Weston 1979; Dulphy and Demarquilly 1983).

Many minor anti-nutritive constituents, such as phenolic compounds, tannins, saponins, alkaloids and allergenic compounds can be found in various species of forage and pasture plants and crop residues (Hegarty 1981; Howarth 1988; Liener 1990). Their concentrations are influenced by factors such as plant maturity and climate (Weston 1979) and could be reduced by plant breeding, crop and animal management, or by secondary processing of the crop (Howarth 1988). These constituents could reduce feed intake and animal appetite by adversely affecting the metabolism of the ruminant itself and of the rumen micro organisms (Weston 1979; Minson 1981).

Diseases and parasitic infections reduce potential feed intake (Weston 1979, 1981; SCA 1990). Climatic factors such as rain, wind, ambient temperature and humidity leading to thermal stress in the animal also have varying effects on feed intake (SCA 1990). For example, feed intake is increased when the ambient temperature falls below the thermo-neutral zone of the particular animal (SCA 1990).

It is important to understand feed intake in ruminants. Recently it has been concluded that the degradation characteristics of roughages in the rumen may have a useful application in predicting voluntary intake (Hovel *et al.* 1986; Ørskov *et al.* 1988).

2.3.3 Digestibility

Chemical analysis methods can indicate the potential value of a particular feed for farm animals, but its actual value can only be arrived at after making allowances for the inevitable but varying losses that occur during digestion, absorption and metabolism. Perhaps the most useful measurement of the nutritional value of livestock feeds is their digestibility

(Corbett 1969). Individual digestibility coefficients refer to that part of a given feed or ration which disappears during passage through the digestive tract (Merchen 1988). A vast number of digestibility determinations have been made upon a great variety of animal feeds and the results have been used as the basis for computing the relative values of livestock feeds.

The conventional method for measuring digestibility is substantially that originally developed by Henneberg and Stohmann (Armsby 1917). In those trials the test feeds were given to the experimental animals in known amounts and the output of faeces measured. Generally, more than one animal is used (Schneider and Flatt 1975; McDonald *et al.*1988; Merchen 1988) in this method. Because faeces contain quantities of material of non-dietary origin the coefficients are known as apparent digestibilities. The digestibility of some feeds such as grain or protein supplements which are seldom fed as the sole ingredient in a ration can be estimated by difference (Merchen 1988).

In some circumstances it may be difficult or impractical to measure directly either total feed intake or total faeces output or both. In such instances the use of inert and completely indigestible substances, known as indicators or markers, can be employed. If the concentrations of these markers in the feed and in small samples of the faeces are determined, the ratio between these concentrations give an estimate of digestibility. The indicators can be grouped into internal markers such as lignin, acid-detergent fibre and acid-insoluble ash, and external markers such as chromic oxide (Maynard and Loosli 1956; McDonald *et al.* 1988; Merchen 1988).

Digestion coefficients of animal feeds are not constant in all situations. They are influenced by a number of factors (Sheehy 1955; Maynard and Loosli 1956; Tyler 1966; McDonald *et al.* 1988; Merchen 1988). Of these factors chemical composition of a feed chiefly affects its digestibility. Generally the fibre fraction has the greatest influence on the overall digestibility of a given feed. Crude fibre tends to exert a protective influence against the digestibility of all other nutrients. For this reason the digestibility of highly-lignified materials such as straws is generally very low. The digestibility of a feed can be influenced by the composition of other ration components, that is various associative effects occur. Thus the

digestibility of a mixture of feedstuffs usually does not equal the sum of the separately-determined digestibilities of its components and most of the time these effects are non-additive (Mould 1988). With increasing levels of intake generally the rate of passage of digesta is increased, therefore feed leaves the reticulo-rumen at a faster rate and less digestion occurs (Joanning *et al.* 1981; Moe 1981). In roughages, particle size reduction also reduces the appearance time of the particles in the rumen before being reduced to a size small enough to exit through the reticulo-omasal orifice. Some feed processing such as chopping, crushing, grinding and pelleting of roughages decreases feed digestibility, but others such as heat treatment improves it. Animal species, their physiological status, age, activity and environmental conditions are the other factors which may influence feed digestion (Sheehy 1955; Corbett 1969; McDonald *et al.* 1988).

Although the conventional method for measuring the digestibility of livestock feeds dates back to the 19th century, it is still the most precise technique for ruminants (Navaratne *et al.*1987). However, because of some obvious limitations with this method, such as high labour requirements, high cost, the large numbers of animals and quantities of feeds needed and the long time involved, there have been numerous alternative analytical systems used to attempt to predict digestibility *in vivo* . These include laboratory procedures such as the Weende system of analysis (McDonald *et al.* 1988), digestion *in vitro* (Tilley and Terry 1963) and detergent fibre fractionation methods (Van Soest 1967). Some of these techniques have a highly significant correlation with digestibility *in vivo* (Deinum and Van Soest 1969; Oddy *et al.* 1983). But the accuracy of any predictions is influenced by many different factors and the techniques must be modified for local conditions.

The most common procedure *in vitro* is the so-called "two-stage *in vitro* technique" which was described first by Tilley and Terry (1963). In the first stage of this method the finely-ground samples of the test feeds are incubated with buffered rumen liquor in glass tubes under anaerobic conditions for 48 hours. In the second stage protein solubilisation is continued with a pepsin-HCl solution. Total dry matter disappearance of the samples is considered to be digestible dry matter or organic matter. Digestibility determined by this

technique is generally slightly lower than that measured *in vivo* and corrective equations may be required (McDonald *et al.* 1988).

In spite of the two-stage technique for *in vitro* digestion being the most accurate laboratory method for predicting digestibility of animal feeds, its requirement for fistulated animals is an important disadvantage of this method. Thus recently many attempts have been made to predict digestive values using various enzymatic preparations (de Boever *et al.* 1988).

One measure of nutritional value for ruminants is the rate of digestion of roughages in the reticulo-rumen. This rate can be measured using a useful, simple, fast and cheap technique, the so-called nylon bag *in sacco* or *in situ* technique (Chenson *et al.* 1970; Mehrez and Orskov 1977; Playne *et al.* 1978; Uden and Van Soest 1984; Kandylis and Nikokyris 1991). The use of the nylon bag technique was first reported by Quin *et al* . (1938) and since then numerous reports have been presented using this technique (e.g. Balch and Johanson 1950; Erwin and Ellison 1959; Van Keuren and Heinemann 1962; Ørskov and Deb Hovel 1978; Mapoon 1980; Nakashima and Ørskov 1992).

In this technique small amounts of the feed samples under test are placed in the nylon (Dacron) bags and incubated in the rumen of fistulated ruminants for pre-defined periods of time. The differences in weight of samples before and after incubation are considered as dry matter or organic matter disappearance. Rumen degradation of feedstuffs as measured *in sacco* correlates well with digestibility *in vivo* (Uden and Van Soest 1984).

Factors affecting the accuracy of the *in sacco* technique can be divided into three categories: (a) bag specifications; (b) sample preparation and (c) experimental animals. Bags for the *in sacco* technique are generally constructed from single pieces of nylon (Dacron or Polyester) cloth (Ørskov *et al.* 1980; Weakley *et al.* 1983; Vik-Mo and Lindberg 1985; Susmel *et al.* 1990). The pore size of the bag cloth regulates the passage of solid particles from and rumen micro organisms into the bags (Ørskov *et al* 1980; Lindberg and Knutsson 1981; Lindberg 1985). Small pore size restricts the flow of rumen liquor and large micro organisms (protozoa) may not be able to enter the bags. There is also a risk of gas formation in this kind of bag (SCA 1990). Uden *et al.* (1974) found dry-matter disappearance of

Guinea grass from nylon bags with a 53 micron pore size was higher than those with 20 or 35 micron pore size. Lindberg (1985) noted that degradability of feed proteins in the rumen was underestimated when pore size was 10 microns and overestimated when it was 36 microns, but Setälä (1985) for the same materials proposed a bag pore size of 40 microns. Nocek (1985) reported that disappearance of soybean meal was higher for bags with pore sizes of 80 and 102 microns compared with 6, 20, 40 or 59 microns. Ibrahim *et al.* (1988) and Gomez Cabrera and Van der Meer (1988) considered a bag pore size of 41 microns as best for estimating the degradability of rice straw and barley straw.

Different sizes of nylon bags have been used. For example, dimensions of 3.5 x 5.5 cm (de Boer *et al.* 1987), 5 x 8 cm (Mehrez and Ørskov 1977), 10 x 11 cm (Michalet-Doreau and Cerneau 1991), 7 x 10 (Flachowsky *et al.* 1991), 8 x 10 (Ørskov and DeB-Hovel 1978), 10 x 12 (Weakley *et al.* 1983), 9 x 17 (Chapman and Norton 1982) and 19 x 10 cm (Gomez Cabrera and Van der Meer 1988) have all been tried. However, SCA (1990) concluded that a suitable size for the bags is 12.5 x 8.0 cm which, after tying, will have an effective surface area of around 150 cm^2. Nylon bags with double-stitch sewing and rounded bottom corners are recommended (Ørskov *et al.* 1980).

Preparation of the samples for this technique is critical. Samples should truly represent the materials which are actually consumed by the animals. The bulk density of feeds probably is the main factor that determines the size of samples. Ørskov *et al.* (1980) reported that 2 g air-dry ground straw, 3 g for good quality hay, 5 g for concentrates and 10-15 g for fresh herbage are suitable sizes. Susmel *et al.* (1990), Ibrahim *et al.* (1988) and Van der Koelen *et al.* (1992) incubated 5 g per bag of air-dried forages, rice straw and a mixed ration per bag respectively. Lindberg (1985) noted that the relationship between amount of sample per unit bag area gave a better estimation of appropriate sample size and he concluded that for bags with a pore size of 20 to 40 microns sample size should be 10-15 mg dry matter/cm^2 bag surface area. Setälä (1985) demonstrated that the size of the sample for feed protein in a bag with internal dimensions of 6x12 cm should be 25-30 mg dry matter per cm^2 for feed protein.

The best particle size of sample would ideally seem to be that as masticated and presented to the rumen, but little information is available to compare particle size of fibrous materials for *in sacco* experimentation (Nocek 1988).

The *in sacco* technique has been used in several animal species including sheep (Ford *et al.* 1987; Pienaar *et al.* 1989), cattle (Van der Koelen *et al.* 1992), including steers (Von Keyserlingk and Mathison 1989) and heifers (Uden and Van Soest 1984), buffalo (Jelan and Kabul 1988) and goats (Uden and Van Soest 1984). Fistulated animals should be fed at maintenance level or slightly higher, and their basal diet should have the same characteristics as the materials under investigation (SCA 1990).

A maximum of six nylon bags may be placed in the rumen of a single sheep at any one time, but more can be incubated in larger animals. Bags should be suspended freely in the rumen and the recommended length of cord from the cannula is about 20-25 cm (Kempton 1980) in sheep. A minimum of three animals or three replications must be used (SCA 1990). The time required for complete degradation varies with the feeds being incubated. As a rough guide, this time is 12-36 hours for concentrates, 24-60 hours for good-quality hay and 48-72 for low-quality roughages (Ørskov *et al.* 1980; Adebowale *et al.* 1989).

It can be concluded that the *in sacco* technique is a rapid and simple method for obtaining basic information about potential digestibility and nutritive value of livestock feedstuffs. Its value for assessing the extent and rate of degradation of low-quality roughages should be investigated further.

2.4 Characteristics and utilisation of lignocellulosic feed stuffs (roughages)

Lignocellulosic resources are either already partly in use (conventional) or are considered to have considerable potential (nonconventional). The nonconventional feedstuffs occupy an important position in the ultimate solution to the world's serious food situation, together with further intensification of agricultural production. The dominant source of unconventional feed resources is lignocellulosic. Lignocellulosic materials contain about 70% complex saccharides, mostly cellulose and hemicellulose. They are produced constantly and have been considered the earth's richest renewable energy source (Boda

1990). Lignin, however, which also occurs in lignocellulosic roughages, possibly rivals cellulose as the most abundant renewable organic resource, i.e in reduced carbon and photosynthetic energy content. Lignin and cellulose, together with hemicellulose, occur primarily as the structural components of the vascular tissues of higher land plants (Kirk 1984).

In general roughages have been defined as rough, coarse and bulky feedstuffs of low nutritive value, containing high levels of crude fibre, which stimulate intestinal muscular movements (Allen 1984; Dalal-Clayton 1985; Webster's dictionary 1988; Macquarie Library 1990). Roughages usually comprise the vegetative parts of plants and are more fibrous, often highly lignified and therefore less digestible (Evans 1979; Castillo 1983; Ellis *et al.* 1988; Hunter 1988). These fibrous feeds are derived from many types of plants, both pasture and crop, and vary in quantity and quality response to many environmental factors.

The cell wall of mature plants is made chiefly of cellulose, hemicellulose and lignin (Han 1978; Bacon 1988). Lignin, which is not a carbohydrate, is nevertheless closely associated with cellulose and hemicellulose in the plant cell wall and confers chemical and biological resistance to this wall and mechanical strength to the plant (Barton 1988; McDonald *et al.* 1988). The cell wall can account for up to 90% of the plant dry matter (Aman and Graham 1990), but there is a considerable variation in its composition, mainly in relation to different stages of growth and maturity (Hogan and Weston 1969; Hogan *et al.* 1969). A high percentage of lignin in sawdust and bark and the very high content of silica in paddy straw and hulls are examples of extreme variation (Jackson 1977). Bacon (1988) has concluded that the amount of cellulose in total-plant cell-wall dry matter ranges broadly from 35 to 60%.

The cellulose molecule is a linear polymer of ß (1-4) glucose units. Hemicelluloses are made up of relatively short chains of a mixture of glucose and mannose linked in ß (1-4) form to the cellulose (Hogan and Leche 1983). The lignin molecule is made up of many phenyl propanoid units associated in a complex, cross-linked structure (McDonald *et al.* 1988).

Fibrous residues are a dominant fibrous material which is used as a feedstuff. Owen (1976) has divided fibrous residues into four broad categories: (a) crop waste produced in the field, which can be further divided into materials with a high dry matter content, such as cereals, legumes and grass straws, and materials with a high moisture content, such as sugar beet and sugar cane leaves (tops) and horticultural by-products; (b) crop wastes produced in industrial processing, such as sugar-cane bagasse, oilseed husks, sugar beet and citrus pulp; (c) fibrous wood waste, such as sawdust, bark, waste wood, pulp and paper-making residues and (d) fibrous animal waste consisting of undigested parts of feed and bedding materials.

The chemical composition of poor-quality roughages varies with species type and variety, with stage of maturity, location, climatic conditions and cultural practices (Jackson 1977; Hogan and Leche 1983). Mature roughages such as cereal straws have been known for their high level of fibre and low amount of crude protein (Dixon 1987). The crude protein contents of legume straws and oilseed hulls are relatively higher, but still their crude fibre contents are as high as cereal straws. Fibrous residues seem to be better sources of the major minerals such as calcium, magnesium, potassium and in some cases sodium than non-fibrous (Coombe 1980).

2.4.1 Availability

Approximately 40% of the biomass produced by photosynthesis every year finishes up as by-products (Boda 1990). Fibrous roughages form the major part of this biomass. One method for estimating the quantity of some of them is from figures for total crop production. In almost all cases the quantity of residues or by-products is equal to or greater than the quantity of products used directly as human food (Kiflewahid 1983).

Grain legumes and grass crops produce some residues, but compared with cereal straws these are quantitatively unimportant (Owen 1976). For example, in the ICARDA region, the overall contribution of grain legume residues to the diets of ruminants is about 1 percent, whereas for cereal straws this value is approximately 30 percent (Capper 1988). The nutritional studies carried out by Allden (1978-1979) in South Australia indicated that grain legume crops and residues are superior in quality to cereals but quantitatively unimportant.

Besides fibrous roughages left in the field, industrial food processing produces localised amounts of such residues as citrus pulp. The quantity of wood wastes is also large and probably about half that of cereal straw (Owen 1976).

Spedding (1971) has calculated that the energy which could be made available from Australian fibrous roughages each year could support about 48 million sheep at maintenance levels. If these roughages were treated by chemical, physical and other methods their potential could be much higher. On a world scale a very large number of ruminant livestock could survive on these materials each year. However, more than 3/4 of these by-products are left on the land (mainly in developed countries) and not harvested (Spedding 1971).

2.4.2 Importance of using lignocellulosic material as animal feeds

The total world population was about 2,400 million at the middle of this century. It reached 5,300 million forty years later (FAO 1990) and is still increasing. The present rate of increase is around 250,000 per day or 90 million per year. The United Nations has predicted that by the year 2050 the world population will reach 10,000 million people (Langer and Hill 1991). This high world population creates enormous pressures and demands for food and other resources. At present, hunger and under-nutrition is the main problem for an increasing proportion of the world's human population (Boda 1990). The problems of feeding the world's livestock is equally great, and both food and feed must be produced against declining resources of soil and vegetation. The main factor hindering the expansion of animal production in the developing countries, however, is the inadequate supply of good-quality roughage (Promma 1988).

The current utilisation of fibrous materials has been classified by Castillo (1983) as follows: (a) ruminant feed; (b) fertiliser or soil improver; (c) fuel; (d) source of building construction materials, kitchen utensils, handicrafts; (e) source of herbal medicines, biologics or chemicals and (f) miscellaneous uses.

Huge amounts of low-quality roughages are produced every year as a result of the production, processing and utilisation of human food (Tannenbaum and Pace 1976). These can of course be converted into useful products by feeding them to ruminants (Coombe

1980) and in many regions of most countries of South East Asia, West Asia and North Africa feeding systems for ruminant animals for at least part of the year are based on such by-products as fibrous crop residues or dried mature plants which have few alternative uses (Jayasuriya 1983; Kiflewahid 1983). Traditionally, farmers have accepted that crop residues constitute the principal supply of roughage for their livestock during the dry period (Promma 1988). Australia and many of the developing tropical countries suffer from a prolonged seasonal drought period which imposes a severe limitation on successful animal production systems. Fibrous crop residues such as cereal straws can be particularly useful during the dry season to supplement poor-quality grazing (Coombe 1980).

Many countries are trying to become self-sufficient in food production. Therefore it is logical to utilise as much as possible of the animal feed resources available and the utilisation of low-quality roughages has an important role in this context (Sundstøl 1984). Modern methods of cereal cropping tends to produce large quantities of fibrous residues (Burrows *et al* 1979). Generally, the disposal of these ligno-cellulosic residues is a big problem for farmers. Burning is regarded as wasteful (it also causes pollution) so a more positive approach is encouraged.

In India, for instance it has been estimated that dry roughages (mainly cereal straws) provide over 50% of the annual feed supply (Coombe 1980). Dixon and Egan (1988) reported that in South East Asia ruminants are usually dependent on diets based on crop residues roughages for at least for part of the year. Many attempts have been made to maximise the use of fibrous crop residues as animal feeds through supplementation or by applying different treatments. In Africa, some proportion of cellulosic materials is traditionally used in livestock feed, as fuel or bedding but large quantities are still wasted (Kiflewahid 1983). In North America a great number of trials have been conducted to use wood waste as an animal feed (Boda 1990).

2.4.3 Limitations to the use of lignocellulosic feedstuffs as animal feeds

The limitations to the use of poor-quality roughages, or crop residues, as ruminant feeds can be summarised as follows: (a) low palatability, intake and digestibility (Han 1978; Dixon

and Egan 1988; Hunter 1988); (b) low concentrations of nitrogen, sulphur and essential minerals for optimal rumen fermentation (Coombe 1980; Dixon and Egan 1988; Hunter 1988); (c) seasonal availability and bulky nature that restrict storage and transportation (Dixon and Egan 1988; Ellis *et al.* 1988) and (d) possible anti-nutritive factors (Van Soest 1981; Dixon and Egan 1988).

It is generally known that low animal productivity on poor-quality residues is due to both low palatability and low intakes of digestible energy and protein, which reflect particularly changes in the chemical composition of the plant as it matures (Han 1978; Coombe 1980). As plant cell walls mature the fibre content (basically lignin) increases, but with a disproportionate decrease in soluble nutrients and fermentability. Lignin, which protects the structural polysaccharides that are combined with it from microbial breakdown, is probably the major factor causing decline in the digestibility of plant cell walls with maturity (Harkin 1973 and Boda 1990). In addition physical factors, such as the morphology of cellulose in the cell wall structure also reduce the usefulness of these materials as animal feed (Van Soest 1981).

In conclusion, when feeding ruminants a diet based predominantly on these materials it is generally impossible to sustain them even at maintenance levels. In such circumstances appropriate treatment and nutrient supplements are required. Improving the feeding value of low-quality roughages will be discussed in the following section.

2.5 The possibility of improving the nutritive value of lignocellulosic roughages

It has been shown that the nutritive value of many lignocellulosic materials can be improved by using various processing methods (Han 1978; Kiflewahid 1983; Sundstøl 1988). Treatment processes can improve the voluntary feed intake, digestible energy content or a combination of these effects (Doyle *et al.* 1986; Sundstøl 1988). Processing methods used can be divided into five categories: (a) physical treatments; (b) chemical treatments; (c) physico-chemical treatments; (d) biological treatments; (e) supplementation

2.5.1 Physical treatments

Many physical treatments have been employed to increase the feeding value of low-quality roughages. Some of these methods, such as chopping and grinding, increase voluntary intake but are unlikely to affect chemical composition. However, others such as soaking, irradiation or steaming under pressure probably do have some effects on the chemical composition.

Long fodders such as hay and straws are sometimes chopped both to reduce wastage and to facilitate feeding. Nevertheless, cutting of long straws and other low-quality roughages into short pieces does not alter the cell structure (Doyle *et al.* 1986) and hence does not necessarily increase digestibility for cattle or sheep (Sheehy 1955).

Grinding low-quality roughages increases the surface area accessible for microbial attack in the rumen (Smith and Broster 1977; Doyle *et al.* 1986). The extent of any increase in surface area depends on the fineness of grinding. It has been established that the major effect of grinding, however, is on increasing voluntary intake (Kiflewahid 1983; Sundstøl 1988), although with a concomitant lowering of fibre digestibility. Reduced digestibility following grinding of low-quality feeds can be attributed to an increase in the rate of passage from the rumen (Minson 1963; Wilkins 1981; Seoane *et al.* 1982; Davis 1983).

Johanson *et al.* (1964) reported that both retention time in the rumen and nutrient digestibilities of ground or pelleted hay were significantly lower than for long or chopped hay. Greenhalgh and Wainman (1972) however concluded that milling roughages increased their voluntary intake. Millett *et al.* (1970) noted that digestibilities *in vitro* can be increased by extreme reduction in particle size and vibratory ball milling was very effective for some wood species. The digestibility *in vitro* of Aspen and Oak wood increased greatly by ball milling for about 30 minutes, but a longer period was no more effective.

In an experiment reported by Walsh (1974) straw ground to the size of 3/8" gave optimum animal performance when fed at the rate of 30 percent in a complete diet. Despite the increased rate of passage due to grinding, digestible dry matter intake usually increased and this occurred to a greater extent for low-quality roughages than for better quality feeds

(Minson 1981). However, the net effect should be assessed in terms of digestible organic matter intake.

Pelleting feed first became popular for poultry and rabbit feeds, because pelleting provides a more edible form for finely ground roughage (Dobie 1959). The pelletised feeds are less dusty, more convenient and pleasant to handle and are not subject to wasting or sorting by the consuming livestock. Feed pellets require for storage space only about 1/5 of that required for chopped or long hay (Bruhn 1955, 1957; Butler and McColly 1959).

Numerous research reports have shown that grinding and pelleting roughages increases the voluntary intake and performance of livestock consuming these feeds (Wallace *et al.* 1961; Johnson *et al.* 1964; Campling and Freer 1966; Greenhalgh and Wainman 1972; Greenhalgh and Reid 1973, 1974; Van Niekerk *et al.* 1973). This has been particularly true for low-quality roughages (Minson 1981) and when roughages constituted most of the diet (Johanson *et al.* 1964; Greenhalgh and Reid 1973). Generally, digestibility *in vivo* of pelleted roughages is low, but increased intakes more than compensate for this, thus the overall effect is increasing the intake of digestible nutrients (Reynolds and Lindahl 1960) and body weight gain (Cate *et al.* 1954; Weir *et al.* 1959; Wallace *et al.* 1961).

Greenhalgh and Reid (1973) observed that the increase in intake in response to grinding and pelleting was greater in young animals (38%) than older animals (18%) and for mature than immature herbage. In another experiment (1974) they found that the effect of pelleting on intake was much greater in short-term (61%) than in long-term (40%) feeding trials. Campling and Freer (1966) showed the voluntary intake of ground, pelleted oat straw was about 26% greater than that of long straw. A simple explanation for increased intake could be that pelleted feeds are more palatable and spend less time in the rumen (Meyer *et al.* 1959; Pearce and Moir 1964; Campling *et al.* 1963). However, economic constraints such as the cost of processing and transportation can restrict the use of this treatment in some countries and regions.

Soaking and wetting livestock feeds has been practised since early times, mainly on peasant farms as a traditional method. Water treatment has been applied for quite different diets,

such as high-concentrate or high-roughage rations (Dalton *et al.* 1953; Hupp and Lewis 1958; Holzer *et al.* 1975; 1976; King 1982). Dalton *et al.* (1953) and Hupp and Lewis (1958) reported that moistening concentrate feeds for dairy cattle significantly increased rate of ingestion. Meyer *et al.* (1959) found that when ground alfalfa was moistened, feed intake and body weight gain of experimental sheep were increased to almost the same extent as by pelleting. Holzer *et al.* (1976) demonstrated that the performance of animals fed moist diets improved. They concluded that it may be attributed to the effects of three factors: (a) increased feed intake (b) improvement in digestibility and (c) increased concentration of propionic acid in the rumen. Similar results in zebu and buffalo calves were reported by Chaturvedi *et al.* (1973). Koes and Pfander (1974) observed a significant increase in digestibility of cellulosic components when water was sprayed over hay prior to feeding to lambs.

Roxas *et al.* (1987) noted that nutrient digestibility and composition of soaked and unsoaked rice straw did not differ significantly, but daily feed intake and body-weight gain resulting from wetted straw was higher than from untreated straw. However, there are two disadvantages in using this treatment; firstly, some limiting of the intake of other nutrients because of the consumption of large amounts of unnecessary water and, secondly, soaked feeds are liable to turn sour when kept for a period more than a few days, especially in warm conditions (King 1982). Therefore, this method of treatment does not appear as suitable as grinding or pelleting.

Irradiation by gamma rays or by high-velocity electrons has been reported to degrade the cell wall structure so that some insoluble carbohydrate components become available for rumen fermentation (Lowton *et al.* 1951; Pritchard *et al.* 1962; McManus *et al.* 1972). This treatment may destroy the natural bonds between lignin and other structural components and reduce the resistance of fibrous feeds to physical degradation without the necessity for fine grinding. Huffman *et al.* (1971) showed that four species of wood that were exposed to $1x10^8$ or $2x10^8$ rads of gamma irradiation had lower cellulose, ADF and ADL content than the un-irradiated samples or those exposed to $1x10^6$ and $1x10^7$ rads. Although this treatment appears to provide an effective means of enhancing the carbohydrate digestibility

of certain roughages (McManus *et al.* 1972), the high costs of the process prohibits its commercial and practical application.

Steam processing and high temperature and pressure treatment of low-grade roughages have been reported to increase feeding value by freeing digestible nutrients from indigestible materials such as lignin or silica (Guggolz *et al.* 1971; Klopfenstein and Bolsen 1971; Oji and Mowat 1978; Rangnekar *et al.* 1982). Under laboratory conditions the effect of steam under pressure on fibrous feeds has been very successful. Hart *et al.* (1981) treated ground rice straw, sugar cane bagasse and sugar cane field trash with steam under pressures ranging from 7 to 42.2 kg/cm^2. The enzymatic digestibility *in vitro* of rice straw and sugar cane bagasse was increased from 26 to 47% and from 17 to 41% respectively after 90 sec. at a pressure of 21.1 kg/cm^2. At this pressure crop residues lost between 5 to 8% dry matter due to volatilisation.

In an experiment *in vivo* Oji and Mowat (1978) treated ground (1.6 cm screen) corn stover at a pressure of 16.2 kg/cm^2 and 205°C for 15 minutes and fed it to wether lambs. This treatment increased dry matter intake (55%) and apparent organic matter digestibility (4 units). However, Garrett *et al.* (1981) showed that steam pressure treatments of rice straw at a pressure of 28 kg/cm^2 for either 20 or 90 sec. did not improve straw digestibility and lamb performance, and they pointed out that there were no animal health problems with steam pressure treated straws, other than body-weight loss.

Heat damage, dry matter losses and the generation of deleterious materials are associative problems with this method. Also, it has no scope in small-scale farming systems due to the cost.

2.5.2 Chemical treatments

Since 1900 chemical treatments have been used for low-quality roughages with the objective of improving the accessibility of cell wall carbohydrates to microbial enzymes in the rumen. A wide variety of chemical treatments has been tested for their potential to improve the nutritive value of these materials (Jones and Klopfenstein 1967; Klopfenstein *et al.* 1972; Jackson 1977; Smith and Broster 1977; Arnason 1979; Hartley 1981; Wilkins 1981; Ibrahim

1983; Kiflewahid 1983; Ben-Ghedalla and Miron 1984; Bunting *et al.* 1984; Lewis *et al.* 1987; Cottyn and DeBoever 1988; Solomon *et al.* 1992;). Although most attention has been given to treatment with alkalis, feeding value of poor-quality feeds may also be increased by treatment with oxidizing agents and acids. Therefore, the chemicals which have been most extensively studied can be classified into three categories: (a) alkalis, (b) oxidative reagents and (c) acids. Alkali treatments usually solubilise hemicellulose and increase the extent and rate of cellulose and hemicellulose digestion. Lignin contents of treated feeds are generally not reduced by alkali treatments (Feist *et al.*1970; Klopfenstein 1978; McManus 1978; Nikolic 1982). Increased digestion is generally attributed to the breaking of bonds between structural carbohydrates and lignin rather than to the removal of lignin (Bunting *et al.* 1984).

Many alkalis have been used in laboratory tests to assess potential to increase digestibility of low-quality feeds. Nevertheless, only four alkalis are being routinely used in experimentation with animals. These are sodium hydroxide, ammonium hydroxide, calcium hydroxide and potassium hydroxide (Gadden 1920; Beckmann 1922; Siebert 1974; Klopfenstein 1978; Sundstøl *et al.* 1979; Lesoing and Klopfenstein 1981; Djajnegara *et al.* 1985).

In 1895, Lehmann reported that dry matter digestibility of oat straw was increased from 37% to 63% and that of wheat husks from 26% to 56% when boiled in a 2% NaOH-solution. Since the beginning of this century many attempts have been made to improve the feeding value of fibrous feeds by chemical treatment and the most prominent among these has been sodium hydroxide treatment. Beckmann (1922) described a practical and on-farm procedure for treating straw with NaOH-solution. In his method chopped straw was soaked for at least 4 h. with 8-fold its weight of 1.5% NaOH-solution at ambient temperature and pressure. Subsequently, the treated straw was washed free of NaOH. The wet-treated straw was fed directly. This treatment increased the organic matter digestibility of straw from 45 to 68%, but resulted in a loss of about 20% of dry matter. Furthermore, the procedure required large amounts of water to wash the NaOH-treated straw and this washing removed soluble nutrients. On a large scale it caused a pollution problem (Wilson and Pigden 1964; Hartley 1981; Kristensen 1982; Sundstøl 1988). It has been estimated, however, that about 15-20%

of Norway's annual straw production is treated on-farm by the Beckmann technique (Owen 1978).

To eliminate the problems associated with the Beckmann method, since the 1960's several dry caustic techniques have been developed. Wilson and Pigden (1964) described a new method the so-called "dry NaOH treatment", based on the use of a much smaller amount of more concentrated alkaline solution. The moist product is fed to animals without being washed (Jackson 1977). Chandra and Jackson (1971) showed rumen dry matter digestibility of ground maize cobs was increased by more than 100% after using a spray method proposed by Wilson and Pigden (1964) at a rate of 10 g per 100 g of roughage (10%). In a feeding experiment with male calves conducted by Singh and Jackson (1971) digestible organic matter intakes of sodium hydroxide spray-treated straw increased with increased concentrations of sodium hydroxide up to 3.3% . Higher concentrations of alkali were less effective in this regard.

The appropriate level of NaOH for dry methods has been the subject of much research. Wilson and Pigden (1964) found that processing wheat straw with up to 9% NaOH caused marked increases in digestibility *in vitro* . Higher NaOH levels gave no further increases. Feist *et al.* (1970) reported maximum digestibilities of Aspen and Red Oak woods was obtained at 5 to 6% alkali, with no further increases observed at higher levels. Mowat and Ololade *et al* (1970), in a sheep feeding trial, observed that dry matter digestibility of barley straw increased by treatment with 4% of sodium hydroxide, but NaOH treatment at higher concentrations was less effective. In general, it is concluded that the best animal performance has been obtained using 3 to 5% alkali.

Sundstøl (1988) has divided treatment methods with NaOH into three categories: (a) wet treatment, a modified method of the Beckmann procedure which has been used by Norwegian farmers for more than four decades and is still in use; (b) semi-wet treatment, in this method fibrous materials are mixed with 3-5% NaOH at a moisture content of 40-70% and ensiled in an air-tight condition for a minimum period of one week and (c) dry treatment, the technique first proposed by Wilson and Pigden (1964). In this treatment a certain

amount of disolved alkali is sprayed on the low quality roughages, mixed and fed to ruminants without washing or ensiling.

However, in spite of the high cost of NaOH processing and the high cost of transportation of fibrous feeds nowadays, in most countries feed prices are steadily increasing and therefore effective methods for treatment of poor-quality roughages can be economically employed in many circumstances, even on a large scale.

The upgrading of low-grade roughages by ammoniation has been known for a long time (Chomyszyn *et al.* 1961; Kernan *et al.* 1981). Ammonia is used in three forms: (a) pure form (anhydrous or gaseous); (b) water solutions (aqueous ammonia or ammonium hydroxide) and (c) solid compounds e.g. urea (Kiangi and Kategile 1981; Davis 1983; Sundstøl 1984; Doyle *et al.* 1986; Cottyn and De Boever 1988).

A simple method for treating straws with anhydrous ammonia has been developed by Norwegian workers. According to their method anhydrous ammonia at the rate of 30 kg per tonne is injected into straw wrapped with polyethylene sheets. Gaseous ammonia penetrates into the stack after evaporating from the liquid. The recommended treatment time is about 8 weeks in northern Europe. Waiss *et al.* (1972) observed that when rice straw was treated with 5% ammonia the optimum moisture content for best results was 30% at ambient temperature.

Generally, ammonia treatment increases both digestibility and nitrogen content of treated straws (Ibrahim 1983; Kiflewahid 1983; Promma *et al.* 1985; Cottyn and De Boever 1988). However, two-thirds of the applied ammonia probably remains unattached and therefore the treated materials must be aerated prior to feeding to improve their palatability and intake (Promma *et al.* 1985). In spite of the several advantages of the ammonia treatment, such as simplicity in application on farms and no residual alkali after treatment (Hartly 1981), it can be a dangerous chemical to handle. The boiling point of anhydrous ammonia is low at atmospheric pressure (-33.4°C) and it must be kept pressurised (Sundstøl 1988).

Aqueous ammonia is a slow-reacting chemical and a closed reaction vessel is necessary to obtain maximum effectiveness (Waiss *et al.* 1972; Ibrahim 1983). The important advantage

of ammonium hydroxide is that it does not need to be pressurised (Kiangi and Kategile 1981). Sundstøl *et al.* (1978) concluded that after mixing roughages with this alkali a reaction time of about 4-6 weeks is needed, but this can be reduced by increasing the temperature.

Urea is a more widely available source of ammonia to farmers and more pleasant chemical to handle than the other forms (Cloete *et al.* 1983; Wongsrikeao and Wanapat *et al.* 1985; Sahnoune *et al.* 1991). Generally, treatment of roughages with fertilizer-grade urea involves spraying these materials with 4-5% urea dissolved in equal amounts of water and storing the treated roughages after mixing for sufficient time (3-4 weeks) for the urea to be hydrolysed (Ibrahim 1985; Dixon and Egan 1988; Sundstøl 1988).

In some cases the intake of ammoniated straw has been reduced to less than for untreated material and this might be due to the residue of ammonia. It is recommended that urea-treated roughages should be aerated for a few hours before feeding (Ibrahim 1983).

Calcium hydroxide is a weaker alkali than NaOH because its solubility is low and, therefore, a longer period of time is needed for a desirable reaction (Ibrahim 1983; Verma 1983; Doyle *et al.* 1986; Kristensen 1982). To compensate for the low solubility of $Ca(OH)_2$, higher concentrations than those used for NaOH and the use of soaking rather than spraying methods are recommended (Djajanegara *et al.* 1985).

Gharib *et al.* (1975) observed that using a longer period (150 days of treatment) $Ca(OH)_2$ treatment was as effective as NaOH in improving the digestibility of poplar bark. Asadpour and Klopfenstein (1979) mixed and ensiled wheat straw with 4 and 5% $Ca(OH)_2$ for 3 weeks prior to feeding to wether lambs. They found the daily gain of experimental lambs was increased from 9 g per day for a control group to 60 and 80 g per day for straw-treated with 4 and 5% $Ca(OH)_2$ respectively. Djajanegara *et al.* (1985) treated hammer-milled (30 mm screen) wheat straw by soaking in a suspension containing 9 g $Ca(OH)_2$/100 g straw. At the end of the soaking time the treated straw was pressed and dried at 55°C for 24 hours and then mixed with other ration ingredients and fed to sheep. Their data showed that organic matter intake increased from 398 g/d for untreated to 685 g/d for $Ca(OH)_2$ treated

straw and digestibility increased from 54% to 62%, without any adverse effects of the high calcium intake.

Using the spray method, $Ca(OH)_2$ has been found to be inferior to sodium hydroxide (Gharib *et al.* 1975). $Ca(OH)_2$ has also been combined with other alkalis to produce better results (Waller and Klopfenstein 1975; Asdpour and Klopfenstein 1979; Kiflewahid 1983). For example, Kiflewahid. (1983) reported that the best results were obtained with 4% NaOH plus 2% $Ca(OH)_2$, when the treated straws were supplemented with 5% molasses and 2% urea.

However, $Ca(OH)_2$ is a cheaper and safer alkali than the other alkalis. It also supplies calcium to the ration without adverse effects, hence it can be applied to farm and village situations, mainly in developing countries. More experiments with $Ca(OH)_2$ are needed.

Potassium hydroxide (KOH) has been as effective as NaOH in treating low-grade roughages by the spray method (Rounds *et al.* 1976; Wilkinson and Gonzalez Santillana 1978; Sundstøl 1988). Generally KOH is more expensive than NaOH and hence it has not been commonly used in an attempt to upgrade the feeding value of low-quality feeds (Doyle *et al.* 1986; Sundstøl 1988).

The polyhydric structure of cellulose and aromatic nuclei of the lignin molecule are susceptible to oxidative attack: therefore, the lignin-cellulose complex may be disrupted by treating the poor-quality roughages with oxidative reagents (Han 1978; Lewis *et al.* 1987).

Gaseous sulphur dioxide (SO_2) has been used to improve the nutritional value of poor-quality feeds (Ben-Ghedalia and Miron 1983; 1984; O'Shea and Baldwin 1986; Ben-Ghedalia *et al.* 1988). In an experiment *in vitro* Ben-Ghedalia and Miron (1983) found that the major and general effect of SO_2 was in solubilising the matrix polysaccharides. In another experiment (1984) they showed that SO_2 treatment of wheat straw at the level of 40 g/kg decreased cell wall content from 79% to 56%. The same pattern was observed in organic matter digestibility.

In the third report from Ben-Ghedalia *et al.* (1988) the authors concluded that SO_2-treated straw, plus poultry litter can replace up to 60% of the concentrate in lamb-growing rations.

It has been shown that ozonation with O_3 gas can also be an effective treatment for improving the nutritive value of fibrous materials (Weakley and Owens 1975; Ben-Ghedalia and Miron 1981; Ben-Ghedalia *et al.* 1982; Ben-Ghedalia and Rubinstein 1986). In addition to solubilisation of the cell wall matrix, O_3 gas oxidises about half of the lignin fraction into organic acids (Solomon *et al.* 1992). In an study *in vitro* Ben-Ghedalia *et al.* (1980) reported organic matter digestibility of cotton straw was increased more than 100% by the ozone treatment (from 30% to 61%). In spite of this method being an effective and rapid treatment the cost is high, mainly because O_3 generation needs sophisticated facilities (Alexander *et al.* 1987).

The effectiveness of some chlorine compounds in improving the nutritive value of straw, both on a laboratory scale (Yu *et al.* 1971, 1975; Cross *et al.* 1974; Ford 1983) and in studies *in vivo* (Miller *et al.* 1979; Ford *et al.* 1987) has been examined. These compounds generally oxidise the aromatic structures of the cell wall matrix. Ford (1978) reported that sodium chloride treatment reduced lignin concentrations of Pangola grass by between 52 and 87%. Similar results were reported by Goering *et al.* (1973) in applying sodium chloride treatment to several straws. However the accumulation of some chemicals, e.g. NaCl could cause environmental problems.

An alkaline solution of hydrogen peroxide (H_2O_2) reacts with lignin and similar compounds in plant cell walls. Low molecular weight, water-soluble oxidation products are the result of this reaction. About 50% of lignin is solubilised by this treatment and the derived products are not toxic (Gould 1985). Lewis *et al.* (1987), in a series of studies *in sacco* concluded that this reagent was more effective in improving dry matter digestibility over that of NaOH treatment alone.

Some of the lignin-carbohydrate bonds of the plant cell wall are very acid labile (Crosthwaite *et al.* 1984). Acid treatment of coarse roughages hydrolyses mainly the hemicellulose portion of the cell wall (Doyle *et al.* 1986). In the experiments which were reported by Han

(1978) acid hydrolysis of fibrous agricultural residues was used as a preliminary step. Dilute sulphuric acid has been tested for improving the nutritive value of woody materials (Boda 1990). Balasubramanya and Bhatawekar (1980) found that the maximum amount of sugar (about 30-34%) was released from rice and wheat straws when heated with 0.5 N H_2SO_4 at 121°C and water : substrate ratio of 3 : 1.

In a series of experiments for improving the feeding value of some fibrous materials with acids, which were carried out by Crosthwaite *et al.* (1984) digestible cellulose digestion in bagasse was increased by about 87% after treatment with 1% sulphuric acid. The maximum effect with sulphuric acid was obtained after about 10 weeks storage at 30°C. Cellulose digestibility of wheat straw was increased from 19% to 34% after treatment with 17% HCl using a storage time of 3-4 weeks at room temperature. Acid treatment of low-quality feed could be a low-cost and feasible method at the village level in under-developed countries. However, some disadvantages are involved such as the danger in handling and transporting acids, also the low pH of the treated materials. More studies *in vitro* and *in vivo* are needed in this area if this method is to be more widely used.

2.5.3 Physico-chemical treatments

The combination of physical and chemical treatments might be expected to be more effective in improving the feeding value of poor-quality roughages (Chandra and Jackson 1971; Guggolz *et al.* 1971; Fernandez Carmona and Greenhalgh 1972; Coombe *et al.* 1979) than either method alone. The surface area of roughages can be increased by reduction in the particle size and it could be expected that chemical treatment of these smaller particles would then give better results. In a feeding trial with beef steers conducted by Coombe *et al.* (1979) alkali treatment of either chopped or pelleted straw increased the digestibility of these components by about 10 units compared with the respective untreated straw.

The effects of chemicals can be influenced by temperature. Ololade *et al.* (1970) reported that the *in vitro* digestibility of alkali-treated barley straw was increased as the treatment temperature increased, with the optimum being about 80°C. Hart *et al.* (1981) found that digestibility of rice straw was improved from 26% to 47% without additives and 64% with NaOH under a pressure of 21.1 kg/cm^2. It is suggested that the combination of different

processing methods has some benefits, but the additional costs this entails must be considered.

2.5.4 Biological treatments

The possibilities of improving feeding value of lignocellulose materials by biological treatments has been studied over many years (Leatherwood *et al.* 1960; Ralston *et al.* 1962; Baker *et al.* 1973; Hartley *et al.* 1974: Burrows *et al.* 1979; Latham 1979; Ramasamy and Veruchtert 1979; Zadrazil 1979; Wilkins 1981; Jung *et al.* 1992). The biological approaches can be grouped as follows: (a) ensilage; (b) fermentation and fungal growth; (c) enzymatic hydrolysis and (d) mushroom cultivation (Han 1978; Doyle *et al.* 1986). Ensilation of green fodder for improving and conserving its feeding quality through anaerobic fermentation has long been understood. In this treatment anaerobic microorganisms, predominantly *Lactobacilli,* rapidly repress the growth of undesirable micro organisms. The lactic acid resultant from this process also gives desirable odour and taste to the mass (Han 1978).

Nitrogen additives such as urea, biuret or ammonium polyphosphates and readily-fermentable carbohydrates, such as molasses, have been added to low-quality, roughage-based silage. As Kifelwahid (1983) reported, mixing urea, molasses, NaOH or even cattle excreta with agroindustrial by-products in many African countries produced good silages. Similar attempts have been made in Asia (Wanapat 1987; Cheva-Isarakul 1988).

Many efforts have been made to increase the protein content of cellulosic materials by aerobic micro organisms (Thomson and Poole 1979). Numerous microbes are capable of using cellulose for their growth, such as *Cellulomonas* (Han and Callihan 1974), *Pseudomonas* sp. (Ramasamy and Verachtert 1979). Most processes involving growing micro organisms, however, have been aimed at the production of protein of microbial origin which can then be used as a feedstuff for non-ruminants (Han and Anderson 1975; Ibrahim 1983).

The main work in this area this been devoted to treatment of lignocellulosic materials with white-rot fungi, which degrade lignin rather than structural carbohydrates (Han 1978; Latham 1979; Jung *et al.* 1992). But as Jung *et al.* (1992) reported, even though straw

treatment with white-rot fungi can improve its quality, the overall loss of dry matter severely limits the practical benefit of this method.

There is interest in the addition of cell-wall-degrading enzymes to fibrous feeds as a means of improving feeding value. Supplementation with these enzymes has given variable and inconclusive results (Leathewood *et al.* 1960; Ralston *et al.* 1962; Willis *et al.* 1980). It seems that at present the cost of suitable enzymes is too high for the technique to be of commercial use.

Some fungi convert lignin and structural carbohydrates into fungal protein (e.g. mushroom) suitable for human food and animal feed (Han 1978). Some changes may occur in the chemical composition and nutritive value of spent lignocellulosic materials but this depends upon the type of fungus, the nature of the starting material and the growth conditions. It is still far from clear which type of mushroom cultivation, if any, can substantially improve the feeding value of fibrous feeds.

2.5.5 Supplementation methods

The major limitations of fibrous feedstuffs are low digestibility, low intake and the deficiency of some essential nutrients. For efficient utilisation of fibrous materials by ruminants the shortage of specific nutrients such as N and S may be corrected by supplementation (Coombe and Tribe 1962; Ernst *et al.* 1975; Schiere *et al.* 1985, 1988; Dixon 1987; Cheva-Isarakul 1988; Aitchison *et al.* 1986; Doyle and Panday 1990; Paduano *et al.* 1990). Cereal grain and molasses are often used as energy supplements for ruminants consuming low-quality roughages. It can be generally concluded that supplementation of fibrous feeds with readily-available energy sources at about 10-15% may increase the intake of poor-quality roughage only when nitrogen and minerals are enough for microbial synthesis (Cheva-Isarakul and Kanjanaprutjipong 1987; Doyle *et al.* 1986). Molasses has been widely used with urea for supplementary feeding of ruminants at maintenance levels during the dry season. Entwistle and Baird (1976) and Niven and Entwistle (1983) reported that feed intake and productivity of sheep can be increased by a molasses supplement as low as 50 g dry matter per day.

Ration supplementation with non-protein nitrogen (NPN) has been known for a long time. Nitrogen supplements only work effectively, however, when N is the primary limiting factor (Egan 1986). In most circumstances it is important to provide a source of readily-available energy, such as molasses, to ensure the efficient use of NPN (mainly urea) due to its rapid degradation (Doyle 1983).

2.6 Using poultry litter as a feed for ruminants

Since protein is one of the important limiting nutrients in livestock feeding the use of poultry wastes in the feeding of animals has been receiving increased attention. Several workers have investigated poultry excreta as a nitrogen source for ruminants (El - Sabban et al. 1970; Lowman and Knight 1970). Tinnimit et al. (1972) reported that the acceptability of dehydrated poultry excreta was excellent when fed to growing sheep at levels up to 80% in a mixed ration. Retention of digested nitrogen was 18% - 72%, depending on rate of intake, and these values compared favorably with values of 16 - 65% for soybean meal rations in the same experiments.

Raising birds on deep litter is a common practice in the most countries. The voided faecal matter is thus mixed with the bedding material which may be sawdust, rice hulls or dry roughages such as wheat straw or chaffed hay. The litter, once fully mixed with excreta, is removed from the poultry house and is normally used primarily as a fertilizer and soil conditioner. Chemical analyses of poultry excreta, as reported by Jakhmola *et al.* (1988) and Parthasarthy and Prasad (1976) indicate that litter may contain 21 - 30% crude protein (12.1% digestible crude protein), 17 - 20% crude fibre, 30 - 35% NFE, 2 - 3% ether extract, 15 - 25% ash, 2.1% calcium, 1.8% phosphorus, and 23.8% total digestible nutrients on a dry matter basis. Many factors such as (i) type of material used for bedding, (ii) density of birds, (iii) age of birds, (iv) age of the litter and (v) method of processing, all affect the chemical composition and nutritive value of poultry litter.

The performance of animals given poultry litter in their rations has been reviewed by Smith and Wheeler (1979) and Leibholz (1983). Dry matter, crude protein and crude fibre digestibility were reduced when 32% poultry manure replaced barley and soya - bean meal in

a sheep diet (Parigi - Bini 1969). The litter can be dried or ensiled prior to feeding to the animals. Poultry litter, because of its physical texture, can be dried quickly with either dehydrators or ovens. To reduce the cost, sun drying is a suitable method. Ensiling of poultry litter has the added advantage of destroying pathogens and parasites (Harmon et al. 1975; McCaskey and Anthony 1979). Where green cereal fodder is available advantage of the high nitrogen content of litter can be taken by ensiling with the green cereal (Rao et al. 1977; Jayal et al; 1981). Molasses may also be added to hasten the process of fermentation (Rao et al. 1977). Neog (1976) found that silage prepared by mixing litter, molasses, green maize and paddy straw (ratio 20 : 20 : 20 : 40 on a dry matter basis) contained 4.2 - 4.5% DCP and 50 - 53% TDN, forming a good maintenance ration for adult cattle. Neog and Pathak (1976) prepared silage with paddy straw, poultry litter, chaffed green maize and molasses (ratio 4 : 4 : 1 : 1 on a dry matter basis) and DCP and TDN were calculated to be 6.3 and 39.8%, respectively. Poultry litter has been was used as a source of nitrogen for fattening steers (Noland et al. 1955). Steers given autoclaved litter gained less rapidly than those fed on cottenseed meal. When the total feed intake of litter-fed steers was increased by 15% to equalise the energy intake of the two groups, the rates of gain were nearly equal (Fontenot *et al.* 1966). Bosman (1973) observed depressed growth and lower feed intake and weight gain on the replacement of maize meal and lucerne hay at the 20 or 40% level with chicken litter. Similar observations were recorded by Barbosa et al. (1980). Other observations show that responses to poultry litter in ruminant rations vary widely, possibly because of the type of animal, quality of ration, nutritional value of ration ingredient being replaced by poultry litter and level of replacement. Tagari et al. (1976) gave pelleted concentrate mixtures containing 0, 15, 25 and 35% broiler litter to steers; differences in weight gain or carcass gain between treatments were not significant . Feed intake was higher on litter - containing rations, but feed efficiency was impaired when the proportion of litter exceeded 25%. Pelleting had a beneficial effect when a ration containing 40% broiler litter was given to fattening calves, as indicated by higher daily gain and higher digestibility of crude protein and organic matter (Kumanov et al. 1969). In growing cattle, body weight gain was higher from a diet containing 25% poultry litter than from a control diet (Borgioli and Tocchini 1969).

Gupta and Verma (1980) studied the effect of replacing a protein concentrate by dried poultry litter on the growth rate of cross-bred calves. They concluded that the replacement of >40% of the concentrate was not economical. A similar level of poultry litter in concentrate mixture (37%) was suggested for growing buffalo heifers by Makkar et al. (1980). Heat-treated poultry litter (135° C for 10 h) could be incorporated to supply 25% of the concentrate protein in the rations of dairy cattle (Taro 1981). Fontenot and Webb (1974) observed significantly greater nitrogen retention for control rations than for rations containing broiler litter. An improvement could, however, be brought about in palatability and nitrogen availability of broiler house litter by ensiling it with crushed barley (Jacobs and Leibholz 1977). Supplementation of other readily available carbohydrate sources such as potato cannery waste, maize or sorghum grains with litter increases the digestibility of dry matter, energy and protein (Daniels et al. 1983).

The lignocellulosic constituents of litter vary with the quantity of bedding material per unit of floor space and the moisture in the litter during the rearing period, which supports the activity of cellulolytic bacteria. The fibre digestibility of deep litter based on wood waste can be extraordinarily high, depending upon the biological activity of the microflora during the rearing period. It was demonstrated in metabolism trials with sheep fed on pine sawdust prior to its use as bedding (Muller et al. 1967) that the organic matter digestibility (OMD) was 11%, while after its use as litter its OMD value increased to 72% (for litter including droppings). Since broiler manure without bedding had 71% OMD, it appears that the potential energy of the sawdust was made available through microbial breakdown during the rearing period. This conclusion was supported by a partial disappearance of lignin, cellulose and other structural carbohydrates (Muller and Drevjany 1967; Muller et al 1968.

The nature of the bedding material has a great influence on both poultry performance and the subsequent nutritive value of deep litter for ruminants (Muller et al. 1968). The quantity of bedding material used per bird has also a pronounced effect on the subsequent protein and vitamin contents and hence any monetary value of the litter (Muller et al 1968).

However, in spite of extensive existing information on the general use of poultry litter in ruminant nutrition, there is no information available concerning either the use of seagrasses as a bed material for poultry nor on the use of seagrass litter as a ruminant feed.

2.7 Ruminants and their characteristics in response to lignocellulosic feedstuffs

A number of animal production systems are used world wide. The cheapest and most common, however, and the one which predominates in Australia, is that involving grazing lands (Jarrige, 1980; Morley, 1981$_b$).

The superiority of the ruminant digestive system , the most advanced herbivore strategy is the real reason for the success of these animals in grazing systems (Williams, 1981; Hume 1984). A dense population of microorganisms (bacteria, protozoa. phycomycetous fungi, mycoplasmas, bacteriophages, viruses) in the reticulo-rumen, is in a symbiotic relationship with the animal and enables it largely to degrade ingested food by fermentation prior to the digesta reaching the true stomach or abomasum. This is one of the major advantages of rumen fermentation over that which occurs in the caecum of other herbivores, since not only is the fermentation itself superior but the products of this fermentation are also able to pass through the intestines for further digestion and absorption, in contrast to the situation in " simple-stomached " herbivores such as horses (Hume and Warner 1980; Hobson and Wallace 1982 Ørskov 1982; Leng 1985; Thomas 1985).

The unique digestive system of ruminants makes them adaptable to a wide range of feeds and is the reason ruminants are the best equipped herbivores for maximal fibre digestion. Fibrous plant material can be retained in the reticulo-rumen for prolonged periods, often exceeding 60 hours, thereby ensuring that further rumination by the animal has sufficient opportunity to break the food mechanically into degradable components and that the resident microflora also have sufficient opportunity to degrade the cellulosic cell-wall constituents (Hobson and Wallace 1982; Hume 1984). Although an advantage in many situations prolonged retention time , however, may also be a problem with high-fibre diets if extreme to the point where feed intake can be limited by rumen distension (Hume, 1984).

The synthetic ability of rumen microorganisms is another useful attribute of the ruminant digestive system, effectively rendering the adult ruminant completely independent of a dietary source of the B-group of vitamins. Furthermore, a wide variety of plant secondary compounds, such as alkaloids, caffeic acid, gossypol, mycotoxins, oxalates and cyanogenic glycosides can be degraded by the ruminal microflora, thus protecting ruminants from these potentially harmful substances in their diet. Detoxification mechanisms however, generally only appear to be effective when the compounds concerned are present at relatively low concentrations (Hegarty 1982; Hume 1984).

2.7.1 Microbiology of the rumen

The most varied and dense microbial populations known in nature can be found in the rumen. They can be dvided into three main groups; (i) bacteria; (ii) protozoa; (iii) fungi. There is an extensive microbiological literature on the subject and up to now more than 200 species of bacteria (flora) and more than 20 species of protozoa (fauna) have been identified (Czerkawski 1986).

Rumen bacteria are numerically the predominant component of the microbial population (Ørskov 1982; Czerkawski 1986) and can be subdivided into groups based on morphological and histochemical differences (cocci, rod, spiral, oval, etc. forms) or gram positive and gram negative forms) or, more appropriately, in terms of the niche (or niches) they fill in the rumen with regards to substrate specificity. Thus, there are ruminal species which digest fibrous material (cellulolytic), starch (amylolytic), fats (lipolytic), soluble sugars (e.g. xylose, glucose, fructose, sucrose) or acids (e.g. lactate, succinate, propionate). There are also ruminal species which produce methane (methanogenic). Many species utilise more than one substrate and are therefore adapted to a number of niches within the rumen (Bryant 1959; Bryant 1963; Hungate 1966; Ogimoto and Imai 1981; Ørskov 1982; Russell 1984).

Cheng *et al.* (1977) showed that the two main types of rumen cellulolytic bacteria initially attack plant material in a similar, tissue-specific way. Gram-positive cocci (*Ruminococcus albus* and *R. flavefaciens*) and gram-negative rods (*Bacteroides succinogens*) attach to plant

particles by the polysaccharide fibers of the bacterial glycocalyx. These fibers are for the most part negatively charged and may form a polar bond with higher cell polysaccharides by way of divalent positive ions or lectins (Costerton et al., 1978). They form a thick slime layer in *Ruminococci* but exteremely fine fibres originate from *B. succinogenes* cells, forming a fine layer around the pliable cell wall, which enables the bacterium to adhere very closely to the substrate, meeting its topographical variations. Although most of the enzymes involved in cell wall degradation are cell associated, some may be excreted into the medium as some degradation of mesophyll and phloem cells is observed without bacterial attachment (Cheng et al. 1977). Epidermal tissue is degraded by attached bacteria, but no digestion and no attachment is observed to vascular and sclerenchyma tissue (Latham et al. 1978).

The rumen protozoa were first obsorved by Gruby and Delafond (1843) and from their dramatic appearance were assumed even then to be of importance in the metabolism and nutrition of the host. These protozoa are principally ciliates and are of two types: the entodiniomorphid protozoa (the oligotrich protozoa) and the holotrichs. The former are characterized by the presence of a firm pellicle and of cilia situated predominately on the peristome and only sometimes elsewhere. In contrast the holitrich protozoa have more flexible pellicles which, in the most commonly occurring species, are almost completely covered in cilia. Flagellate protozoa are also present in most rumens, although some of the organisms that were earlier described as flagellates are now known to be phycomycete fungi. The entodiniomorphid protozoa are well adapted to the environment and utilise particulate rather than soluble food materials. In contrast, the holitrichs can use soluble food materials and are more aero-tolerant (Williams et al. 1992).

Boyne *et al.* (1957) first showed that there could be up to a threefold variation in protozoal population density in an animal kept on a constant ration under constant condition. More recently Clarke *et al.* (1982) have measured protozoal population densities in each of a series of four animals fed and sampled differently. Results showed that while the animals in some groups behaved similarly, there could be large density differences (e.g. 9 g to 58 g protozoa per rumen) and either small or large differences when individual protozoal genera

were considered. Results in which the effects of different feeding regimes have been determined using very few animals must be treated with care.

Almost all rumen ciliates feed on starch grains and, within limits, any increase in the amount of starch fed increases the protozoal population density (Abe *et al.* 1973), due mainly to an increase in the numbers of entodinia (Lyle *et al.*, 1981; Dennis *et al.*, 1983). If animals are fed to appetite on starch-rich rations, however, then rumen acidity increases and all the ciliate protozoa disappear (Lyle *et al.*1981). The entodinia are the least sensitive to low pH and are the last to disappear. Purser and Moir (1959) found a linear regression of mean daily protozoal population density and minimum daily pH with, under their conditions, 670 x 10^3/ml at rumen pH 5.9 and 290 x 10^3/ml at rumen pH 5.2 In contrast, the feeding of a restricted high-grain ration (80% of appetite) produced very high (>2 x 10^6/ml) protozoal population densities (Christiansen *et al.* 1964; Eadie *et al.* 1970) and over 95% of these could be *Entodinium spp* . A wider range of protozoal genera and species are found in animals fed on hay rather than starch (Van der Wath and Myburgh 1941).

Aerobic fungi and yeasts have long been known to be normal inhabitants of the rumen (Clarke and Dimenna 1961; Lund 1974) but most species isolated are considered to be transient and non-functional, entering the rumen with the feed. Some aerobic fungi are capable of growth under anaerobic conditions, and Brewer *et al.* (1972) concluded that *Aspergillus fumigatus*, *Mucor rouxii* and all of those which are implicated as causative agents in ovine ill-thrift, could survive in the rumen. Two other groups of fungi are now known to occur, one group parasitic upon ciliate protozoa, the other saprophytic on plant tissues. Anaerobic fungi saprophytic on ruminal digesta have been discovered in the rumen (Bauchop 1979).

The rumen fungi so far examined produce a wide range of enzymes that can digest the major structural carbohydrates of plant cell walls (Lowe *et al.* 1987).

2.7.2 Factors affecting population size and composition of rumen bacteria

Diurnal changes: Warner (1966a), using direct counting procedures found that for sheep fed once daily the concentration of total rumen bacteria decreased from 1 to 4 hours after

feeding, increased slowly to a maximum between 12 and 20 hours, and then gradually decreased till the next feeding. The concentrations of 3 morphologically distinct bacterial groups (Selenomonads, Eadie's ovals and Peptostreptococci) were also determined, and in general they followed similar growth patterns. It was concluded that these concentration patterns reflected an initial dilution by feed, water and saliva, then an increase in growth rate and finally a depletion of nutrients with a corresponding decrease in growth rate until this was less than the dilution rate. It is of interest that this pattern was very similar to the changes in viable bacterial concentrations observed earlier by Bryant and Robinson (1961).

The concentrations of rumen bacteria in sheep fed to appetite in pens or on pastures followed similar growth patterns to those in animals fed once daily (Warner 1966b). This appeared to be the result of the animals consuming most of their daily intake in one period of continuous eating. In contrast, rumen bacterial concentrations were fairly stable with time in sheep fed on a limited ration every 3 hours (Warner 1966c). Bryant and Robinson (1968) investigated the effects of diet and sampling site within the rumen on bacterial concentrations in cattle at various times after feeding. Four diets were used (chopped hay, pelleted hay, hay-grain and silage) and the animals were fed at 12 hour intervals. For all diets, concentrations were lowest at 1 hour after feeding, and increased significantly between 1 and 2.5 and 5.5 hours. Concentrations did not differ between 5.5 and 10 hours. Bacterial concentrations were highest in samples taken from the dorsal rumen, with lower numbers occurring in samples taken from the ventral rumen and reticulum.

Leedle et al. (1982) used direct counts and viable counts to study the diurnal variations in bacterial numbers in cattle fed on maintenance levels of high-forage or high-concentrate diets once daily. Direct and viable counts decreased after feeding; the lowest values were observed at 2 hours and 4 hours after feeding with the high-concentrate and high-forage diets respectively. Concentrations then increased steadily, reaching their highest values at 16 hours. These data would support the previous observation of Warner (1966a) with sheep. The lowest viable proportion of direct-count bacterial populations occurred at 2 hours after feeding (14.6% and 14.1%), while the highest values (48.6% and 73.5% on the high-forage and high-concentrate diets, respectively) were found at 16 hours. Both Bryant and Burkey

(1953) and Maki and Foster (1957) had previously observed a higher percentage of viable bacteria when animals were fed on concentrate diets. Because of the marked differences in bacteria viability with time after feeding, the magnitudes of concentration changes are greater with viable counts than with direct counts. It was suggested that, in addition to dilution and increased passage from the rumen, the loss of viable bacteria after feeding may be due to rapidly changing rumen conditions such as osmotic shock effects, temperature changes, pH changes, entrance of oxygen, and attachment of organisms to incoming feed particles (Bryant and Burkey 1953; Maki and Foster 1957).

Diet: A number of studies can be found in the literature which compare total viable bacterial concentrations in different species fed on either high-forage or high-concentrate diets. In general, bacterial concentrations are higher in those animals receiving a high-concentrate diet (Grubb and Dehority 1976; Leedle and Hespell 1980). However, there are also several reports in which numbers are equal or higher in animals fed on high-roughage diets (Bryant and Robinson 1968; Latham *et al.* 1971; Leedle *et al.* 1986). Differences between such factors as percentage of concentrate in the diet, feeding frequency, feeding level and sampling time, and individual animal variation, all appear to influence bacterial concentrations and in turn make comparisons difficult. Data indicate that bacterial concentration do tend to increase with an increased intake of available energy (Mackie and Gilchrist 1979). However, rumen volume can also be influenced by the type of ration. Data for individual sheep from the study by Grubb and Dehority (1975) showed that although bacteria concentrations on high roughage or high-concentrate diets were different in three experimental animals, adjusting for rumen volume eliminated the difference between diets.

Thorley et al. (1968) compared bacterial concentrations in two cows fed *ad libitum* either long grass or the same grass ground and pelleted. Mean colony counts were significantly higher when the animals were given ground grass (15.7×10^9 /g) rather than long grass (10.5×10^9 /g). Such factors as pH and rate of fluid and particulate matter turnover could have affected these values.

Level and frequency of feeding: Studies which focus on either of these two parameters specifically are quite limited. Moir and Somers (1957) found that rumen bacterial concentrations were similar in sheep fed 1, 2, or 4 times daily. In a later study by Warner (1966c) bacterial numbers did not show much fluctuation with time in animals fed every 3 hours. Using a poor quality teff hay diet, Gilchrist and Kistner (1962) fed 3 sheep on 1200, 800 and 300g /day for three consecutive periods of 42, 60, and 109 days. They found no differences in viable counts of bacteria fermenting cellulose, starch, glucose, xylose or lactate between feed intake levels. Warner (1962b) reached a similar conclusion when comparing total bacterial concentrations in sheep fed with a similar range of intakes.

In a more controlled study, Dearth *et al.* (1974) determined total bacterial concentrations in animals fed on the same diet at maintenance or 1.8 x maintenance level. with 8 sheep in a Latin square design, bacterial concentrations were significantly increased by the higher feed intake.

2.7.3 Factors affecting population size and composition of rumen protozoa

Diurnal variation: Marked diurnal variations have been noted in the concentration of rumen protozoa. Purser and Moir (1959) first reported a distinct diurnal cycle for *Entodinium* in sheep. Numbers decreased for 6 - 8 hours after feeding and then gradually rose to prefeeding levels by 20 - 24 hours. Subsequent work by Purser (1961) established that a diurnal cycle also existed for the holotrichs, though differed from that for *Entodinium.* Peak concentrations occurred at feeding time (animals fed once daily) and then numbers gradually diminished until 20 hours after feeding when a rapid increase occurred up to feeding time. These concentration cycles were confirmed by Warner (1962b).

In subsequent work Warner (1966a,b,c) studied diurnal changes in protozoan concentrations in sheep fed a limited diet once daily, fed to appetite in pens or pasture, and fed a limited diet every three hours. His results for animals fed once daily were in agreement with previous data and expanded the observed diurnal cycle for *Entodinium* to include almost all the entodiniomorphs. Diurnal changes in protozoan concentrations for sheep fed to appetite were similar to those in animals fed once daily (Warner 1966b). From monitoring of time

spent eating, it appeared that almost all of the daily intake was consumed during one major period. Studies on sheep given small amounts of feed every three hours suggested a three - hour cycle of concentration changes (Warner 1966c); however, a gradual decline in concentration occurred over time, possibly an effect of the repeated sampling.

Warner's (1966a) conclusions, based upon measurements of dilution rate, were that the diurnal fluctuation in entodiniomorph concentrations was the end result of changes in dilution rate associated with eating (increase saliva flow and drinking) and changes in protozoan growth rate in response to incoming nutrients. This explanation appears to be in agreement with the results obtained with cattle by Clarke (1965) who found that total numbers of entodiniomorphs in the rumen-reticulum did not decrease after feeding.

Purser (1961) and Warner (1966a) both observed a marked increase in holotrich concentrations in sheep just prior to feeding. For lack of a better explanation, Warner (1966a) suggested a very rapid multiplication of the holotrichs within a 4 - 8 hour period around feeding time, with no further division for 16 - 18 hours. However, the numbers of dividing cells observed during the time of rapid increase in numbers did not substantiate this explanation (Warner 1966a; Michalowski 1977; Dehority and Mattos 1978).

Somewhat in contrast to the previous data on holotrichs, Clarke (1965) did not observe a rise in total holotrich numbers in cattle until feeding time, with numbers peaking in the first several hours after feeding. Similar cycles for holotrich concentrations in cattle were later noted by Abe *et al.* (1981) and Murphy *et al.* (1985). Visual and microscopic observations of the inner walls of the rumen and reticulum by Abe et al. (1981) suggested that the holotrichs sequester on the reticulum wall a few hours after feeding, and migrate into the rumen again at the next feeding. On the basis of the known chemotaxis of *Isotricha* to soluble carbohydrates Orpin and Letcher (1978) proposed that this migration at feeding could be a chemotactic response to soluble sugars in the incoming feed. Their studies also suggested that the quantity of feed and the act of ingesting feed could be additional stimuli for migration. Murphy et al. (1985) were able to show that glucose solution infused into the reticulum stimulated migration of the holotrich protozoa into the rumen whereas water, artificial saliva, NaCl or starch solutions had no effect. In addition, they found that

bypassing the act of feed ingestion by placing chopped straw directly into the rumen also elicited migration of the holotrichs. The rapid decrease in holotrich numbers after feeding would appear to be the result of their return to the reticulum wall, although the factors controlling this sequestration remain to be studied.

In general the prefeeding rise in holotrich concentrations has primarily been observed in sheep, while increases in cattle occur immediately after feeding. However, it should be noted that Michalowski (1975) did observe a prefeeding increase in holotrich numbers before feeding. Any explanation for the rise in holotrich concentrations based on composition of feed or act of feeding would apply only to an increase after feeding. Data presented by Warner (1966a) and Dehority (1970) indicate that, although holotrich concentrations begin to rise before feeding, there is an additional increase after feeding, presumably a chemotactic response to incoming feed. Other unknown factors, unique to the small ruminant, could be responsible for the prefeeding increase in holotrich concentrations in sheep.

The diurnal curves for protozoan concentrations presented by Michalowski (1977), Warner (1966a,b,c) and others indicate that the percentage generic distribution varies considerably during a 24 hour period. Little information is available on species distribution during this same time period; however data presented by Clarcke (1965) and Dehority (1970) suggest that the proportion of *Dasytricha ruminantium* to *Isotricha* changes with time after feeding.

Diet effect: The influence of diet on protozoan concentrations in sheep has been studied particularly by Nakamura and Kanegasaki (1969) and by Grubb and Dehority (1975). In the study by Nakamura and Kanegasaki (1969), sheep were changed from a diet of 1500 g orchard grass hay plus 600 g of concentrates (28.5% concentrate) per day to 1500 g orchard grass hay per day. The ration were fed in equal portions twice a day. Protozoan concentrations were in the range 7 - 12 x 10^5 per ml on the hay-concentrate diet and 2 - 4 x 10^5 per ml on hay alone. Grubb and Dehority (1975) abruptly changed their sheep from an all-roughage diet to a 60% corn - 40% roughage diet, with 800 g of diet being fed once daily. Concentrations ranged between 4 and 6 x 10^5 protozoa per ml on 100% orchard grass hay, rose markedly during the five days following the diet change, and then stabilized

between 10 and 18 x 10^5 protozoa per ml. Although there were differences in the experimental design of these two studies, they both used similar types of diets and, when the amount of available energy in the ration increased, protozoan concentrations increased. Similar increases in protozoan concentrations have been observed in cattle and water buffalo when concentrates were added to the diet (Abe et al. 1973; Michalowski 1975; Dehority and Mattos 1978; Dennis et al. 1983).

As the percentage of concentrates in the diet increases to 60% or more, there is generally a corresponding decrease in minimum rumen pH values (Abe et al. 1973; Mackie et al. 1978; Wedekind et al. 1986). This can result in a decrease in protozoan concentrations, a shift towards *Entodinium* species and, in some cases, even complete disappearance of the protozoa (Latham et al. 1971; Vance et al. 1972; Abe et al. 1973; Mackie et al. 1978). The type of grain also influences rumen pH and protozoan concentrations (Slyter et al. 1970). It would appear that rations containing about 40 - 50% roughage will support maximal protozoan numbers with a diverse fauna containing species of most of the genera.

Experiments by Czerkawski and Breckeridge (1979), using continuous *in vitro* fermentation systems, have demonstrated the importance of solid digesta in the maintenance of protozoan numbers. The protozoa apparently sequester in the solid digesta and concentrations in the effluent are only 10 - 20% of these associated with the particulate matter. An inert solid matrix plus a balanced soluble substrate did not provide adequate conditions for maintenance of the protozoa (Czerkawski and Breckenridge 1979b). Regular addition of solid digestible hay was necessary to stimulate a normal rumen fermentation.

Straining the rumen contents can markedly affect measurements of generic composition, particularly numbers of *Entodinium* (Dehority 1984). In several studies where distribution has been determined in whole rumen contents, proportions of *Entodinium* have ranged from about 90 - 98% on concentrate - type diets to 40 - 90% on hay or pasture diets (Michalowski 1975; Dehority 1978, 1979). The majority of remaining ciliates were from genera in the subfamily *Diplodininae* and, as would be expected, constituted about 2 - 10% on concentrates to 10 - 55% of the population on hay or pasture. One exception would be the

high incidence of *Epidinium* (20 - 25%) which has been observed in New Zealand cattle grazing on fresh red - clover (Clarke 1964).

Level of intake: When sheep were fed a pelleted high-concentrate ration to appetite, rumen protozoa were eliminated in most cases or reduced to a very low concentration. However, relatively high concentrations of protozoa were obtained when different physical forms of a ration were fed. Protozoan numbers were inversely related to particle size and rate of passage of feed through the rumen (Christiansen et al. 1964).

Warner (1962b) fed the same diet to two sheep at levels ranging from 300 to 1200 g per day. Some decrease in protozoan concentrations was observed at the 300 g intake level, although, he concluded that the level of given diet above a certain minimum has little effect on protozoan concentrations. These observations were later substantiated in a more comprehensive study by Potter and Dehority (1973). Their data indicated that energy may be the important factor controlling protozoan concentrations at low intake levels, whereas feed passage rate becomes the controlling factor at higher intakes.

Dearth *et al.* (1974) fed the same diet to sheep at either 1.0 or 1.8 times their daily maintenance energy requirement, and found that protozoan numbers were significantly decreased at the 1.8 times maintenance intake. The concentration decrease occurred primarily in the genera *Dasytricha*, *Entodinium* and *Ophryoscolex*.

Dehority (1978) fed 3 sheep on 800 g of a roughage diet and 3 sheep on 1400 g of a concentrate diet. Mean protozoan concentrations were 38.9×10^4 per ml for the roughage fed animals and 118.4×10^4 per ml for the concentrate fed animals. Average liquid rumen volumes were 6.37 and 2.57 litres for the roughage and concentrate sheep, respectively, while fluid turnover rates were similar on both diets. The differences in protozoan concentrations and rumen volumes were both significant. Although the amount of dry matter in the rumen contents may vary slightly (3 - 5%) between roughage and concentrate feeds, multiplication of volume by concentration should give an estimate of total protozoa in the rumen. The resulting values, 2.43×10^9 and 2.98×10^9 protozoa in the rumens of the roughage and concentrate fed animals were not significantly different. Thus rumen volume,

as influenced by level and type of diet, can be of major importance when evaluating protozoan populations.

Frequency of feeding: The effect of multiple feedings upon rumen protozoan concentrations was first demonstrated by Moir and Somers (1956). In a Latin-square design experiment with sheep they found that feeding the same quantity of feed 4 times daily instead of once a day resulted in a doubling of protozoan concentrations. If the same quantity of diet was fed twice daily, protozoan concentrations were intermediate but still significantly higher than those of the once-a day feeding.

The most plausible explanation for this increase in numbers would be that multiple feeding prevents the drastic fluctuations in rumen pH which can be inhibitory to protozoa. For example, when a given level of concentrates was fed to cows twice a day, rumen pH ranged from about 5.58 to 6.65; however when it was fed 6 times daily, rumen pH fluctuated only between 6.15 and 6.4 (Kaufmann et al. 1980). In a more recent study Bragg et al. (1986) determined the diurnal pattern for rumen pH and protozoan concentrations of steers fed on corn silage concentrate diets (40 : 60) either 2 or 8 times a day. Using a Latin - square design, minimum pH values when the animals were fed twice daily were 5.45 compared to 5.8 when they were fed 8 times. This was reflected in slightly higher protozoan concentrations and considerably less fluctuation over the day in those animals fed 8 times daily.

Clarke *et al.* (1982) fed two levels of chaffed alfalfa hay, either hourly or once a day, to 32 sheep. The sheep were slaughtered at the end of the experiment to measure weight of rumen contents. In general, protozoan concentrations were highest in those animals fed the high level of hay at hourly intervals. However, calculated total protozoan dry matters varied as as much as from 14 to 70 g in two sheep in the same group. Therefore it can be concluded that there was marked variability in numbers, sizes and masses of ciliate protozoa in the rumens of individual sheep fed the same diet. These data would suggest that an experimental design like the Latin square, where each animal is on all treatments, is almost essential in these types of studies.

Seasonal differences: For animals grazing native pastures seasonal changes can cause marked variation in protozoan numbers. The two principal seasonal changes, i.e. hot to cold and wet to dry, both inhibit or slow down plant growth and result in a decrease in energy available to the animal. Pearson (1965,1969) observed a marked decrease in rumen protozoan concentrations in mule deer from Utah and white-tailed deer from Texas during the winter months. Similar winter decreases in numbers occurred in red deer and sheep in the Scottish Highlands (Hobson et al. 1976). Westerling (1970) has reported a 45% decrease in protozoan numbers in Finnish reindeer between August and November, the latter samples taken about 2 weeks after snow cover.

A compilation of protozoan concentrations to illustrate the effect of the feeding variables discussed above is extremely difficult. Variation in type of feed, diet composition, intake level, time of feeding, time of sampling, number of feedings per day, and season and animal species make conclusions somewhat questionable. In general, from the references cited, protozoan concentrations in domestic ruminants fed on mostly roughage diets or pasture range from about 10 to 50 x 10^4 per ml. Values for animals fed concentrate type rations are usually 50 - 150 x 10^4 protozoa per ml; however, concentrations up to 300 x 10^4 per ml are occasionally reported (Dehority 1978).

2.7.4 Interrelationships between rumen bacterial and protozoal populations

In the literature most attention has been given to the question of whether the protozoa are essential to the ruminant animal, probably because it is possible to establish and study protozoa-free or defaunated ruminants. Beginning with the experiments of Becker et al. (1930), it has been established that the rumen protozoa are not essential to the host. In his review Veria (1986) has summarized and evaluated most of the experiments comparing faunated and defaunated animals, and he concluded that the major nutritional effect of rumen protozoa is upon the protein/energy ratio of nutrients available for absorption in the small intestine. This would offer a possible explanation for the major areas in which the rumen protozoa appear to be involved: affecting animal growth rate, feed intake and feed digestibility, the effects varying with diet and physiological age; exerting a levelling or

buffering effect on the rumen, resulting in a more stable rumen fermentation; and influencing the quality and quantity of protein passing down the digestive tract (Becker *et al.* 1930).

In general the metabolic capabilities of the bacteria and the protozoa appear to be similar (Prins 1977; Coleman 1980). Bacterial concentrations have been found to be much higher in defaunated than faunated animals, and to decrease markedly following re-faunation (Eadie and Gill 1971). Thus it would appear that, if protozoa are absent, the fermentation of feedstuffs is taken over by an increased bacterial population. Orpin (1974) observed a 327% increase in rumen fluid bacterial concentrations after defaunation, which because of volume changes represented a 480% increase in total rumen bacterial numbers. Total microbial protein concentrations in rumen samples (liquid-small particle phase) were measured by Teather *et al.* (1984). They found that the variation among animals was less for total microbial protein concentrations than for either the bacterial or protozoal protein levels. A highly significant negative correlation was found between concentrations of the bacteria and protozoa when the ratio of these concentrations ranged from 0.08 to > 1000.

In summary, different levels of energy intake would be expected to support different amounts of total microbial protoplasm. The proportions of bacteria and protozoa contributing to this total, as well as the different species involved, could be influenced by a wide variety of factors.

2.7.3 Rumen fermentation

Rumen fermentation may be characterized by the following parameters: the total amount and relative proportions of volatile fatty acids (VFA) formed; the amounts of methane formed and organic matter (OM) fermented; the amount of microbial matter synthesized; and the efficiency of the latter process, which is most often expressed as g of N incorporated into microbial matter per Kg of OM fermented (g N_i / Kg OM_f) (Demeyer and Van Nevel 1986).

Perhaps the most important aspect of rumen fermentation is the degradation of structural carbohydrates including cellulose, hemicellulose and xylan. These compounds are almost undegradable in monogastric animals. Even in the rumen they can not be fully digested. The digestibility of these structural carbohydrates varies from 30% to 90%, depending on

total energy levels in the diet. Soluble sugars and starch are almost completely fermented in the rumen. Overall, only about 70% of all carbohydrates can contribute to significant increases in ruminant productivity (Czerkawski 1986).

Microbial fermentation of plant materials produces a number of end products. The main energy products are volatile fatty acids (VFA's), mainly acetate, propionate, and butyrate. Although different diets can greatly influence the bacterial population in the rumen, the relative ruminal VFA concentration are usually very stable, with molar ratio of acetate:propionate:butyrate being near 65:25:10 with roughage and 50:40:10 for concentrate diets. The efficiency of food energy utilisation generally depends upon the relative proportion of the major VFA's that are absorbed through the rumen wall into the blood stream of the animal and are utilised by the animal as carbon and energy sources (Church, 1988). It is estimated that VFA's constitute 50-70% of the energy requirement of the ruminant animal (Church 1988).

Ruminal VFA concentrations represent the balance between rates of production and rates of removal for each VFA, as well as their interconversions (Owens and Goetsch, 1988). The rate of VFA absorption is influenced by concentration, osmolality, chain length of the individual acids and ruminal pH. Reduced ruminal pH increases VFA absorption (Black and Kennedy, 1984). Increasing chain length also results in increased absorption rates with relative rates of absorption of undissociated acids as follows: butyric>propionic>acetic (Arnold, 1970). The rate of VFA metabolism by cells of the rumen wall is in order of butyrate>propionate>acetate. Acetate enters the blood in the greatest quantity because it is present in the greatest concentration within the rumen and it is metabolised at the slowest rate by the epithelial cell of the rumen walls. Of the absorbed VFA's propionate is used for the synthesis of glucose; acetate and butyrate mainly contribute to fat synthesis, particularly in milk (Czerkawski 1986), i.e. as well as their direct metabolism for energy production.

The main nitrogen source for the ruminant animal is derived from the breakdown of bacterial cells, as well as some undigested plant protein, in the abomasum (Hungate, 1966; Jarrige, 1988). It is estimated that up to 80% of the ultimate protein nitrogen supply to the ruminant animal is derived from bacterial protein. Another feature of ruminants is that they can

survive on a diet low in protein because of nitrogen recycling via saliva and the activity of a urea cycle (McDougall, 1948). The amount of urea nitrogen transfered to the rumen is determined by the rate of salivary secretion and by the plasma urea concentration.

Rumen fermentation patterns are greatly influenced by feed supplied to the animal. The microbial processing of cereal grains differs profoundly from that of forage materials. The feeding of cereal grains sets in motion an ecological succession that gives rise to a fermentation in which the production of acids and other products may exceed the absorptive and digestive capacity of the animal, and may predispose the ruminant to digestive disturbance (McAllister *et al.* 1990).

2.7.4 Microbial nutrients

For their own growth rumen microbes need C, P, N, S, H, as well as numerous specific compounds e.g. heme for *Bacteroides ruminicola*, vitamin K for *B. melaninogenicus*, methionine for *Ruminococcoccus flavefacines*, etc. In the mixed rumen population microorganisms may supply each other with such compounds or they are made available by death and lysis of cells (Prins and Van den Vorstenbosch 1975). A lack of material for microbial synthesis (e.g. N) will be reflected in either impaired fermentation or, as seems to occur more often, in uncoupled fermentation. In the latter case the extent and rate of fermentation are not affected but microbial growth is impaired (Demeyer 1981).

Nitrogen. For incorporation into cellular N cellulolytic bacteria require ammonia as their N source. Ammonia N is supplied by deamination in the rumen of feed protein amino acids or of urea recycled to the rumen via the saliva or from the blood (Demeyer 1981).

A lack of fermentable N for the rumen microbes may be reflected in a lowered extent of overall fermentation (Mehrez *et al.* 1977). The need of microorganisms for N may be reflected in a low rumen ammonia concentration and, according to Satter & Slyter (1974), a concentration below 50 mg NH_3-N/L indicates a shortage of N. Using limited barely-fed animals, Merhez et al. (1977) argued that optimal NH_3-N concentration for maximal fermentation, 200 mg NH_3-N/L, is not necessarily identical to optimal concentration for growth. It should be realized, however, that the static ammonia concentration is the resultant

balance of both production and utilisation. Hespell and Bryant (1979) point out that it is unlikely that rumen ammonia concentration would be limiting for bacterial growth as ammonia K_S values (concentration at which 50% of optimal growth is reached) for rumen bacteria are around 1 mg NH_3-N/L. Urea is rapidly degraded in the rumen, whereas cell wall carbohydrates are slowly fermented. This has led to speculation on the necessity to provide fermentable N (ammonia) to the microbes at the same rate as crude fibre is fermented (Johnson, 1976). Various " slow release " non-protein-nitrogen sources have been introduced, such as biuret, isobutyl diurea, acetylurea, treated mixtures of molasses and urea and glucosyl- or lactosylurea. Most of these compounds, however, are degraded too slowly or even not at all by rumen microbes or require careful adaptation to the animal (Males *et al.* 1979).

Phosphorus. It is evident that microbial growth and metabolism necessitates a constant supply of P for nucleic acid and ATP synthesis. Rumen microbes requires 3.6 - 5.5 g/Kg OM_f. This requirement is normally met by salivary P supply. Phosphorus in tropical hay is only partly released in the rumen and microbial P deficiency with poor roughages may occur, especially in the presence of high Ca and Mg. Such deficiency may result in impaired microbial growth (Durnad & Kawashia, 1979: Harrison & McAllah, 1979). A stimulation of rumen cellulose activity by phosphate concentrations up to 50 mM (about 1.5 g P/L) has been reported (Francis *et al.*, 1978). Rumen concentrations are normally below that value (Van Nevel and Demeyer 1977a)

Sulphur. Although large amounts of sulphur amino acids can be incorporated directly into microbial protein, microbial needs can be supplied entirely from rumen S. A microbial requirement of 1.5 g S/kg OM_f can be estimated. Rumen cellulose degradation in a poor roughage ration can be stimulated by S addition, up to 0.32% in the diet (Harrison and McAllan 1979).

Macro-minerals. Rumen microbial deficiency of Ca has not yet been established, whereas suggested tentative microbial requirements for Mg and K were 0.8 and 5 g/Kg DMf respectively. Rumen microorganisms normally receive an adequate supply of Na (Durian and Kawashima 1979).

Easily-degradable carbohydrates. Low quantities of easily degradable carbohydrates (5 - 10% of the substrates) can stimulate cellulolysis. This effect may be related to a stimulation of the rate of microbial glycocalyx formation (Cheng *et al.* 1977). Higher amounts of starch or soluble carbohydrates, however, can inhibit cell wall carbohydrate degradation, apparently related to a decrease in pH. Cellulolysis *in vitro* is very low at pH 6 and optimal between pH 6.7 and 7 (Stewart, 1977). Other factors than pH must be involved in the inhibition of rumen crude fibre digestion by starch, as shown by Gilchrist *et al.* (1979). Cellobiose and, to a lesser extent, glucose, were inhibitory to the activity of *R. albus* cellulases (Smith *et al.*, 1973) and cellobiose inhibited the attachment of *B. succinogenes* to cellulose (Minato and Suto 1978). Despite the widely used combination of low-quality roughages and urea-molasses, there are few data to suggest that molasses alone has any important role in stimulating digestion or feed intake. It may well be that the principle effect of molasses is to ensure a slow continuous supply of urea and to attract the animal's interest (Loosli and McDonald 1968).

Lipids: Lipids inhibit crude fibre digestion in ruminants and concentrations over 7% in the feed are not advised (Demeyer 1973). The inhibitory effect has been attributed to a coating of the fibres with lipid but recent evidence suggests that the toxicity of free fatty acids for protozoa may be a reason for the decreased fibre digestibility.

Toxic substances: Some substances can be toxic for rumen microbes. Terpenoid hydrocarbons present in certain plant species, pesticide residues, high concentration of some trace elements, all inhibit rumen microbial fermentation, bacterial growth and protozoa activity and therefore can be toxic for sheep (Warner 1962; Schwartz *et al.* 1973; Fontenot and Webb 1974).

2.7.5 Rumen pH

When ruminanants are fed forages, particularly those of high cell-wall content, ruminal pH is usually near neutral. Evidence suggests that when the pH is reduced below 6.0 digestion of cell-wall constituents is inhibited (Egan *et al.* 1987). Laboratory studies have shown that, for a range of straw diets supplemented with single meals of cereal grain, the rate of digestion of fibre in nylon bags is negatively correlated with the proportion of time over

which pH is below 6.0 (Egan *et al* 1987). However, this is dependent upon the timing of pH depression relative to the stage of microbial attack. Bacterial colony action and lag phase colonies seem more susceptible than are established colonies in log-phase, unless NH_3 concentration is also depressed. Egan (1990) stated that the digestion rate of straw particles in nylon bags continues with little alteration when pH is depressed if there is a well established anaerobic fungal invasion. On the other hand, material newly introduced during a period of low pH fails to initiate a fungal population. Changes in ruminal ammonia concentration have little effect or rate of fibre digestion where substantial fungal growth has been established (Egan 1990).

The rumen ecosystem is like a series of micro-environments in which stage of growth of different organisms is a major factor in determining the rate of local digestion and the effects on this of changing pH and level of dissolved nutrients. The rate of digestion over set periods of time (24-48 hrs) can vary widely and in particular there is little likelihood that the more refractory cell-wall constituents are rendered more digestible by changing ruminal conditions cyclically (Egan *et al.*, 1987).

2.7.6 Rumen ammonia

Ammonia is the most important source of N for protein synthesis in the rumen. Its concentration in the rumen fluctuates markedly, from less than 1 mM observed in some animals on extremely-low-protein roughages to perhaps 40 mM, transiently after feeding, in animals receiving rapidly-degraded protein or urea. There are several different enzymic mechanisms for ammonia uptake into amino acids, each with a different affinity for its substrate. The most important mechanisms, and perhaps different organisms as well, therefore probably vary in their response to ruminal NH3 concentration changes. The mechanism of ammonia uptake, because it is the central pathway for protein synthesis, is of great interest to microbiologist and nutritionists dealing with ruminants (Wallace 1961)

Extensive interactions are involved in converting dietary protein to NH_4^+ as this major source of microbial N. Valine, leucine and isoleucine in particular are deaminated to form isobutyric, 2-methylbutyric and isovaleric acids respectively. Many rumen species require

one or more of these acids for synthesis of branched chain amino acids and fatty acids (Wallace 1961).

In summary, in this section the most important factors in the performance of the rumen ecosystem in relation to the lignocellulosic feedstuffs have been reviewed, but still there may be other numerous factors which play important roles in rumen ecosystem characteristics, but little is known of them.

The next section of this review covers the lot-feeding industry especially as it relates to the last experimental chapter. All practical attempts at improved nutrition, including improving chemical composition, increasing voluntary intake and digestibility of feed, and improving ruminal conditions, all aiming to produce more efficient nutrient utilization are designed primarily for promoting high performance in terms of final animal production. Lot-feeding is one of the more practical and useful systems for this purpose.

2.8 Feedlotting in the sheep industry

Lot-feeding on a commercial scale originated in the 1950's in the United State of America, primarily for converting surplus grain into high-quality meat from beef cattle (Moore 1990). Although the first feedlots were established at that same time in Australia (Howard and Plasto 1991) it is generally considered that the feedlot industry in Australia effectively began in the early 1960's (Tucker *et al.* 1991). At present the total Australia beef cattle feedlot capacity is about 2.3% of the national herd of 22 million. Increasing demand from both domestic and overseas markets positively affects the feedlot industry in Australia (Callow 1992). Clark (1985a) noted that in South Australia commercial beef cattle feedlots were used: (a) for finishing stock that could not be finished on pastures; (b) in specialised feeding for a specific market; (c) as opportunity feedlots when store cattle are cheap or surplus grain could not be sold economically; (d) to take advantage of local industry by-products and finally (e) as an alternative to feeding at maintenance level in droughts.

Lot-feeding of sheep in Australia, in contrast to cattle, has became a common practice only recently (Hall and Mulholland 1982). In South Australia sheep lot-feeding has been undertaken for various reasons:

(a) In the Mediterranean-type environment of this State generally there is a 3-5 month dry period during summer and early autumn (Allden 1959). In these months paddock feed may not support the flock, so lot-feeding can maintain livestock numbers. The dramatic effects of heavy grazing on medic pastures, as reported by Carter (1981a, 1982) and Carter *et al.* (1982), can be reduced by lot-feeding of sheep during the dry period of year. In other words, lot-feeding can be used to protect both the soil and the pasture legume seed supply (Carter *et al.* 1993).

(b) In continuous sheep grazing over the summer months soil is at risk of wind erosion. Lot-feeding can be considered as an integral part of the "risk management program" on farms (Morbey and Ashton 1990).

(c) In seasons where paddock feeds are inadequate and cheap supplies of energy sources are available, lot-feeding of sheep can be considered for producing marketable meat of high quality (Hack *et al.* 1988c).

(d) When fodder or hay contains weed seeds, farmers could restrict the spreading of these weeds by lot-feeding where weeds can be more easily monitored and controlled (Morbey and Ashton 1990; Ashton 1990).

(e) Lot-feeding at the break of the season, coupled with deferred grazing, reduces the grazing pressure on the young pasture plants and improves pasture establishment (Ashton 1989).

(f) The live sheep export from Australia to international markets has developed as a major trade over the past 20 years. These sheep are lot-fed before and during shipping (McDonald *et al.* 1990).

2.8.1 Systems

Ashton (1989) has divided lot-feeding of sheep into three different classes as follows:

(a) Lot-feeding for maintenance purposes. This category involves removing the sheep from the paddocks before they become bare during the dry period. These sheep are kept in yards

and fed at maintenance or survival levels. It can be a useful practice for maintaining sheep numbers during droughts.

(b) Lot-feeding after the break of the season. This type of lot-feeding has to be done during wet and cold weather, hence the yards and feeds can become wet and boggy. Therefore, it is more difficult than lot-feeding during the dry weather.

(c) Lot-feeding for finishing or production. The aim of this management is to produce marketable meat or live sheep. Therefore, sheep requirements, growth rate and feed conversion are the most important factors which must be considered. This type of lot-feeding is different from the other types and the animals should be fed by balanced least-cost, complete rations with a high energy content. Computer programs are useful tools in this task for producing the maximum benefits.

2.8.2 Structures

Many factors affect the selection of a site for a feedlot. Some are associated with economic considerations and others involved with animal health and environmental performances. Generally the chosen site for a feedlot should have a hard clay or stony base to reduce dust. Ideally, the site should be dry, well drained, sheltered from the wind and be away from sources of frequent disturbance (Ashton 1986, 1990; Bell *et al.* 1986; Hack *et al.* 1988a).

Different yard sizes can be considered, but generally 5 to 10 m^2 per sheep could be a good guide, although the yard size itself is not a critical point. In a larger area, sheep will tend to walk more and raise dust, cause soil erosion and waste energy. In very sandy conditions a reduced area of 1-2 m^2 per sheep can be used to minimise soil disturbance and consequent dust problems. In wet conditions a larger area, e.g. 20 m^2 per sheep, is preferred. Group sizes can also vary, but poor results have been experienced with large mob sizes in the feedlots (Bell *et al.* 1986). It is preferable to build a number of smaller lots if more than 500 sheep are to be fed (Hack *et al.* 1988a).

A supply of fresh, cool and clean water is essential. Water should be available at all times, with a daily allowance of 3 to 6 litres for an adult sheep and one metre of trough space for

every 100 lambs. However, the speed at which a water trough fills is more important than its size. If the water troughs are located close to the feeding system these should be cleaned regularly to prevent fouling of the water by feed particles and dust (Ashton 1986; Bell *et al.* 1986; Hack *et al.* 1988a; Tucker *et al* 1991).

In feedlots, whole grain and other feeds with small particle size can be spread on selected clay pans, hard bare ground or other suitable areas, or alternatively fed in troughs. In sandy, muddy or deeply cracked soils feeding animals in troughs is essential. Feed troughs prevent feed wastage and other problems such as sand impaction (Hack *et al.* 1988a; Ashton 1990). Sometimes self-feeders have been used instead of traditional feed troughs. Self-feeders are convenient and more flexible and by eliminating the need for daily feeding they can reduce labour requirements and the amount of trough space. Also, self-feeders can provide an *ad lib* feeding system (Bell *et al* 1986). Tucker *et al.* (1991) however, considered that self-feeders were an undesirable feeding system, because the accumulated grain and manure under these feeders creates a bad odour, attracts flies and is difficult to remove. Also, beside the higher cost, feeding grain in self-feeders increases the risk of grain poisoning. As a rough guide about 5 cm of trough space per sheep is needed when self-feeders are used (Bell *et al.* 1986).

Feed troughs should be easy to load and clean and designed to minimise spoilage and fouling by animals. Where sheep have access to both sides of the feed troughs a minimum of 12 cm per sheep is needed (Morbey and Ashton 1990). If sheep are fed *ad lib.* about 5 cm (Fels 1980) feed trough is required but on restricted rations more space, around 30 cm per sheep, is needed (Ashton 1986). Bell *et al.* (1986) noted that the width and depth of troughs for lot-feeding of lambs ideally should be 30 and 25 cm respectively. These types of troughs are suitable for feeding grain alone, grain/hammer milled roughage rations or a grain/whole hay mixture. Troughing should be 15 to 30 cm above ground level. Whole hay can be fed from hay racks. Hay racks can be simply made up from 10 cm square mesh held up by steel posts.

2.8.3 Principles of nutrition

Generally sheep rations have two major ingredients, grains and roughages. Sheep in a feedlot or in the paddocks, as with all ruminants, need roughage to ensure the efficient functioning of the digestive tract. Similarly roughage should comprise about 20-40% of the ration for sheep and lambs in feedlots (Bell *et al.* 1991a). Cereal and grain-legume straws are produced in abundant amounts in the crop-growing areas of Australia and can be used in place of hay for sheep in feedlots (Ashton 1990).

Rations comprising straw and cereal grain are suitable for maintenance feeding of adult sheep (Ashton 1990). However, many grains and straws have a low protein content for growing animals, and rations often need to be supplemented with small amount of materials with higher crude protein contents. For example, grain legume seeds such as lupins have been added or low-quality roughages sprayed with urea (Dunlop and McDonald 1986). Furthermore, the palatability of low-quality roughages is generally low. A small quantity of good quality lucerne hay or molasses is needed to ensure the initial acceptance (Bell *et al.* 1991a; Tucker *et al.* 1991). In feedlots, sheep should be introduced gradually to high levels of grain in the ration to reduce the risk of grain poisoning (Bell *et al.* 1991b).

Feed accounts for a large proportion of the variable costs of running a feedlot and hence ration quality and quantity and feeding management have the greatest effects on animal performance and the overall efficiency of the feedlot (FAU 1990). Therefore, it is necessary to provide a balanced and economical ration for animals in feedlots. Many methods have been proposed for ration formulation of farm animals (Crampton and Harris 1969).

Computer programs have been developed for calculating the least-cost ration for intensive livestock industries. Nevertheless, there has been little application in Australia of these programs for formulation of the optimum diets for feedlot animals (Shaw and Thornton 1974). Recently, the Department of Primary Industries in South Australia has produced a computer program (TAKE-AWAY) that calculates least-cost rations for sheep and cattle. This program can formulate suitable rations for different physiological conditions such as maintenance, growth, pregnancy or lactation feeding (Barber 1990).

2.8.4 Animal health

In a well-managed sheep feedlot health problems rarely occur and the death rate should be less than 1% over about 3 months (Ashton 1990; Langman *et al.* 1990). In a survey carried out by the South Australian Department of Primary Industries on Eyre Peninsula after the 1988 drought the average death rate in farm feedlots was 1.4% (Moreby and Ashton 1990). The main causes of death in these feedlots were: grain poisoning (19%), pregnancy toxaemia (13%), accidents (9%), enterotoxaemia (8%), shy feeders (6%), suffocation (6%) and fly strike (5%). Feedlot health problems can be grouped into nutritional and non-nutritional categories.

Grain poisoning and pregnancy toxaemia are the most serious nutritional health problems. Grain poisoning (lactic acidosis) probably is the main cause of death in feedlots. This illness results from a sudden increase in the diet of highly-digestible concentrates, such as cereal grains. The inclusion of a large proportion of readily-fermentable grain rapidly reduces rumen pH and increase the population of potentially-pathogenic micro-organisms within the digestive tract. Some bacteria can migrate to the liver through the inflamed rumen wall and cause abscesses (Hack *et al.* 1988b; FAU 1990; Langman *et al.* 1990). Sheep should be slowly introduced to grain or concentrate and changes to the ration must be made over about two weeks. Adequate roughage must be provided and preferably fed before grain (FAU 1990; Langman *et al.* 1990).

Pregnancy toxaemia is a ewe disease caused by insufficient energy intake during the last 4-6 weeks of pregnancy. The ration should be increased in late pregnancy, about eight weeks prior to the start of lambing. A full ration for pregnant ewes should be given 6 weeks prior to lambing (Langman *et al.* 1990). Urea poisoning and sand impaction resulting from feeding sheep on sandy soils are other feed-related illnesses (Clark 1985b; Hack *et al.* 1988b; Langman *et al.* 1990).

The main non-nutritional health problems in lot-fed sheep are enterotoxaemia and pinkeye. Enterotoxaemia, a bacterial disease, occurs primarily when sheep are fed highly-digestible concentrates, although it is usually associated with grazing lush pasture. A regular enterotoxaemia vaccination program must be employed.

Pinkeye can be caused by dust or sharp roughage particles and husks. Extra care is needed for severely-affected sheep. Preferably these sheep should be placed in a separate yard in feedlots.

Fly strike, heat stress and accidental death are other non-nutritional health problems which often need attention (Langman *et al.* 1990; Morbey and Ashton 1990).

2.8.5 Management practices in feedlots

A small proportion of sheep may not adapt to feedlot conditions or miss their share of feed and therefore perform poorly. These sheep, known as "shy feeders" or "poor doers", may eventually die of starvation or become susceptible to stress-related illnesses. Up to 5 percent of shy feeders is common when feeding rations with high amounts of grain. These sheep must be managed separately. The proportion of shy feeders can often be reduced by using more feed troughs (greater length per sheep), self-feeders and giving detailed attention to other management aspects (Hack *et al.* 1988b; Langman *et al.* 1990).

Sheep must be vaccinated against enterotoxaemia. Two injections for sheep which have never been vaccinated with an interval of 4 to 6 weeks gives sufficient protection. Endo-parasites can cause significant body weight and production losses (Langman 1988), and thus sheep must be drenched against these parasites before going into feedlots (Ashton 1984 1990).

Daily checking of sheep can help prevent problems before they occur. Feed must be given at the same time every day and detailed records kept. Any dirty section of troughs need to be cleaned. Observation of sheep behaviour and general feedlot conditions are the other daily routine tasks that should be considered (Fels 1980).

At the end of lot-feeding, breeding sheep should be released to the paddock immediately after they have eaten their normal feed. It is recommended that sheep be fed hay for a few days in the paddock. Ewes in late pregnancy should be fed with good quality hay and grain for at least the first week in the paddock, especially if the paddock feed is poor (Morbey and Ashton 1990).

For most farmers in South Australia however, sheep lot-feeding is still new and to make this practice profitable detailed attention must be given to all aspects detailed above.

CHAPTER 3

Selection of seagrass *Posidonia australis* from among aquatic plants by chemical estimation of their nutritive value

Christianson and Allender (1988)

"There are thousands of species of aquatic plant throughout the world with different potential nutritive value. For the utilisation of any aquatic plants two important overall issues, environmental effects and economic realities, should be considered." (Author).

CHAPTER 3

Selection of seagrass *Posidonia australis* from among aquatic plants by chemical estimation of their nutritive value

3.1 Experiment 1: Selection of *posidonia australis* from among marine plants

3.1.1 Introduction

The principle long term purpose of the current project is to examine possible commercial utilisation of aquatic plants native to South Australia, so that at the time of drought, pasture deterioration and feed shortage one or more species might replace conventional feedstuffs as a ruminant feed in general, for sheep nutrition in particular.

In order to choose appropriate aquatic plants from amongst the hundreds of species available in South Australia (Section 2.1.1), Kingston beaches were first sampled based on information from the Department of Botany, the University of Adelaide and local sources. Thirteen species of plentiful and readily available aquatic plants (12 species of seaweed, 1 species of seagrass) were collected and identified. Chemical analysis and digestibility *in vitro*, as simple and fast methods, were applied to estimate the possible nutritive value of collected species.

3.1.2 Material and Methods

Experimental plants: Twelve species of fresh seaweeds from the water and 1 species of dry seagrass from the beach were collected at Kingston, South Australia. The genera and species of the collected aquatic plants were identified by the Department of Botany, the University of Adelaide as follows:

(i) Seaweeds:

1) *Acrocarpia paniculata* (AP)
2) *Cystophora platylobium* (CP)
3) *Cystophora moniliformis* (CM)
4) *Cystophora retorta* (CR)
5) *Cystophora subfarcinata* (CS)
6) *Ecklonia radiata* (ER)
7) *Seirococcus axillaris* (SA)
8) *Sargassum bracteolosum* (SB)
9) *Sargassum dicipens* (SD)
10) *Sargassum lineafolium* (SL)
11) *Sargassum varians* (Sva)
12) *Sargassum vervuculosum* (SVe)

(ii): Seagrass

1) *Posidonia australis* (PA)

Examples of some of the experimental species of seaweeds (AP, CR, ER, SB) and seagrass (PA) are shown in plates 3.1, 3.2 and 3.3 (source: Christianson *et al.* 1988).

Lucerne chaff (with particle length of 2-4 cm), obtained from stocks at the Waite Institute, was used as a comparison throughout these studies.

Sample preparation for chemical analysis: All fresh aquatic plants after collection and identification were separately sun dried over 24 hrs, further dried in a force-draught oven at 60° C for 24 hrs and then allowed to come to equilibrium with the moisture in room air. About 500 g of each dried plant was ground through a 1 mm screen, further mixed and a 200 g sub-sample was placed in an air-tight plastic container for later chemical analyses. Sample preparation and handling procedures are outlined diagramatically in Figure 3.1.

Analytical techniques: Ground samples were analysed for dry matter (DM), ash, organic matter (OM), Crude protein (CP), crude fibre (CF), ether extract (EE) and dry matter digestibility (DMD).

Plate 3.1: *Acrocarpia paniculata* (top) and *Cystophora retorta* (bottom)

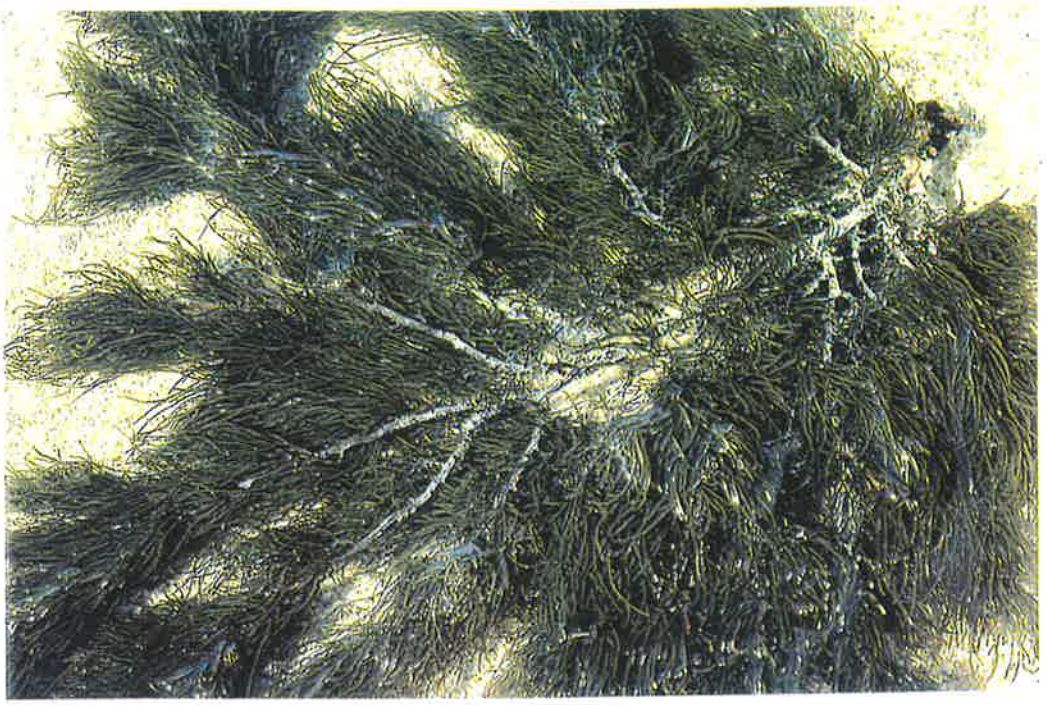


Plate 3.2: *Sargassum bracteolosum* (top) and *Ecklonia radiata* (bottom)

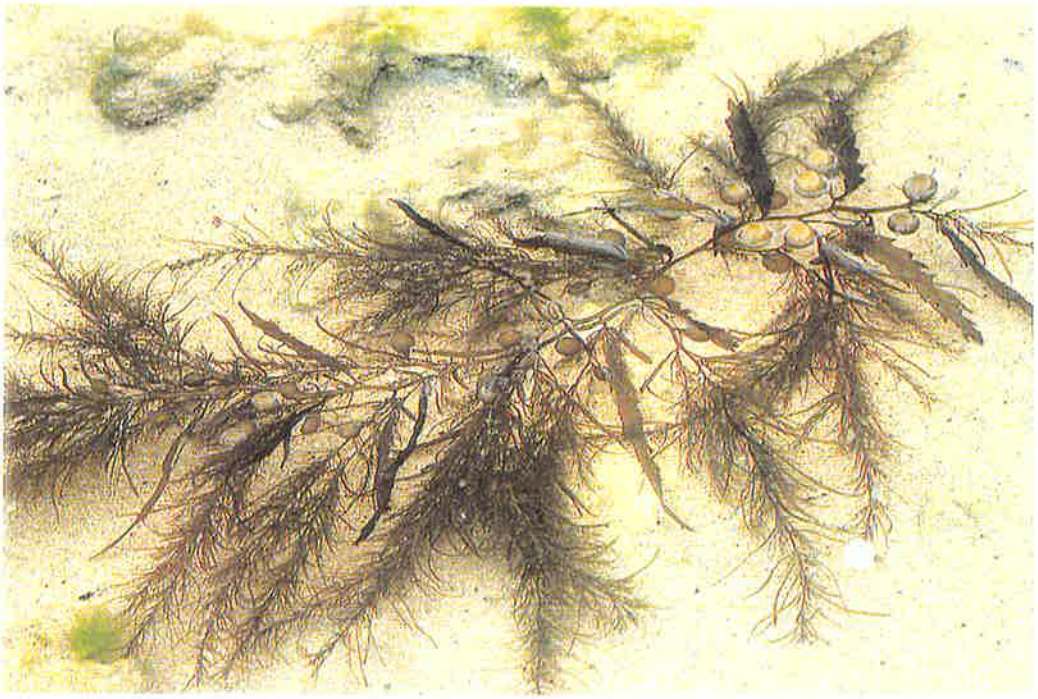


Plate 3.3: Green seagrass (top) and dry seagrass on the beach (bottom)

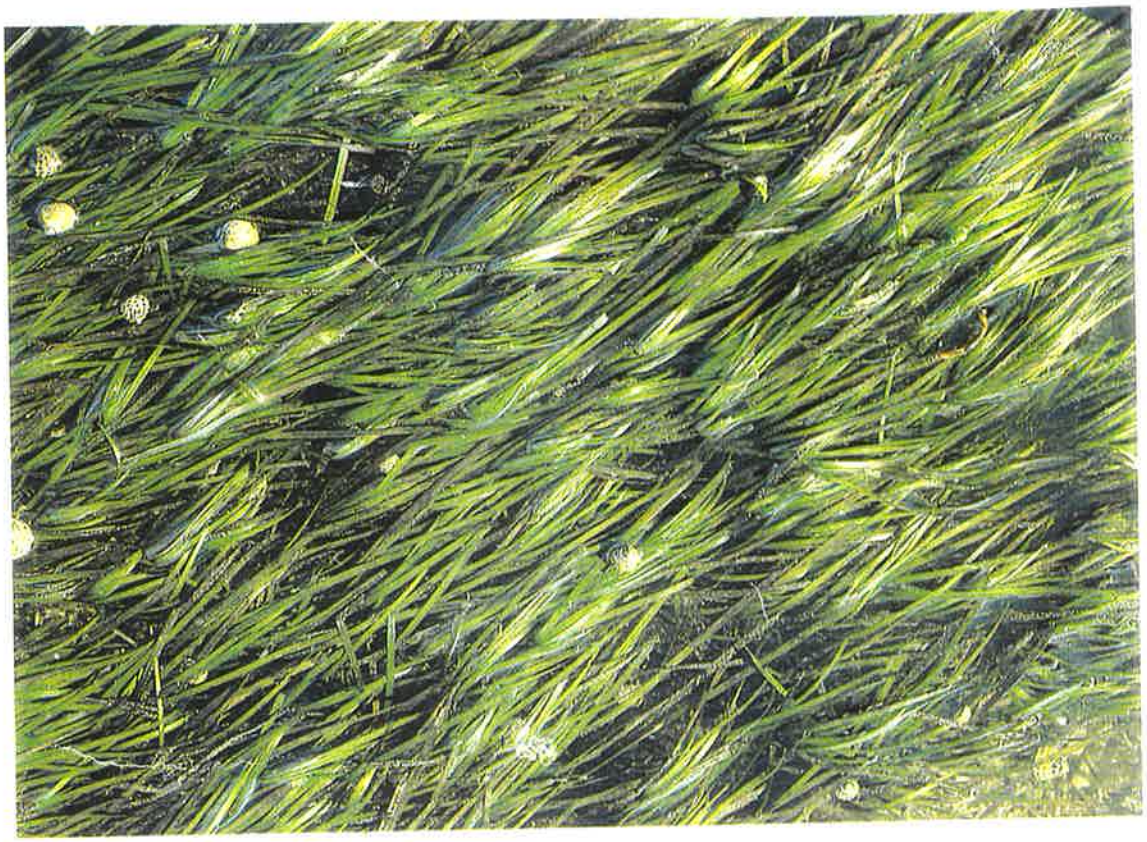


Plate 3.4: Drawing rumen liquor from fistulated sheep for determination of digestibility *in vitro*

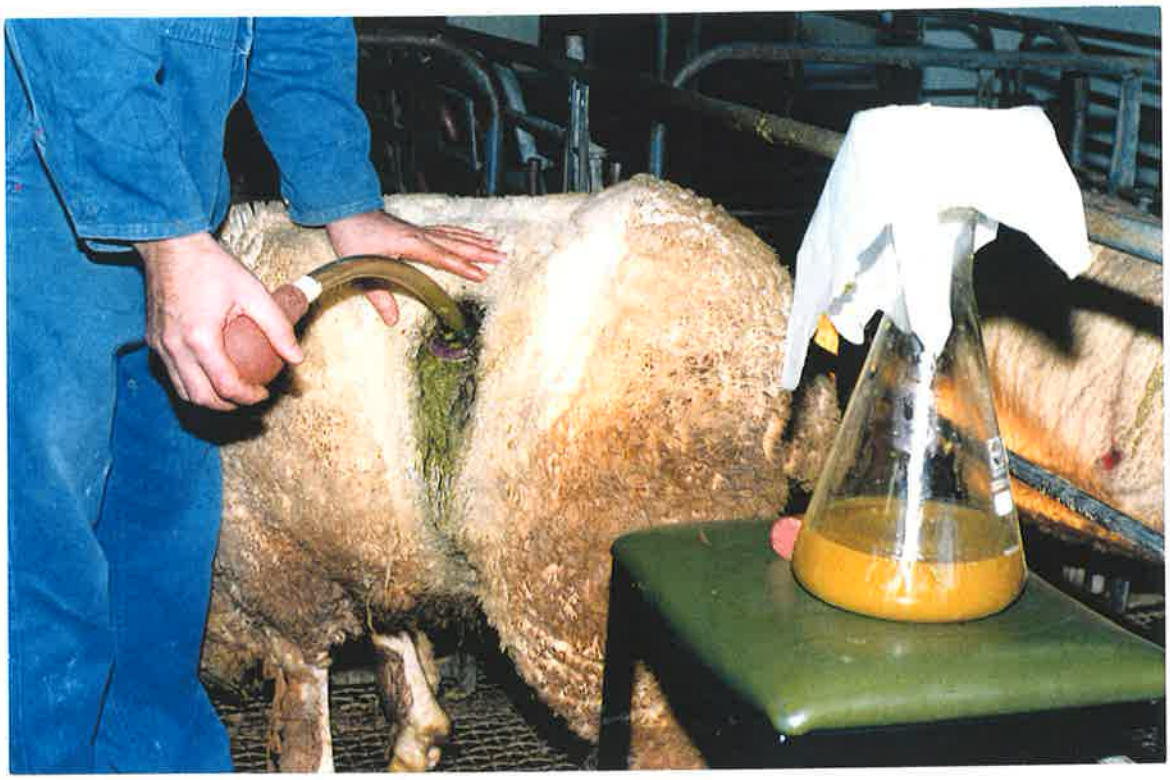
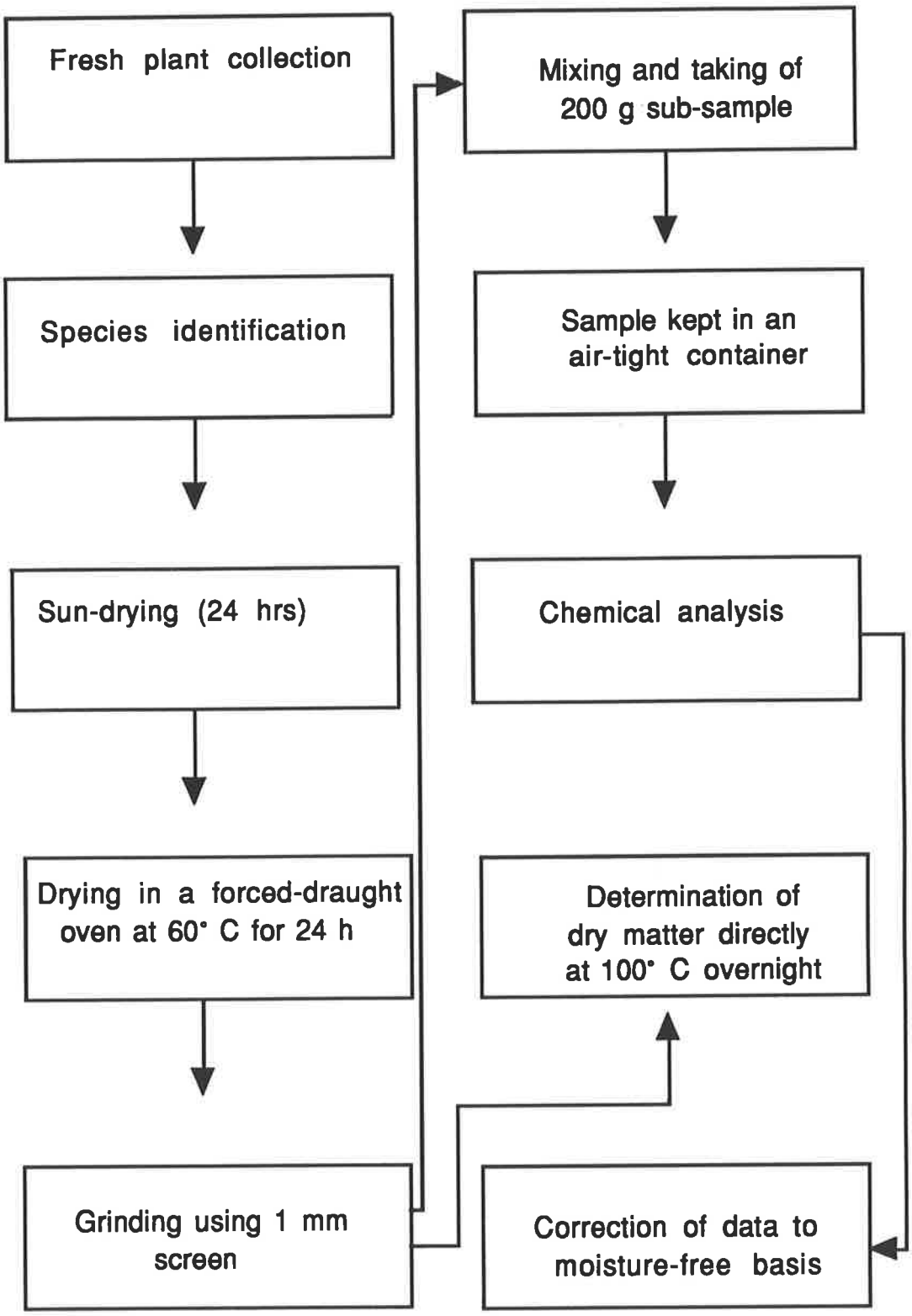


Figure 3.1: Steps in sample preparation of aquatic plants



Dry matter: Dry matter content of aquatic plant samples was determined by a method only slightly different from that described by Harris (1970).

Ash and organic matter: Two grams of ground samples were weighed into tared crucibles and placed in an electric muffle furnace at 600° C for at least 4 hours (AOAC 1990) and the residue weighted. Organic matter was determined by subtracting the ash value from 100.

Crude protein: The CP content of the samples was determined by Kjeldahl analysis, using the Kjeltec Auto System. Samples of around 1 g were weighed into nitrogen free-paper (Schleicher and Schuell) and digested with concentrated sulphuric acid in the preheated digester (Digestion System 40 1016 digester, Tecator). The digested samples were analysed by the Kjeltec Auto 1030. The conversion factor of nitrogen to CP was the standard 6.25.

Ether extract: A Soxhlet apparatus was used for lipid extraction. A 2 g sample was weighed into a numbered paper thimble (Whatman 30x10 mm). The thimbles containing samples were placed in the Soxhlet extraction unit and Shell X-55 was used as solvent. Extraction was continued for at least 7 hours. At the end of extraction and solvent volatilisation the extracted lipid was dried in an electric oven overnight at 100° C and then weighed.

Crude fibre: Solvent-extracted and moisture-free samples were quantitatively (1.5-2 g) transfered to tall beakers and boiled with pre-heated 1.25% (0.255 N) sulphuric acid for 30 minutes. After boiling with acid the contents of the beakers were filtered. The residues on the filter papers were returned to the beakers and boiled with pre-heated 1.25% (0.312 N) sodium hydroxide for another 30 minutes. After digestion with sodium hydroxide the contents of the beakers were filtered and washed with distilled water and ethyl alcohol. The residues were dried at 100° C overnight. Dried residues then were ignited at 600° C in an electric muffle furnace for at least 4 hours. The loss of weight on ignition was regarded as the weight of crude fibre.

Nitrogen free-extract: NFE was determined by difference after the analyses had been completed for ash, CP, CF and EE as follows (all on a moisture-free basis) (Crampton and Harris 1969):

NFE (%) = 100 - (Ash% + CP% + CF% + EE%)

Dry matter and organic matter digestibility: A modification of Tilley and Terry's two-stage technique (1963) was used for the determination of dry matter and organic matter digestion *in vitro*. Sub-samples of c. 0.55 g were weighed into 100 ml numbered glass tubes. The samples were incubated with buffered rumen liquor under anaerobic conditions for 48 h in an incubator at 38° C. Tubes were shaken periodically.

The rumen liquor had been collected from four fistulated sheep before they received their daily feed ration of equal proportions of chaffed oaten hay and commercial pellets (Appendix 3.1). The liquor was strained through six layers of cheese cloth and added to a buffer solution in the ratio of 1:4 (v/v). The composition of the buffer solution is shown in Table 3.1.

Table 3.1: Buffer solution used for digestibility *in vitro* (modified from McDougall 1948)

Component	g/L (in de-ionized H_2O)
$NaHCO_3$	9.8
Na_2HPO_4, $12H_2O$	9.3
NaCL	0.47
KCl	0.57
$MgCl_2$, $6H_2O$	0.09
$CaCl_2$	0.05

At the end of the first stage of digestion samples were centrifuged and after discarding the supernatant a hydrochloric acid-pepsin (1:2500) solution (1000 ml RO water + 8.9 ml concentrated HCl + 0.5 g pepsin) was added. Digestion of samples in this second stage continued for 48 h under the same conditions. After digestion the second-stage samples were centrifuged and the final residues transferred to weighed crucibles and dried in an oven at 100° C for 24 h. In order to determine organic matter digestibility dried residues were burnt in a muffle furnace for at least 2 h at 600° C (AOAC 1990).

With every run for digestion *in vitro* two standard samples of known high and low digestibility *in vivo* (lucerne and wheat straw) and four blank glass tubes containing the rumen liquor buffer mixture were also included. The following equations were used for calculating the dry matter and organic matter digestibilities.

Dry matter digestibility = wt of sample - (wt of undigested residue - wt of residue from blank) / wt of sample x 100

Organic matter digestibility (on dry matter basis) = wt of sample organic matter - (wt of undigested organic matter - wt of organic matter from blank / wt of sample dry matter x 100

In order to estimate digestible and metabolisable energy, the equations described respectively by Heaney and Pigden (1963) and ADAS (1984) were used as follows:

DE (Mj/Kg DM)= 19.66 DMD - 0.70

ME (Mj/Kg DM)= 0.80

Because of its general simplicity and practicality this technique for digestibility *in vitro* was also used later for the determination of the digestibility of the constituents of chemically and biologically treated experimental feeds (see chapter 5).

Statistical analysis: Data obtained for chemical composition and digestibility *in vitro* were analysed using analysis of variance and means were compared by Fisher's protected-LSD method at the 0.05 probability level or less.

3.1.3 Results

Chemical composition: Figures 3.2, 3.3, 3.4, 3.5 and appendix 3.2 show the chemical composition of seaweeds (12 species), seagrass (1 species) and lucerne chaff. It is evident that there were wide variations in chemical composition amongst the various aquatic plants.

The crude protein content of the seaweed species (Figure 3.2 and Appendix 3.1) is low and ranged from 4.4% in *Seirococcus axillaris* to 7.3% in *Acrocarpia paniculata*. This value for seagrass *Posidonia australis* was 5.5% but for lucerne chaff was 17.9%, i.e. significantly greater.

The crude fibre content of the experimental plants is shown in Figure 3.3 and Appendix 3.2. It can be observed that crude fibre in seagrass *Posidonia australis* (34.4%) is considerably greater that in all of the seaweed species and in lucerne (29.8%). Crude fibre in the seaweeds ranged from 3.7% in *Cystophora retorta* to 10.1% in *Cystophora moniliformis*.

The lipid (ether extract) content (Figure 3.4 and Appendix 3.2) of both seaweeds and seagrass was very low. This value in seaweeds ranged from 1.1% to 1.7% and in seagrass and lucerne was 1.1% and 2.0% respectively. Lucerne was significantly higher than seaweeds and seagrass.

NFE content (Figure 3.5 and Appendix 3.2) of seagrass was 39.2%, which was significantly lower than all seaweeds (46.7% to 67.5%). The NFE content of lucerne was higher than seagrass (42.0 vs 39.2%) and lower than seaweeds. The difference in NFE of seaweeds, seagrass and lucerne was significant ($P < 0.01$).

The Ash content (Figure 3.6 and Appendix 3.2) of seaweeds ranged from 19.1% in *Cystophora moniliformis* to 40% in *Sargassum lineaifolium*; this value for seagrass was 19.8%. The ash content of all seaweeds and of seagrass was significantly higher than for lucerne.

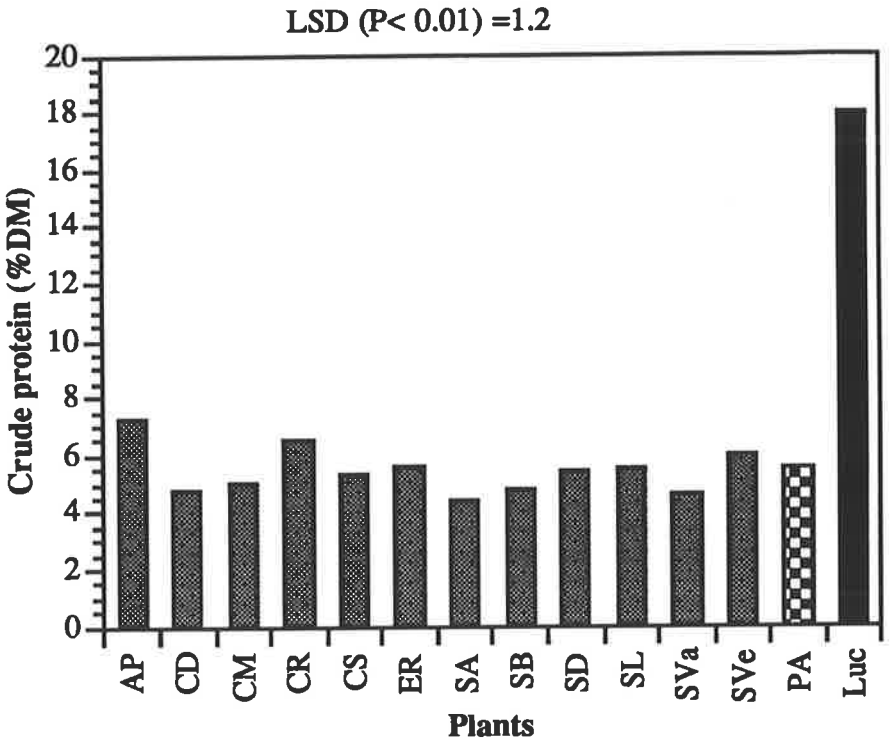


Figure 3.2: Crude protein content of experimental plants (see abbreviation on section 3.1.2)

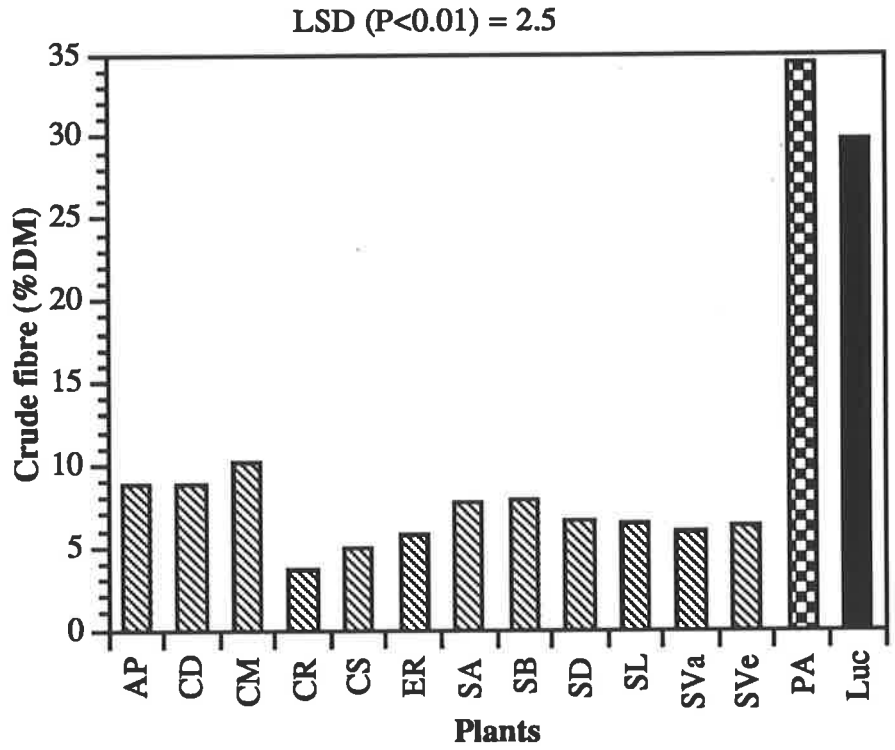


Figure 3.3: Crude fibre content of experimental plants (see abbreviation on section 3.1.2)

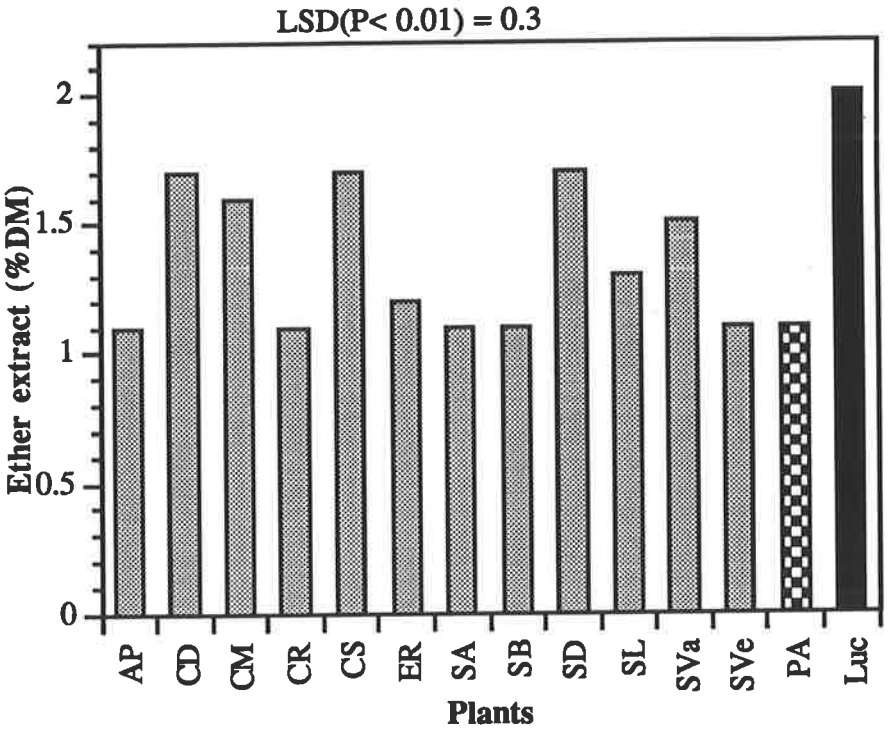


Figure 3.4: Ether extract of experimental plants (see abbreviation on section 3.1.2)

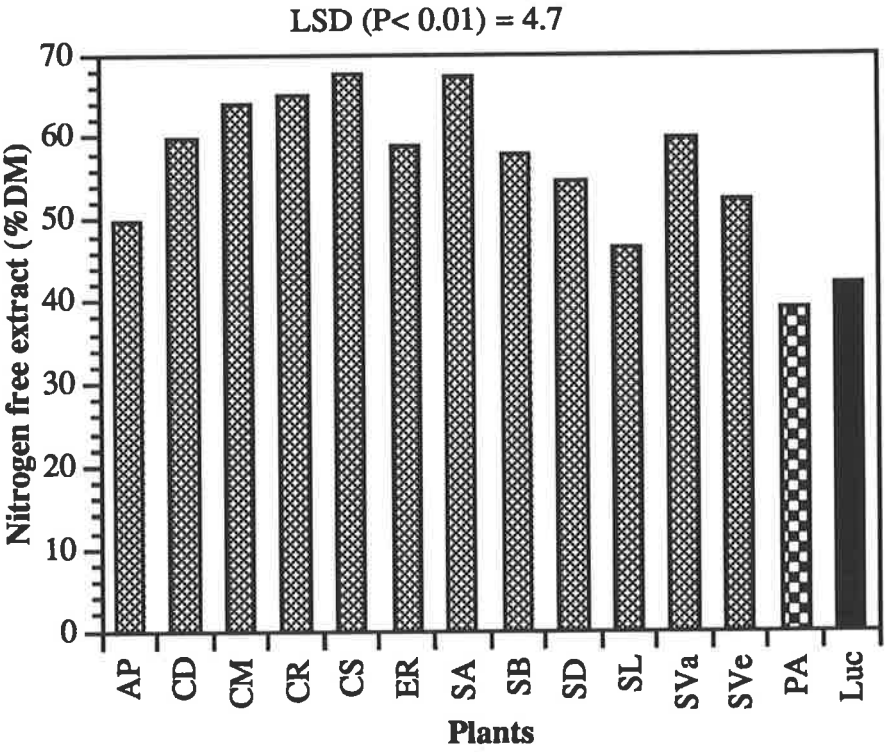


Figure 3.5: Nitrogen-free-extract of experimental plants (see abbreviation on section 3.1.2)

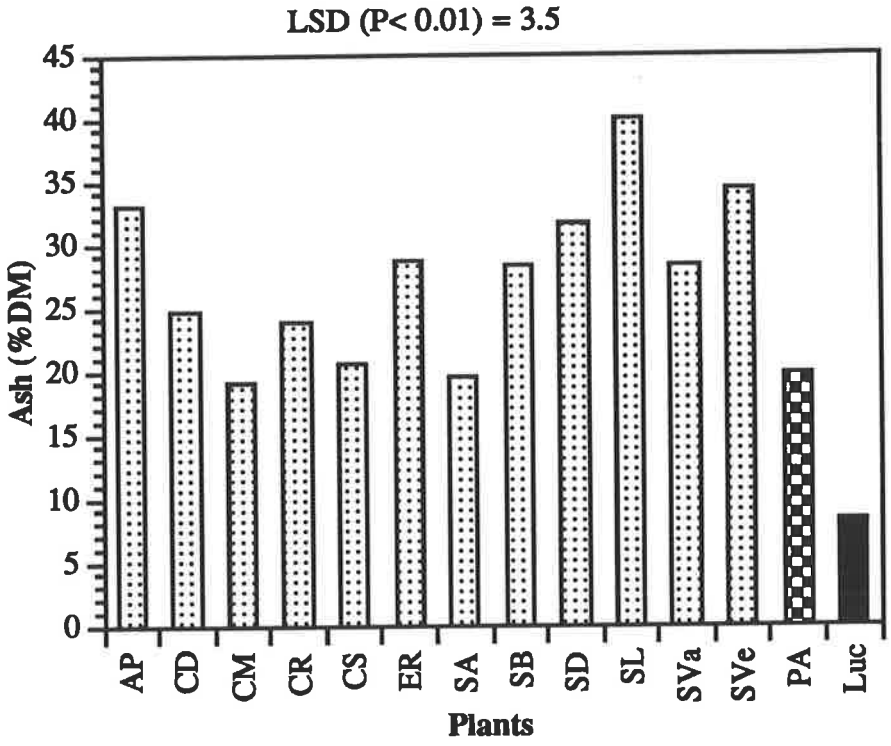


Figure 3.6: Ash content of experimental plants (see abbreviation on section 3.1.2)

Digestibility : Dry-matter and organic-matter digestibility and digestible and metabolisable energy of the experimental plants are shown in Table 3.2. DMD and OMD of seaweeds ranged from 34.1% to 51.5% and from 21.8 to 40.3% respectively; these values for seagrass were 34.7% and 20.1%. DMD and OMD of lucerne chaff were substantially higher than for the aquatic plants (67.7% and 64.9%).

Data in Table 3.2 also show that the values for DE and ME of aquatic plants compared with lucerne are very low with minimum values of DE and ME respectively of 6.0 and 4.9 MJ/Kg dry matter in *Cystophora moniliformis* and maximum values of 9.4 and 7.6 in *Ecklonia radiata*. Seagrass had values of 6.1 and 5.0 MJ /Kg dry matter in this regard.

Table 3.2: Digestibility and energy content of experimental plants (Dry matter basis)

Sample*		DMD (%)	OMD (%)	DE (MJ/Kg DM)	ME (MJ/Kg DM)
AP		48.1	30.1	8.7	7.1
CD		34.7	21.8	6.1	5.0
CM		34.1	24.1	6.0	4.9
CR		38.8	33.2	6.9	5.6
CS		36.5	25.0	6.5	5.2
ER		51.5	40.3	9.4	7.6
SA		37.2	31.7	6.6	5.4
SB		42.6	25.0	7.7	6.2
SD		45.8	28.1	8.3	6.7
SL		50.0	31.9	9.1	7.4
SVa		41.8	29.8	7.5	6.1
SVe		41.2	24.4	7.4	6.0
PA		34.7	20.1	6.1	5.0
Luc		67.7	64.9	12.6	10.2
LSD	0.01	3.4	4.1	0.7	0.5
	0.05	2.6	3.1	0.5	0.4

*** See abbreviation on section 3.1.2**

3.1.4 Discussion

The general purpose of this first experiment described in this chapter was two fold:

(i) An estimation and comparison of the possible nutritive value of widely available aquatic plants in South Australia;

(ii) The screening of one appropriate species to utilise further in sheep nutrition studies, ultimately from a commercial point of view.

The results of the chemical composition studies of the thirteen aquatic plants examined show that both all seaweeds and seagrass contain a very high ash content. This result is in agreement with work by Black (1955) and Durako and Dawes (1980), the latter reporting ash content of aquatic plants up to 35%.

It was found that the protein content of both seaweeds and seagrass is so low so that they could not realistically be regarded as a significant feed source of protein. Although there are some exceptional species of aquatic plant which contain high level of protein, the literature shows that mostly seaweeds and seagrass are poor in protein (Harrison and Mann 1975, Suberkropp *et al.* 1976, Augier *et al.* 1982, Price 1985).

Seagrass *Posidonia australis* contains a very high amount of crude fibre, significantly higher than all species of seaweed and lucerne as well. This high value of seagrass in crude fibre is in agreement with report by Bjorndal (1980), Klumpp and Van der Valk (1984) and Pirc (1985).

The apparent dry-matter and organic-matter digestibilities and digestible and metabolisable energy values of seaweeds and seagrass are very low. It is apparent from Table 3.1 that the digestibility of lucerne is considerably higher than that of these aquatic plants.

In short, according to the data presented here aquatic plants, including seaweeds and seagrass, are low in protein, ether extract, energy and digestibility. In spite of the low content of crude fibre in seaweeds the amount of this nutrient in seagrass, however, is very high.

Finally, from amongst the aquatic species examined the seagrass, *Posidonia australis* , was selected to be studied further as a possible feedstuff because of its lower content of ash, its high quantity and ready availability in South Australia.

There are possibly hundreds, even thousands of tonnes of the seagrass *Posidonia australis* massed each year by the action of waves on southern Australian beaches. Local residents generally consider that this causes severe environmental problems. While seaweeds are readily available in South Australian waters from a commercial point of view the harvesting

of aquatic plants from sea water entails such large costs that utilisation that as a feed for animals may never be economic. Therefore one of the more important reason to prefer seagrass as a possible alternative feedstuff for ruminant in South Australia is the fact of its already being washed up on the beach and is readily harvested.

In addition, data shows that the crude fibre content of seagrass in about 3 times greater than in the seaweed species. Seagrass can thus be regarded as possibly a rich source of polysaccharide carbohydrates for ruminants.

In order to understand further the carbohydrate constituents of seagrass, *Posidonia australis,* and its other important structural nutrients a second experiment was conducted.

3.2 Experiment 2: Determination of structural carbohydrates and cell-wall constituents of four different physical forms of *Posidonia australis*

The work described in this section was carried out with advise and assistance from Dr. G. Anison, CSIRO Division of Human Nutrition, South Australia. The authour is grateful for his valuable co-operation.

3.2.1 Introduction

From the first experiment it was concluded that amongst marine plants available in South Australia the seagrass, *P. australis*, because of its ready availability in great quantity and its high content of crude fibre, might be regarded as a potential alternative animal feedstuff. This high crude fibre content may be rich in structural polysaccharides, a potential useful source of energy for ruminants.

Therefore in this second experiment samples of four different physical forms of this plant were collected from the water (green and fresh) and on the beach (washed and un-washed) and were examined and compared for chemical composition, including nonstarch-structural polysaccharides (NSP) and other important constituents using advanced methods.

The hypothesis was that the green and fresh plant of *P. australis* might be more valuable in terms of chemical composition than material that had been massed and weathered on the beach for a long time.

3.2.2 Material and Methods

Experimental plants: Four different physical forms of *P. australis* were collected from the same area of beach at Kingston, South Australia in mid-summer, as follows:

(i) Green *P. australis* (GP): This form of the plant, which was green in colour was collected from the sea at a maximum depth of one meter (Plate 3.3 top).

(ii): Fresh *p. australis* (FP): This was collected from the edge of the beach, as near as possible to the water; it seemed to have been massed by wave action very recently. The colour of this was mostly brown.

(iii) Dry and washed *P. australis* (DWP) was collected on the beach back the water's edge and had probably been exposured to the weather for a long time (Plate 3.3 bottom). The collected plants were washed 3 times in tap water the day after collection in order to remove surface sand, dirt and other contaminants.

(iv) Dry but unwashed *P. australis* (DUP): was just as collected, i.e.(iii), but not washed (Plate 3.3 bottom).

Sample preparation: All samples were prepared for analysis essentially as previously outlined in Figure 3.1.

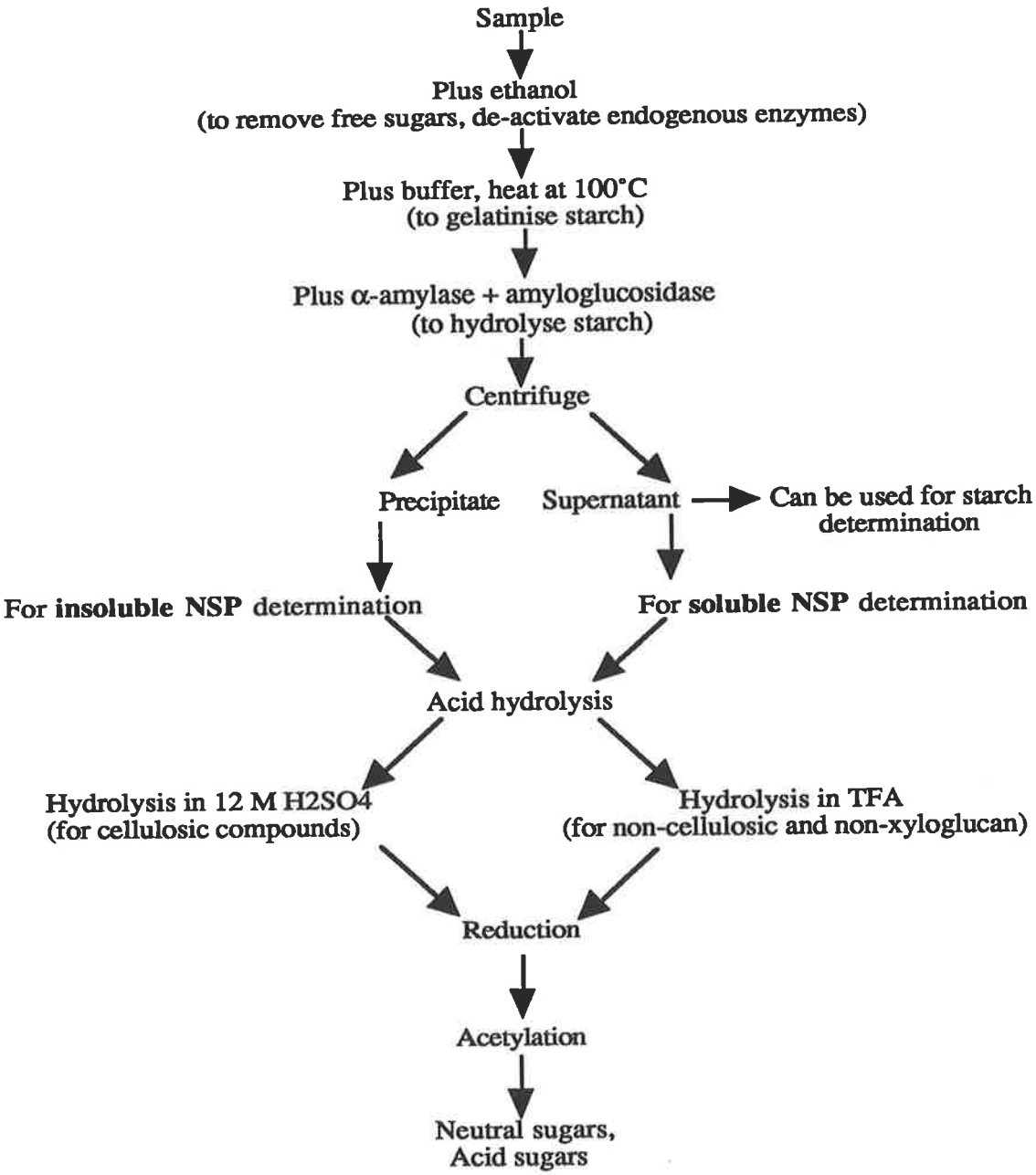
Analytical techniques: Ground samples were analysed for non-starch polysaccharides (NSP), uronic acids (UA), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose (C), hemicellulose (HC), amino acids (AA), crude protein (CP), tannin, ether extract (EE) and ash (soluble and insoluble) with the following procedures.

Determination of non-starch polysaccharides (NSP) :

(i) Principles :

Firstly polysaccharides present in the sample must be converted to monosaccharides and then to volatile derivatives (Sawardeker *et al.* 1965; Blakeney 1983). The essential steps in the proceedure that separate the various constituent are shown Diagramatically in Figure 3.7.

Figure 3.7: Essential steps in determination of NSP (modified from Englyst *et al.*1982)



Although a number of alternative methods exist the most widely used technique for determining the composition of monosaccharide mixtures, including those resulting from the hydrolysis of polysaccharides, involves the preparation of aditol acetate derivatives of the sugars. This analysis of the sugars in polysaccharides involves three steps, namely hydrolysis, Reduction and acetylation.

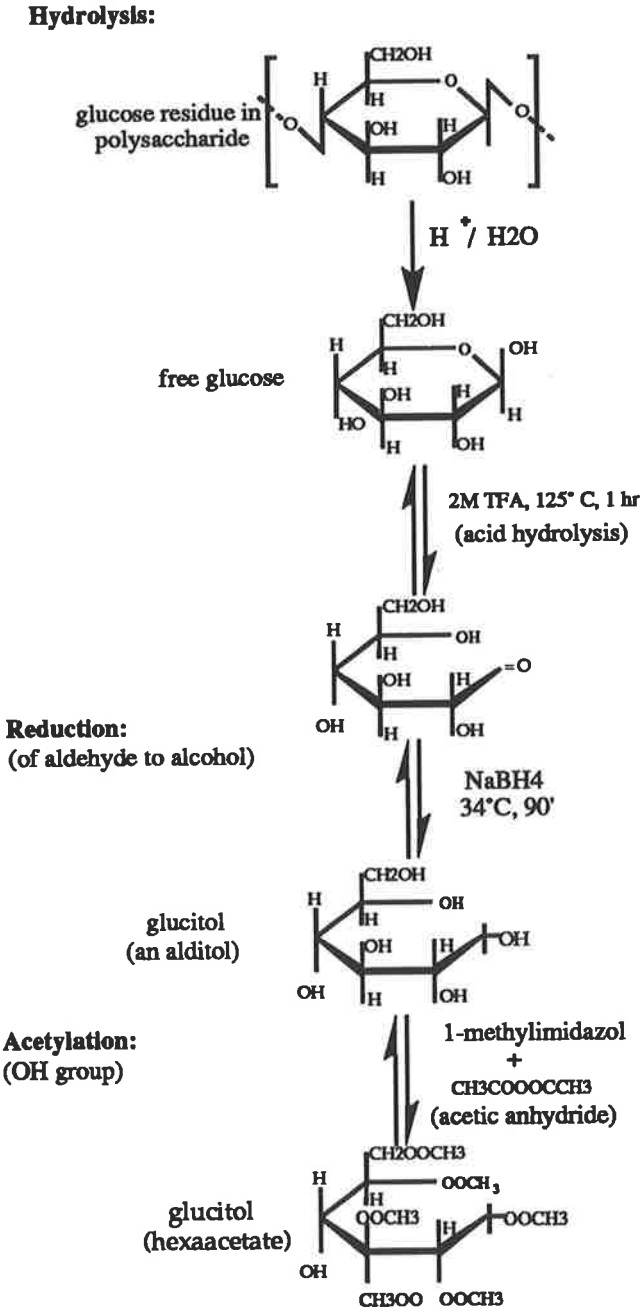
In this experiment **hydrolysis** of polysaccharides present in the samples was done by using 2N trifluoroacetic acid (TFA) for 1 hour at 125° C. The TFA analysis was first described by Albersheim *et al.* (1975). Commenting on this procedure Talmadge et al. (1973) noted that TFA fails to hydrolyse polysaccharides with the glycosidic link of cellulose and also fails to hydrolyse quantitatively the ß- (1-4) -glucosyl linkages present in the backbone of xyloglucan. This procedure also results in some degradation products and thus results in the loss of most of the uronosyle of the cell-wall polymers. On the other hand, the procedure does release intact all of the other neutral sugar residues, including those which are present in the cell-wall polymers in aldobiuronic acid linkages.

In the **reduction** step a monosaccharide is converted to a polyhydroxy alcohol when its aldehyde group (reducing end) is reduced. For the purpose of GC analysis, monosaccharides are usually reduced by $NaBH_4$. Most workers use a large excess of $NaBH_4$ which is decomposed subsequently with glacial acetic acid (Blakeney et al. (1983). It should be borne in mind, however, that an excess amount of acid, as well as that of $NaBH_4$, may interfere with acetylation (Blackeney et al. 1983).

In the **acetylation** step the reaction between polyhydroxy alcohols and acetic anhydride results in the formation of their volatile esters. This acetylation process depends on the reaction conditions. In particular the type of catalyst used. When pyridine is used as a catalyst, acetylation of alditols is usually complete in 20 minutes at 120° C (Selvendran *et al.* 1979), whereas acetylation is complete after 10 minutes at room temperature when using 1-methylimidazole as a catalyst (Blakeney et al. 1983). Since the acetylated sugars are more volatile, extra care may be needed to prevent loss of sugars if samples are to be evaporated to dryness prior to injection into the GC column.

The determination of the sugar composition of food materials and polysaccharides requires quantitative hydrolysis, reduction and acetylation. It should be remembered that this is probably never completely achieved due to the diversity of sugars and linkages present. The manipulation of reduction conditions, particularly the hydrolytic conditions, however, usually gives satisfactory results.

An example of the three-above mentioned reactions are as follow:



The end hexaacetates for galactose, mannose and xylose in above reaction are galactitol, manitol and xylital respectively. These alditols are volatile and resolved and determined by G.L.C.

(ii) Methods (Modified from Englyst et al. 1982):

Sample preparation: Each sample was ground so that it could pass through a 0.5 mm screen and its moisture content was equilibrated by placing it in a desiccator with silicon get for 24 h. A 2 g sub-sample was taken to determine dry matter content for future calculations. Precisely 50 mg of sample were weighed and placed in an 8-ml screw-capped glass tube, 5 ml of 80% ethanol added and the tube heated to 80° C for 10 minutes and then centrifuged at 2000 g for 10 minutes and the supernatant removed by suction. This step was to remove free sugars and to de-activate endogenous enzymes. The sample was then dried at 40° C under N2, 4.75 ml of acetate buffer (pH 5.2) added and then heated at 100° C for 30 minutes. This procedure was to gelatinise the starch so that subsequently-added enzymes could attack it more efficiently. The preparation was cooled to 85° C, and after 0.05 ml of α–amylase (Sigma) was added it was held at 85° C for 1 hr with vigorous mixing in every 20 minutes. It was then cooled to 55° C, 0.2 ml of amyloglucosidase added and incubated at 55° C for 4 hr with shaking. After incubation it was centrifuged at 2000 g for 30 minutes and 1 ml of supernatant taken for soluble NSP and precipitant was used for insoluble NSP measurement (Figure 3.7).

Determination of soluble NSP:

(1) 1 ml of supernatant was transferred into a 8-ml screw-capped vial, 4 ml of absolute ethanol added and the tube centrifuged at 2000 g for 20 minutes. The supernatant was discard and the residue washed with absolute ethanol several times. This step was to remove the sugars released by the α-amylase and amyloglucosidase.

(2) The precipitate was dried under a nitrogen stream and after 1 ml 2M Trifloroacetic acid (made by adding 2 ml TFA to 11 ml H2O) was added.

(3) It was heated at 125° C for 1 hr, with constant stirring to make sure all of the sample was all exposed to the acid.

(4) It was then cooled to room temperature, and 0.2 ml internal standards (inositol, 1mg; allose, 1mg/ml) was added.

(5) Following further mixing it was evaporated to dryness at 45° C under nitrogen it was repeated by adding 0.2 ml H_2O.

(6) The final residue was dissolved in 2.0 ml of water and transferred to a 50-ml tube.

(7) 1 ml of freshly prepared $NaBH_4$ (2 g sodium borohydride in 100 ml DMSO) was added.

(8) Then 0.1 ml glacial acetic acid was added to decompose the excess amount of $NaBH_4$.

(9) 0.2 ml 1-methylimidazole and 2 ml acetic anhydride were added and left 10 minutes at room temperature.

(10) 30 ml H_2 was added to decompose excess amount of acetic anhydride.

(11) When the mixture cooled, 1 ml dichloromethane was added and mixed vigourously.

(12) After the phases had separated the top layer was removed by suction and discarded.

(13) Step 12 was repeated several times and finally the bottom layer was transferred into a 3.5-ml vial and evaporated to dryness at 35° C under nitrogen.

(14) The residue were taken up in about 1.0 ml ethyl acetate and a small amount anhydrous sodium sulphate was added to removed any traces of water that might still be present in the sample.

(15) As much as possible of the ethyl acetatewas removed with a pasture pipette into another 3.5 ml vial and again evaporated to dryness under nitrogen. Then 0.5 ml of ethyl acetate was added and injected into the GLC.

The amount of polysaccharides in each sample was calculated as follows;

Polysaccharide (g/Kg) = [(peak of relevant sugar) / (peak of internal standard i.e. inositol)] x amount of internal standard x total volume of supernatant (5 ml) x 1000/sample weight.

Polymerisation factors: pentoses 0.88, hexoses 0.9 and deoxisugars 0.89.

Insoluble NSP:

(1) The residue from last step of sample preparation (see Figure 3.7) was washed with water (4-5 ml) and centrifuged at 2000 g for 15 minute and the supernatant discarded; this washing was repeated several times to make sure all glucose released from the starch digestion was completely removed. 2 ml of acetone was then added and the mixture vortexed and centrifuged. The supernatant was discard and the residue dried under nitrogen.

(2) 0.5 ml of 12M H2SO4 was added and the sample stirred at 35° C for 1 hr.

(3) 5.5 ml of water was added and the preparation held at 100° C for 2 hr.

(4) Following cooling to room temperature and centrifugation to sediment insoluble materials an aliquot of 0.2 ml was transferred to a 3.5-ml reaction vial and 0.05 ml of 28% NH_3 added, plus 0.2 ml of inositol (1mg/ml) and 0.2 ml of allose (1 mg/ml).

(5) From this an 0.2 ml aliquot was taken, transferred to a 50-ml tube and the sugars reduced and acetylated as described above.

The amount of polysaccharide in each sample was calculated as follows:

Polysaccharide (g/Kg) = {(peak of relevant sugar) / (peak of internal standard)} x amount of internal standard x 1000 x total volume of supernatant (6 ml) / 0.2 ml (aliquot amount for derivation) x sample weight

And the same polymerization factors of soluble NSP were used to calculate.

Uronic acid determination (modified from Blumenkrantz and Asboe-Hansen 1973): Oxidation of sugars at the CH_2OH group, but not at the aldehyde group, yields uronic acids. Uronic acids are the components of the repeating unit of all acid mucoplysaccharides (glycosaminoglycans) with the exception of keratosulphate, in which galactose replaces the uronic acid moiety. Determination of poly uronic acids in the samples followed three steps:

depolymerization, determination by spectrophotometer and calculation from a calibration curve.

Sample was ground through a 0.5 mm screen. 25 mg of ground sample were solubilized in 72% w/w H_2SO_4 and diluted with water to 1M H_2SO_4. This acidic solution was heated for 1 hour at 100° C and then filtered through glass fibre paper. The uronic acid content of the residue on the filter paper was determined as follows:

0.1 ml solution of orthohydroxy diphenyle (OHDP) was diluted with 0.5 ml of water to make 0.6 ml solution. This amount was pipetted into a capped test tube (16 mm x 150 mm) containing the above mentioned residue and was vortex-mixed with a 0.00125 M solution of sodium tetraborate in concentrated H_2SO_4 (3.6 ml). The test tube was cooled in an ice bath for 10 minutes, heated in a boiling water bath for a further 10 minutes. A 0.15% w/v solution of (OHDP) in 0.5% 1N NaOH (60 µl) for 5 minutes. The absorbance of the solution was measured at 476.8 nm against a reagent blank. A lambda 5 UV/ is spectrophotometer with quartz UV cells (10 mm x 45 mm, Vovibond W 1100) was used. A sample blank was also prepared similarly and its absorbance was measured.

Six D-glucuronic acid standards (20, 40, 60, 80, 100 and 200 µg/ml) were prepared and treated by the same procedure described above. Maximum absorbances were measured at 476.8 nm and calibration curves drawn.

Determination of neutral detergent fibre (NDF) and acid detergent fibre (ADF): The NDF, which is the residue after extraction with boiling neutral solutions of sodium lauryl sulphate and ethylenediaminetetraacetic acid (EDTA), consist mainly of lignin, cellulose and hemicellulose and can be regarded as a measure of the plant cell-wall material. The acid-detergent fibre (ADF) is the residue after refluxing with 0.5 M sulphuric and cetyltrimethylammonium bromide and represents essentially the crude lignin and cellulose fraction of plant material but also include silica (Mc Donald *et al* 1988).

(i) Neutral detergent fibre:

(1) 1 g of sample was weighed into a beaker of a refluxing apparatus.

(2) 100 ml of neutral detergent solution (Table 3.2), 2 ml decahydronaphthalene and 0.5 g sodium sulphuric added.

(3) This was all heated to begin boiling (about 10 minutes) and then refluxed for 60 minutes.

(4) The beaker contents were swirled to suspend solids and poured into tarred Gooch crucibles on a filter manifold and vacuum dried.

(5) Samples in the crucibles were rinsed twice with hot-distilled water (90° - 100° C) and twice with acetone.

(6) Crucibles were dried at 100° C overnight and the residue weight was reported as NDF.

(7) The residue in the crucible was then ignited for at least 3 hours at 550° C and final weight reported as ash in the cell wall.

Table 3.2 Composition of neutral detergent fibre solution*

Component	Amount (g)
Sodium lauryl sulphate	30
Di-sodium ethylene-diaminetetraacetate (EDTA) (dehydrate crystal, reagent grade)	18.61
Sodium borate decahydrate (reagent grade)	6.81
Di-sodium hydrogen phosphate (anhydrous reagent)	4.56
2-ethoxyethanol (Ethylene glycol monoethyl ether) (purified grade)	10 ml
Distilled water	1000 ml

* The EDTA and $Na_2B_4O_7.10\ H_2O$ were put in a large beaker, some of the distilled water was added, and heated until dissolved; This was then added to solution (in another beaker) containing sodium lauryl sulphate and 2-ethoxyethanol (ethylene glycol monoethyl ether). Na_2HPO_4 was put in another beaker, some of the distilled water was added, heated until dissolved and then added to the solution containing the other ingredients. The final pH of the solution was checked to be in the range 6.9 to 7.1.

(ii) Acid detergent fibre: This procedure was essentially the same as that for NDF except that an acid solution of detergent was used (Table 3.3)

Table 3.3: Composition of acid detergent solution*

Component	Amount (Per litre)
Sulphuric acid, reagent grade, standardised to 1 N	49.04 g
Cetyl trimethylammonium bromide (CTAB), technical grade	20 g
Distilled water	1000 ml

* The sulphuric acid was weighed and made up to 1 litter with distilled water at 20° C; then CTAB was added and stirred.

Acid detergent lignin: In the acid detergent lignin procedure the initial ADF procedure is used as a preparatory step. The detergent helps remove the protein and other acid-soluble material that would interfere with the lignin determination. The ADF residue consist of cellulose, lignin, cutin and acid-insoluble ash (mainly silica). Treatment with 72% H_2SO_4 dissolves the cellulose. Ashing of the residue will determine the crude (organic) lignin fraction (AOAC 1980).

Triplicate samples of about 1 g of oven dried ADF in sintered glass crucibles (previously tared) were covered with cooled (15° C) 72% H_2SO_4 and stirred with a glass rod to a smooth paste. The crucibles were then filled about half full with more 72% H_2SO_4 and stirred at hourly intervals as acid slowly drained away. They were so treated for 3 hours at lab temperature (20° - 23° C). After this time as much acid as possible was drawn off under vacuum and then the contents were washed with hot water until free of acid. Crucibles were dried at 100° C, weighed, ignited in a muffle furnace at 500° C for minimum 3 hours, cooled and weighed. Acid-detergent lignin was calculated as follow:

ADL = Loss upon ignition / Oven-dry sample weight x 100

Cellulose and hemicellulose were calculated using the values obtained for NDF, ADF and ADL as follows:

Cellulose = ADF - ADL

Hemicellulose = NDF - ADF

Amino acids: The amino acids were determined at the South Australia Research and Development Institute (SARDI) following the successive stages of pre-oxidation of the sample, hydrolysis and separation of amino acids by chromatography. All samples prepared well and all internal standards fell within normal limits (± 0.025 of the batch mean). Figure 3.7 shows some examples of the amino acid peaks obtained.

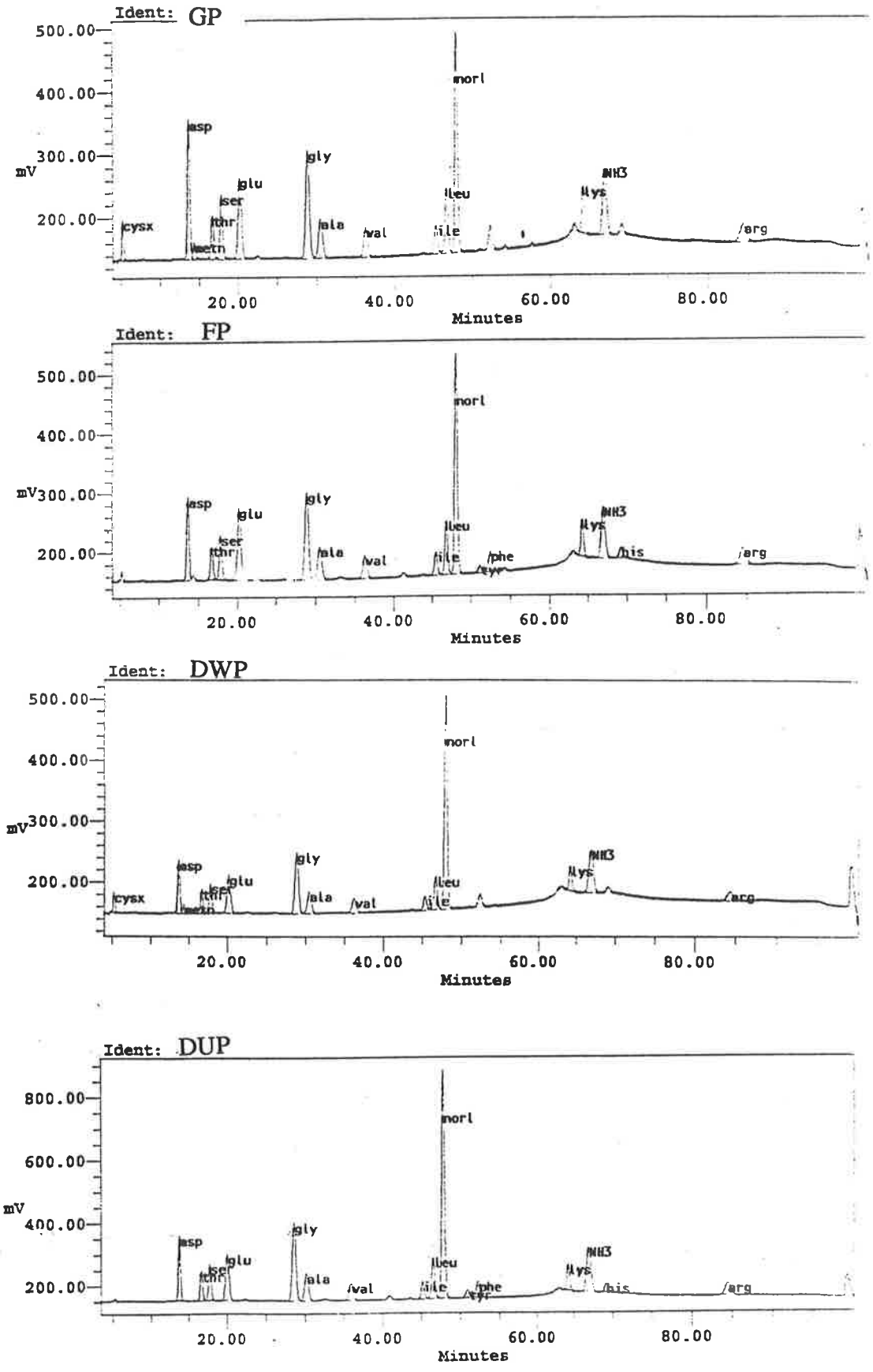
Tannin: The tannin content of the samples was determined using the vanillin/HCl method of Burns (1963), by Dr. H.S. Saini, Wollongbar Agricultural Institute, New South Wales. I take this opportunity to thank him for this.

Minerals: Approximately 1g of sample was digested in 10 mls of nitric acid (Zarcinas et al. 1982) initially at 120°C and then at 140°C until 1 ml of solution remained. This sample was diluted to 20 ml and analysed using an Inductively-Coupled Plasma Spectrometer (ICP) at the Department of Soil Science, the University of Adelaide.

Crude protein, ether extract and total ash content of samples were determined by procedures already described in section 3.1.2.

Statistical analysis: Data were analysed statistically by methods already mentioned in section 3.1.2.

Figure 3.8: Amino acids peak seperation of four collection of *Posidonia australis*



3.2.3 Results

Non-starch polysaccharides (NSP): The contents of soluble, insoluble and total NSP of the four samples of *P. australis* are shown in Table 3.4 and Figures 3.9, 3.10 and 3.11 respectively. In all forms of seagrass collected glucose, galactose and mannose were dominant sugars in the soluble fraction of NSP (more than 1% of dry matter), ribose and rhamnose were present in the least amounts.

Insoluble constituents of NSP were dramatically greater than those soluble NSP. Among these glucose and rhamnose revealed most and least values respectively. All insoluble NSP constituent of four different samples were significantly different each other ($P< 0.01$) (Table 3.4).

The data in Figure 3.9 show that total soluble NSP of GP and FP were higher than that of DWP and DUP ($P< 0.01$). This value for all samples was less than 6% of dry matter content.

Figure 3.9 shows the insoluble NSP content of the samples. Overall insoluble NSP in samples was high, when compared with soluble NSP (> 20% vs < 6%). Among the different samples dry washed *Posidonia* (DUP) showed significantly less insoluble NSP than the other forms ($P< 0.01$). Total NSP (soluble + insoluble) is shown in Figure 3.10. Values for GP, FP, DWP and DUP were 28.8, 28.7, 26.9 and 24.4% of dry matter respectively.

Uronic acids: Figure 3.12 shows the uronic acid content of the four different samples. The amount of uronic acid in GP, FP, DWP and DUP was 17.2, 17.7, 18.4 and 18.6% respectively. Although both dry forms contained more uronic acid than did the green and fresh forms in overall there were no significant differences among different samples in this regard.

Table 3.4: NSP constituents of four collections of *Posidonia australis* by Englyst method (1982) (Data show mean and SE)

Constituent	Different colections *Posidonia**				
	GP	GF	DWP	DUP	LSD** (P<0.01)
Soluble:					
Xylose	0.49 ± 0.01	0.53 ± 0.02	0.80 ± 0.00	0.63 ± 0.01	0.05
Mannose	1.39 ± 0.01	1.71 ± 0.01	1.39 ± 0.05	1.99 ± 0.01	0.1
Galactose	1.01 ± 0.03	1.05 ± 0.09	0.15 ± 0.00	0.09 ± 0.02	0.2
Glucose	1.42 ± 0.19	1.14 ± 0.01	1.71 ± 0.01	1.33 ± 0.01	0.4
Rhamnose	0.08 ± 0.00	0.32 ± 0.02	0.15 ± 0.01	0.12 ± 0.01	0.5
Fucose	0.12 ± 0.01	0.14 ± 0.00	0.18 ± 0.02	0.14 ± 0.01	0.04
Ribose	0.03 ± 0.00	0.11 ± 0.07	0.24 ± 0.19	0.02 ± 0.00	0.4
Arabinose	0.17 ± 0.00	0.17 ± 0.01	0.27 ± 0.02	0.19 ± 0.01	0.05
Insoluble:					
Xylose	5.75 ± 0.05	5.94 ± 0.09	5.31 ± 0.08	4.13 ± 0.01	0.49
Mannose	0.52 ± 0.03	0.55 ± 0.08	0.42 ± 0.06	0.71 ± 0.01	0.23
Galactose	0.71 ± 0.04	1.09 ± 0.19	0.39 ± 0.07	0.67 ± 0.03	0.46
Glucose	15.70 ± 1.54	15.06 ± 0.10	14.03 ± 0.66	12.20 ± 0.06	2.6
Rhamnose	0.22 ± 0.01	0.20 ± 0.01	0.14 ± 0.00	0.16 ± 0.00	0.3
Fucose	0.36 ± 0.01	0.30 ± 0.00	0.33 ± 0.01	0.30 ± 0.01	0.4
Ribose	0.33 ± 0.01	0.32 ± 0.00	0.26 ± 0.01	0.25 ± 0.00	0.3
Arabinose	0.47 ± 0.01	0.37 ± 0.01	0.30 ± 0.01	0.50 ± 0.01	0.4

*: See section 3.2.2 for description of forms collected
**: Least significant difference

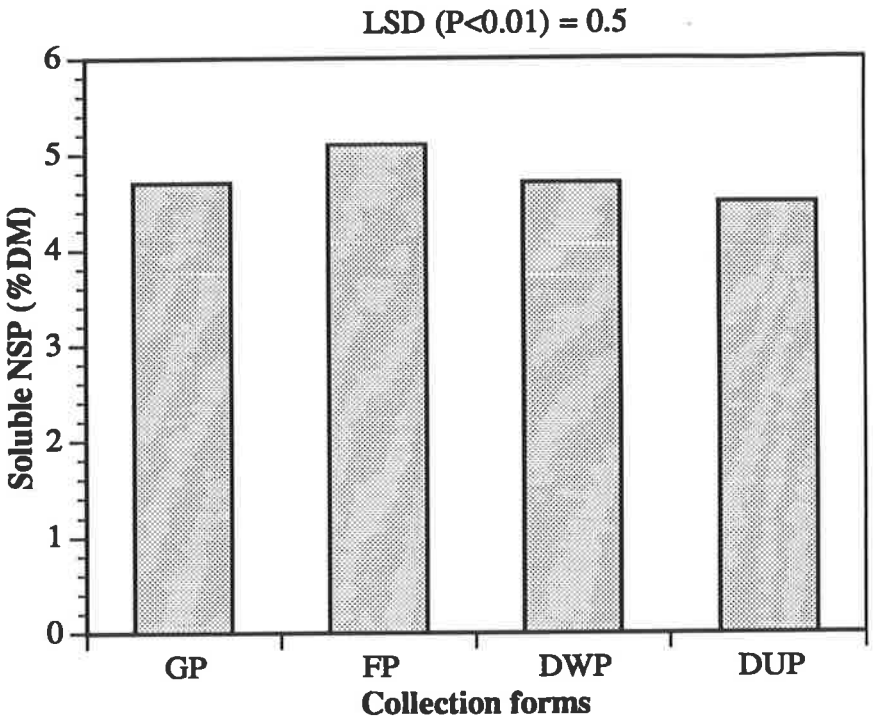


Figure 3.9: Soluble NSP in different collection forms seagrass

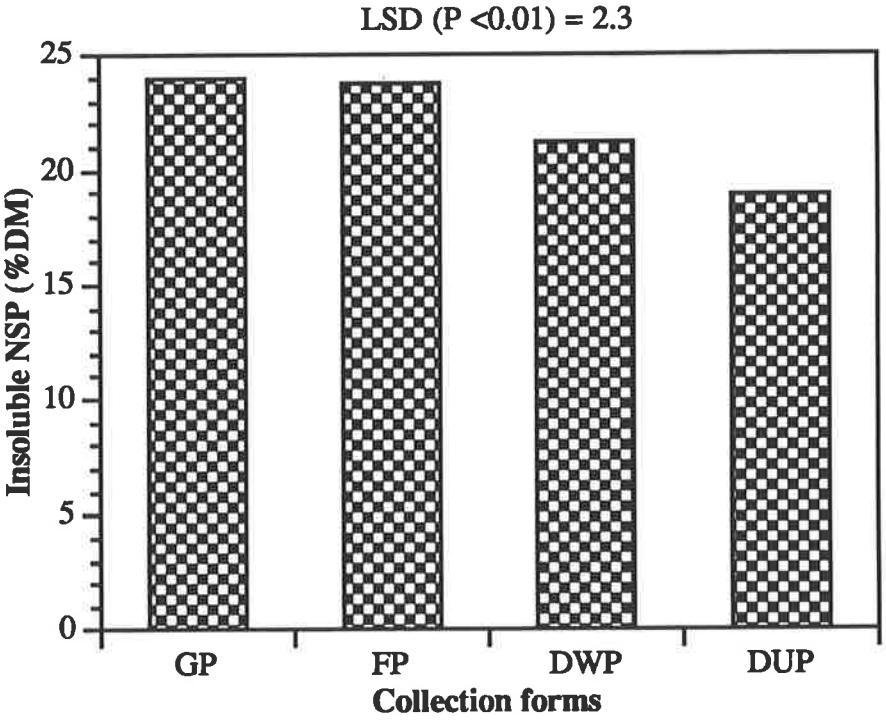


Figure 3.10: Insoluble NSP in different collection forms of seagrass

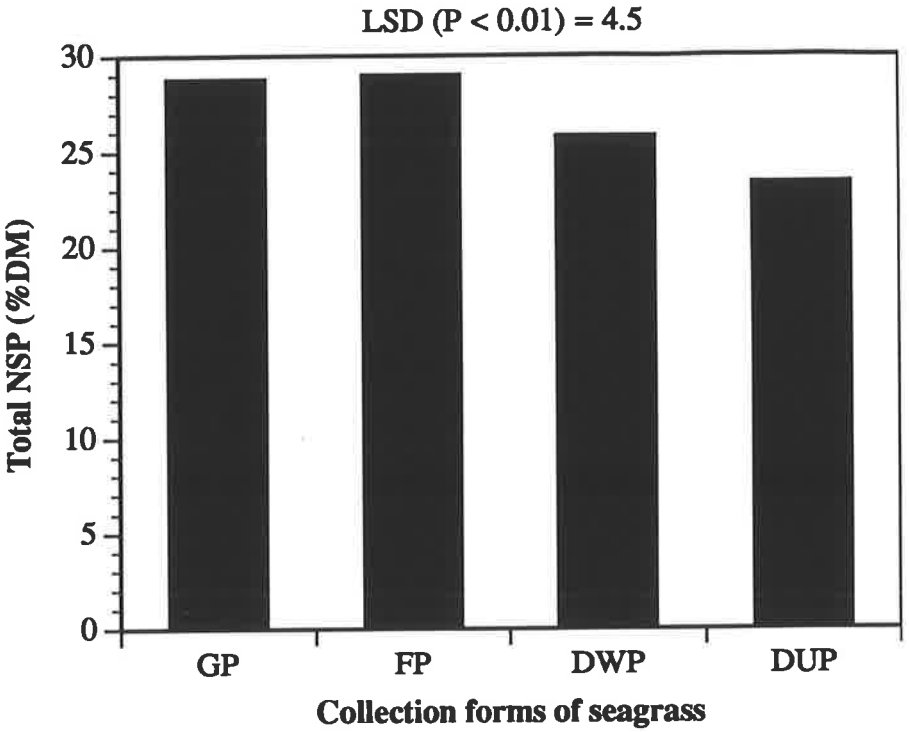


Figure 3.11: Total NSP in different collection forms of seagrass

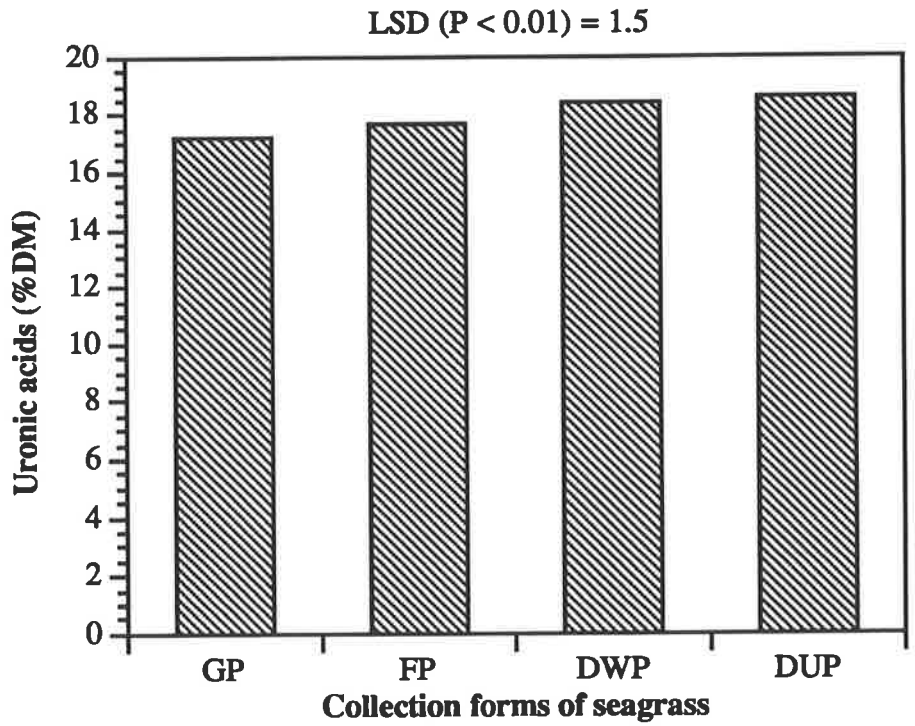


Figure 3.12: Uronic acid in four different collection form of seagrass

Cell-wall contents (Van Soest method): Table 3.5 shows the cell-wall contents of the experimental samples. The NDF content of green, fresh, dry-washed and dry unwashed seagrass was 46.8, 46.5, 47.3 and 45.2% of dry matter, respectively. The value of NDF in dry-unwashed *Posidonia* was less than in the other samples, but there were no significant differences between the other samples. The cellulose, hemicellulose and lignin contents of the samples varied from 19 to 20.9%, 11.2 to 11.7 and 4.5 to 15.4%, respectively. Generally it is evident that the major constituents of *Posidonia* cell wall are cellulose and lignin.

Table 3.5: Cell-wall constituents of four collection forms of *P. australis* (%DM)

Sample	Constituent				
	NDF	ADF	ADL	Cellulose	Hemicelluloe
GP	46.8	35.1	14.9	20.2	11.7
FP	46.5	35.3	15.4	19.9	11.2
DWP	47.3	35.9	15.1	20.9	11.4
DUP	45.2	33.5	14.5	19.0	11.7
LSD(P<0.01)	1.0	0.4	1.6	2.4	1.1

Amino acids: Table 3.6 shows the amino acids constituents (protein) and crude protein content of samples. Glutamic acid, aspartic acid, leucine, serine, valine and arginine are respectively the six dominant amino acids in the experimental samples while histidine, methionine, tyrosine and cystein were in lowest amount respectively. The crude protein content of samples (GP, FP, DWP and DUP) was 6.1, 5.4, 4.8, 5.6% of dry matter respectively.

Tannin: The tannin content of samples (GP, FP, DWP and DUP) was 1.74, 1.74, 1.85 and 1.82% of DM respectively (Table 3.7).

Ash and Minerals: The ash content of samples is shown in table 3.8. Some 30% of total ash content is insoluble. Although the total ash content of dry-unwashed *Posidonia* (DUP) is in highest amount (20% of DM) its insoluble ash content is only approximately equal to that of the other collection forms.

Table 3.6: Amino acid constituents (true protein) and crude protein content of four collections of *Posidonia australis* (on DM basis)

Constituent		GP	FP	DWP	DUP
alanine	(g/Kg)	3.06 ± 0.30	2.93 ± 0.19	2.31 ± 0.14	2.50 ± 0.01
arginine		3.13 ± 0.13	2.76 ± 0.43	2.47 ± 0.09	2.25 ± 0.07
aspartic acid		7.08 ± 2.06	5.72 ± 0.39	4.16 ± 0.20	4.49 ± 0.18
cystein		1.62 ± 0.00	1.16 ± 0.00	0.98 ± 0.00	1.23 ± 0.00
glutamic acid		7.43 ± 1.02	7.01 ± 1.36	4.86 ± 0.27	5.08 ± 0.16
glycine		3.70 ± 0.29	3.46 ± 0.19	2.78 ± 0.16	3.03± 0.04
histidine		0.82 ± 0.00	0.84 ± 0.00	0.64 ± 0.00	0.65 ± 0.00
isoleucine		2.84 ± 0.33	2.65 ± 0.25	2.00 ± 0.00	1.95 ± 0.07
leucine		4.06 ± 0.33	3.65 ± 0.31	2.80 ± 0.06	2.90 ± 0.10
lysine		2.50 ± 0.49	2.38 ± 0.33	1.80 ± 0.09	1.88 ± 0.09
methionine		1.02 ± 0.00	0.78 ± 0.00	0.72 ± 0.00	0.66 ± 0.00
phenylalanine		2.38 ± 0.00	2.49 ± 0.00	2.00 ± 0.00	2.06 ± 0.00
proline		2.83 ± 0.30	2.55 ± 0.19	2.05 ± 0.12	2.08 ± 0.06
serine		3.50 ± 0.32	2.77 ± 0.16	2.10 ± 0.06	2.32 ± 0.04
threonine		2.79 ± 0.23	2.39 ± 0.17	2.05 ± 0.06	2.16 ± 0.04
tyrosine		1.03 ± 0.00	0.89 ± 0.00	0.88 ± 0.00	0.99 ± 0.00
valine		3.38 ± 0.55	4.69 ± 1.78	2.49 ± 0.06	2.49 ± 0.02
Total	(%)	5.3 ± 0.63	4.9 ± 0.57	3.7 ± 0.14	3.9 ± 0.07
Crude protein	(%)	6.1	5.4	4.8	5.6

Table 3.7: Tannin content of four collection of *Posidonia australis* (%DM)

GP	FP	DWP	DUP
1.74	1.74	1.85	1.82

Table 3.8 shows some important element of dry-unwashed *Posidonia* (DUP). The quantity of Ca, P and Na as three important elements in animal nutrition are 4.1, 0.06 and % of DM respectively. Among the element, Ca, Na and Mg (4.1, 2.6, 1.3% DM) , respectively are in highest quality; while Cu, Ma and Ni were in lowst quantity.

Ether extract: The ether extract of the four collection forms of *Posidonia* (GP, FP, DWP and DUP) was 1.20, 1.22, 1.17 and 1.10% of dry matter, respectively.

Table 3.8: Ash content of four collection forms of *Posidonia australis* (%DM)

Constituents	GP	FP	DWP	DUP
Soluble ash	9.8	9.4	10.2	14.6
Insoluble ash	5.5	5.7	5.4	5.4
Total	15.3	15.1	15.6	20.0

Table 3.9: Mineral constituents of dry-unwashed *Posidonia australis* (DUP) (ppm)

Fe	Mn	B	Cu	Mo	Co	Ni	Zn	Ca	Mg	Na	K	P	S	Al
662	36	3512	1.2	1.4	0.24	4.2	10	41420	12905	25637	2763	585	5343	575

In summary some of the important chemical constituents of the experimental samples are compared in Figure 3.13.

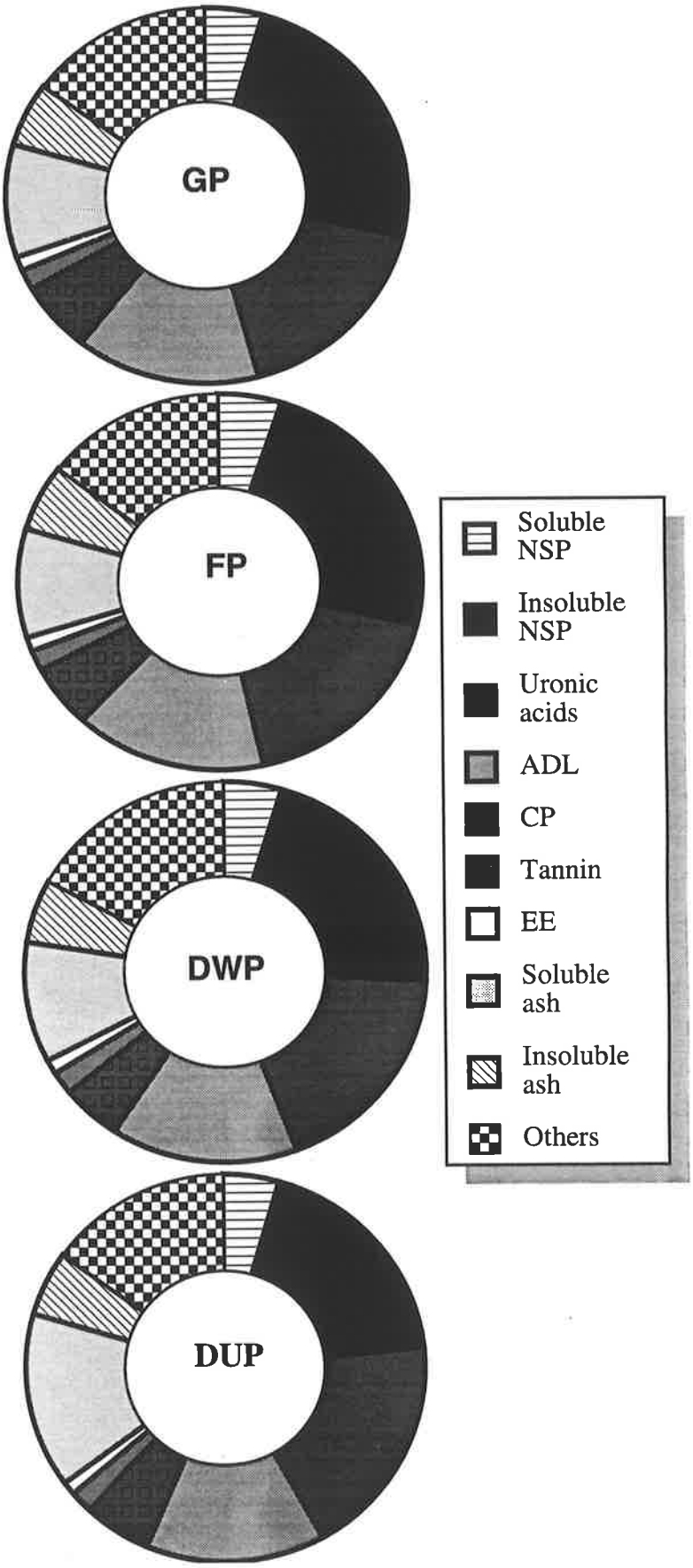


Figure 3.13: Chemical composition of four different physical forms of *Posidonia* (% Dry matter)

3.2.4: Discussion

Leading on from the first experiment in this chapter the purpose of this second experiment was two fold:

(i) to estimate and compare the chemical composition and cell-wall constituents of four different physical forms of seagrass *P. australis* available on South Australia beaches.

(ii) to screen the best physical form of this plant in terms of its valuable nutrients and potential commercial importance so that the selected form might be used for further study of its possible value in sheep nutrition.

Various factors influence the quality of animal feed but undoubtedly crude fibre is one of the most important (Van Soest 1981). Several methods are available for determination of dietary fibre. Defining dietary fibre solely as NSP, as proposed by Englyst et al. (1982), gives the best index of the plant cell-wall polysaccharides which is chemically precise and in keeping with the original concept of dietary fibre. The method presented in this chapter however measured dietary fibre as the sum of monosaccharides released by hydrolysis of NSP. This not only gives separate values for total, soluble and insoluble dietary fibre, but also characterises the various types of fibre by giving values of cellulose and the constituent sugars of the non-cellulosic polysaccharides (Table 3.4). In general the soluble-NSP of all four samples were the same, ranging from 4.5 - 4.7% of dry matter. This concentration of soluble NSP is roughly similar to the results reported by Pirc (1989). Insoluble-NSP of two samples of GP and FP were slightly different from DWP and DUP but this is probably due to the higher content of soluble ash in DWP and DUP, which in turn affects the proportion of insoluble-NSP. All samples contained high amounts of uronic acids.

From NDF determination as representative of the fibre content of plants, lignin, cellulose and hemicellulose were similar in all samples. The lignin content of the cell wall ranged from 14.5 in DUP to 15.4 in FP. This amount of lignin in seagrass seems to be very high when compared with traditional lignocellulosic feedstuffs. The high proportion of fibre, especially lignin, in seagrass samples is in agreement with Bjorndal (1990).

This experiment also identified 17 amino acids contained in the four collected samples. More detailed analysis would certainly added to this list. The amino acids analyses show differences with the results obtained by other researchers e.g. Augier *et al.* (1982), but these variations could be due to many factors, such the place and depth where the *Posidonia* was collected, degree of development of the plants, seasonal variations and so on.

The crude protein and total ash content of the experimental samples are generally in agreement with results presented by Klumpp and Van der Valk (1984) and Klumpp and Nichols (1983) respectively. Insoluble ash content of samples varied from 5.4 to 5.7% of dry matter, which is proportionally high in comparison with other plants.

Tannin is one important constituent of the experimental samples. The amounts detected are high in comparison with other grasses, so the associated effect of tannin on protein/carbohydrate digestion could be important. In this experiment the tannin content of different collection forms of *Posidonia* was the same. This result is in contrast with the statement of O'Donovan (1992) that stated level of tannin is higher in plant growing in the sun relative to shade. The explanation of this contrast might be that of the limitations associated with total tannin measurements. Although the same total results for different collection forms were obtained could be due to possible oxidation reactions different form of tannins in *Posidonia* collected on the beaches.

In summary the substantially variation of nutrients between species of marine plants and also within any one species could be the result of their different original location, time of year and lifecycle form (age). All four different form of *Posidonia australis* can be characterised as high in fibre, including both cellulose and lignin, and poor in protein. They can thus be placed in the general category of lignocellulosic feedstuffs, most of which are poor in protein as well.

Generally the results from this experiment show that there are no considerably differences between the four different collected physical forms of seagrass in terms of their most important chemical constituents; this result lead the project to select the dry-unwashed

seagrass, which is readily available in large quantities and easily harvested as possible important from a commercial point of view.

CHAPTER 4

Nutritive evaluation *in vivo* of both fresh and partially decomposed *Posidonia australis*, both before and after their use as litter for broiler chicken

(Author)

"Voluntary intake as the amount of feed that an animal consumes in a given time and digestibility are two important aspects of feed evaluation." (Garnsworthy and Cole 1990).

CHAPTER 4

Nutritive evaluation *in vivo* of both fresh and partially decomposed *Posidonia australis*, both before and after their use as litter for broiler chickens

4.1 General introduction

On the results of section 3.1, *Posidonia* was screened from amongst some available aquatic plants in South Australia as a possible ruminant feed source. Four physical forms of *P. australis* were then compared in a series of laboratory analyses (Section 3.2). Finally, as a result of the studies presented in chapter 3 *Posidonia* in the dry form, as massed on the beach at Kingston, South Australia, beaches was selected for further nutritional study.

The experiments of this current chapter were based on two main objectives:

(i) Determination of voluntary intake and digestibility *in vivo* of the selected *Posidonia* (experiment 1)

(ii) Studies on the possibility of improving the nutritive value of *P. australis* by physical, biological and supplementation methods including a) decomposition, b) using both fresh and decomposed as a chicken litter and c) supplementation with molasses (Experiments 2, 3, 4 and 5).

4.2 Materials and methods

Feeds: The major experimental diets in the different experiments were:

Experiment 3) *Posidonia* (P), was collected on the beach up to 100 m from the water's edge, at Kingston, South Australia (see section 3.2.2-iv). It had been exposed to the weather for a long time (Plates 3.3, bottom and 4.1). Its particle length was about 3-5 cm.

Experiment 4) Partly-decomposed *Posidonia* (DP) was collected from Kingston beach 150 m back from the water's edge, opposite where the more recently deposited *Posidonia*

(P) had been collected. It had been removed from the beach anywhere from two to ten years earlier. Particle size was between 2 to 10 mm in length.

Experiment 5) *Posidonia*-litter (PL). *Posidonia* as described above (Experiment 1) was used as a bedding material for broiler chickens. 3500 day old broiler chickens were housed on this material in a closed house (approx. 40 x 8 m) situated at Maclaren Flat, South Australia. The depth of bedding was approximately 5 cm and the chickens were raised on this for 6 weeks. General views of the chicken farm (outside and inside) are shown in Plate 4.1. When the chicken house was emptied the litter, a mixture of the original bedding material and chicken manure, was delivered to the Waite Institute. Throughout this section this material is referred to as *Possidonia* litter (PL).

Experiment 6) Decomposed *Posidonia* litter (DPL). Partially decomposed *Posidonia* (Expt. 2) was used as bedding material for broiler chicken (as described above) and at the end of rearing period (6 weeks) the litter (DPAL) was delivered to the Waite Institute.

Chemical composition of the above experimental feeds is shown in Table 4.1.

Table 4.1: Chemical composition of major experimental feed (experiments 1, 2, 3, and 4)

Relevant experiment	Feed	Ash	CP	CF	EE	NFE
		% dry matter				
1	*Posidonia* (P)	20.5	5.6	34.0	1.2	38.7
2	Decomposed *Posidonia* (DP)	40.5	5.2	8.2	1.0	45.1
3	*Posidonia* litter (PL)	16.9	18.0	19.5	2.5	43.1
4	Decomposed *Posidonia* litter (DPL)	36.4	16.3	11.9	2.0	33.4

The control experimental feed for the above experiments was lucerne, *Medicago sativa* (see section 3.1.2) that was mixed in different proportions (0, 25, 50, 75 and 100%) with each of above major experimental diets in each experiment.

Experiment 7) Molasses-treated *Posidonia* (PM). *Posidonia* (P), as described above, was sprayed with sugar cane molasses/water mixture (1:3) at rates of 0, 5, 10, 15 and 20% (W/W). These diets were prepared daily on the afternoon of the day before feeding.

Experimental design and time table: All five experiments were conducted in the Department of Animal Science, Waite Institute animal house (Plate 4.2). Twenty Merino wethers for each experiment, averaging respectively 67.5, 65.0, 64.8, 63.6 and 60.6 Kg body weight and about three years old, were selected from the same flock. The animals, which had previously been dewormed and treated for enterotoxaemia, were allocated randomly to the experimental treatments. Each experiment was carried out based on a Completely Randomised Block Design (CRBD), consisting of five diet treatments and four replicate sheep for each treatment. Diet treatments for each experimet consisted of a mixture of the relative feed and 0, 25, 50, 75 and 100% lucerne (except experiment 5 in which, 0, 5, 10, 15 and 20% molasses were mixed with the posidonia of experiment 1.

The modified method of Moore (1969) was applied to determine voluntary intake and digestibility *in vivo*. The sheep were kept in individual pens throughout the experiment. Water was available at all times. Each experiment was conducted using three periods, as follows:

(a) Adaptation period: For the sheep to adapt to pens, indoor conditions and to the experimental diets.

(b) Preliminary period: Test diets were fed *ad libitum* in order to measure voluntary intake and to ensure that undigested residues of previously-consumed feedstuffs had been eliminated from the digestive tract (Church 1988). To avoid selection of more platable feed by sheep in mixed diets, feed were properly mixed five times during the day.

(c) Collection period: Total feed intake and faecal output for each sheep was measured to determine the digestibility *in vivo* of the experimental diets.

All animals were fed once daily at 0900 hr, just after collection of feed residues from the previous day's feeding.

The experimental time table that was followed is detailed below.

(a) Adaptation period (7 days)

Day 1. All sheep were weighed perior to feeding, placed in individual pens and given a diet of 90% chaffed oaten hay and 10% commercial pellets at the level of 2.5% of body weight.

Days 2-7. Sheep were allocated to their dietary treatments. Experimental feeds were gradually introduced into the diet. On day 5 all sheep were fitted with harnesses for faecal collection.

(b) Preliminary period (13 days)

Days 8-20. Voluntary intake was measured during this period, including daily adjustment of the quantity of feed offered to each sheep in order to provide between 100 and 200 g residue per day. Residues were collected and weighed every day. If a sheep left less than 100 g on any given day, the amount offered was increased by 100 g, but if no residues were left, the amount offered was increased 200 g over the amount offered the day before. If more than 400 g was left, the amount offered was reduced by 200 g and if the amount of residue was between 200 and 400 g, the offering was reduced by 100 g each day. However, the minimum amount offered (100% *posidon*ia or other major feed) was not less than 400 g/d. Residues (refused diets) were never refed. The above-mentioned procedure was followed throughout the period.

On day 16 all harnesses on the sheep were fitted with faecal-collection bags.

Every day, duplicate samples of approximately 100 g were taken from each of the diets and residues (if any), then dried at 100°C overnight to constant weight for determination of dry matter content.

(c) Collection period (12 days)

Day 21. All sheep were weighed and fed the average amount of diet eaten during the previous period. Feeds were sampled (200 g samples) and stored at room temperature.

Day 22. Sheep were fed experimental diets at the same level as day 21. Faecal-collection bags were changed daily at 0830 hr, after removing the feed residues.

Faeces were weight and sampled for dry matter determination. Every day duplicate samples of fresh faeces were taken and dried at 100°C for 24 hours for determination of dry matter. Samples were kept for measurement of organic matter.

Day 30. This was the final day for measurement of dietary intake.

Day 31. This was the final day for feed residue collection. Faecal collection and sampling was done as for previous days.

Day 32. This was last day for faecal collection and sampling. All sheep were weighed and released to the paddock.

Dry matter digestibility (DMD) and organic matter digestibility (OMD) of the diets were calculated as follows:

$$DMD = \frac{\text{Feed eaten - Faecal wt}}{\text{Feed eaten}} \times 100$$

$$OMD = \frac{\text{Organic matter eaten - Organic matter voided}}{\text{Organic matter eaten}} \times 100$$

DE and ME were calculated as already mentioned in section 3.1.3.

The dry matter digestibility of *Posidonia*, decomposed *Posidonia, Posidonia litter* and decomposed *Posidonia litter* (experiments 1, 2, 3, and 4 respectively) in the mixed diets was calculated using the equation described by Crampton and Harris (1969) as follows:

$$S = \frac{100\,(T - B)}{s} + B$$

Where: S = Digestibility of e.g. *Posidonia* in mixed diet

T = Digestibility of mixed diet

B = Digestibility of lucerne

s= Proportion of e.g. *Posidonia* used in mixture diet

Sample preparation for chemical analysis

All samples were prepared for analysis essentially as already outlined in section 3 (Figure 3.1)

Analytical techniques

Feed sub-samples were analysed for dry matter, organic matter, crude protein, crude fibre, ether extract. Faeces sub-samples were analysed for dry matter and organic matter. The methods which already were mentioned in section 3.1.2 were employed to measure above constituents of feed and faeces samples.

Plate 4.1: Close-up view of experimental *Posidonia australis*

Plate 4.2: General view of the sheep with faecal-collection harnesses used in the digestion experiments *in vivo* .

Plate 4.3: General views of experimental chicken farm, outside (top) and inside (bottom)

4.3 Experiment 3: Voluntary intake and digestibility *in vivo* of fresh *Posidonia Australis* (P)

4.3.1 Introduction

The potential value of a feed for supplying a particular nutrient can be determined by chemical analysis, but the actual value of the feed to an animal can be arrived at only after making sure how much is voluntarily eaten by the animal and after making allowances for the inevitable losses that occur during digestion, absorption and metabolism. Two important taxes imposed on a feed are the amount of intake and that represented by the part of it which is not absorbed and is excreted in the faeces.

The prediction of feed intake, in particular of fibrous roughage, is one important aspect of ruminant nutrition. Perhaps the most important factor controlling animal production and the utilisation of roughages by ruminants is the voluntary intake of the material by the animals concerned (Hovell *et al.* 1986; Minson 1987; Garnsworthy and Cole 1990). Also, as Ketelaars and Tolkamp (1992) mentioned, that amount of feed which ruminants voluntarily consume is the first step in the process of converson of feed into valuable products, so that knowledge of feed values is often of little interest without knowledge of how much the animals will consume.

The digestibility of livestock feeds is another useful measure of nutritional value (Corbett 1969). Animals are not able to obtain all of the potential nutrients from the food they eat so that possibly the most important and also most variable difference between feeds is in how completely they are digested.

This section is a study on these two important aspects of nutritive value (voluntary intake and digestibility) of *Posidonia* as massed in dry form at Kingston, South Australia.

4.3.2 Results

Chemical composition: Table 4.2 shows the chemical composition of pure *Posidonia* (P) and its mixture with different proportions of lucerne. It is evident that there was a wide

variation in both ash and crude protein contents of the diets but no marked differences in crude fibre, ether extract nor nitrogen-free extract.

Table 4.2: Chemical composition of *Posidonia* (P) mixed with different proportions of lucerne (Luc)

Diet PA : Luc	Ash (%)	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Nitrogen free extract (%)
100 : 0	20.5	5.6	34.0	1.2	38.7
75 : 25	17.5	8.8	32.9	1.4	39.4
50 : 50	14.4	12.0	31.8	1.6	40.2
25 : 75	11.3	15.1	30.6	1.9	41.1
0 : 100	8.2	18.3	29.5	2.1	41.9

As expected the crude protein content of the experimental diets increased with increasing proportion of lucerne (from 5.6 to 18.3%) and the ash content decreased correspondingly (from 20.5 to 8.2%). The crude fibre, ether extract and nitrogen-free extract values ranged from 34.0 to 29.5%, 1.2 to 2.1% and 38.7 to 41.9% respectively in diets with 0 to 100% lucerne.

Voluntary intake: Mean voluntary intakes of dry matter, organic matter and the different nutrients are presented in Tables 4.3 and 4.4.

There were clearly significant differences between the intakes of the various diets. As expected voluntary intake increased with increasing proportion of lucerne. As a measure of feed intake used for eliminating the effects of differences in body size results have often been reported in terms of grams of dry matter eaten per unit metabolic weight, where the 0.75 power of body weight is regarded as metabolic weight (Minson 1980). On this basis (g/Kg $W^{0.75}$/d) intake increased from 8 g for 100% *Posidonia* to 56.7 g for 100% lucerne. Statistical analysis for the data expressed in this manner also showed significant difference in the dry matter intake of straight *Posidonia* diet and the mixed diets. The mean organic matter

consumption followed the same pattern, i.e. lowest at 100% *Posidonia* and increasing, with increasing proportions of lucerne (Table 4.3).

Table 4.3: Voluntary dry-matter and organic-matter intakes (Mean ± SE) of *Posidonia* (P) mixed with different proportion of lucerne (Luc) and sheep body weight changes (Mean).

Diet	Dry matter		Organic matter		Bodyweight changes
P : Luc	(g/sheep/d)	(g/KgW0.75/d)	(g/sheep/d)	(g/KgW$^{0.75}$/d)	(g/sheep/d)
100 : 0	165 ± 10.9	8.0 ± 0.3	131 ± 9	6.4 ± 0.24	-177
75 : 25	593 ± 29.0	26.8 ± 0.5	490 ± 24	22.1 ± 0.4	-77
50 : 50	678 ± 10.4	29.8 ± 0.6	581 ± 9	25.5 ±0.50	+23
25 : 75	815 ± 42.8	36.6 ± 1.4	723 ± 38	32.5 ± 1.20	+162
0 : 100	1310 ± 26.5	56.7 ± 1.4	1202 ± 24	52.0 ± 1.31	+392
Significance	**	**	**	**	
LSD (5%)	77	3	67	2.7	

** = P <0.01

The calculated dry-matter voluntary intake of just *Posidonia* is shown in figurer 4.1. It is evident that the highest intake occurred when the diet included 75% *Posidonia* and 25% lucerne.

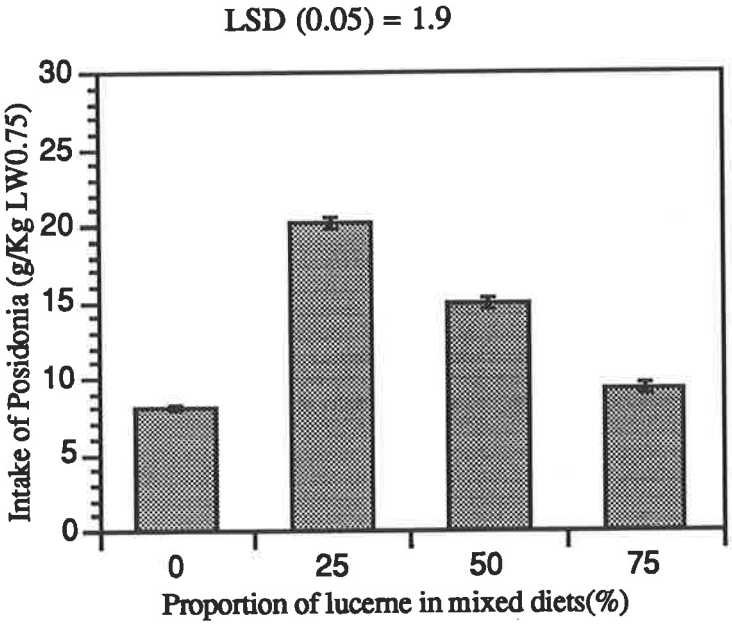


Figure 4.1: Voluntary dry-matter intake of *Posidonia* in mixed diets

The average index of organic matter intake (Kg DM/100 Kg body weight) was highest with sheep fed 100% lucerne. For organic matter intake of mixed diets however, as for dry matter intake, the measured means were lower than the recommended index for maintenance (ARC 1980).

The major reason for higher nutrient intakes as the proportion of lucerne increased was higher intake (Table 4.4). The higher CP for the lucerne accentuated this.

Table 4.4: Voluntary intake by penned sheep of individual nutrients from diets of *Posidonia* (P) mixed with different proportion of lucerne (Luc). Data show means ± SE

Diet	g/sheep/day			$g/KgW^{0.75}$/day		
P : Luc	CP	CF	NFE	CP	CF	NFE
100:0	9.2 ± 0.61	109 ± 7	101 ± 7	0.45 ± 0.2	5.3 ± 0.20	4.9 ± 0.19
75:25	52 ± 2.5	195 ± 9	234 ± 12	2.4 ± 0.0	8.8 ± 0.17	10.6 ± 0.21
50:50	81 ± 1.3	216 ± 3	273 ± 4	3.6 ± 0.1	9.5 ± 0.19	12.0 ± 0.24
25:75	123 ± 3.1	249 ± 13	335 + 18	5.5 ± 0.2	11.2 ± 0.41	15.0 ± 0.60
0:100	240 ± 5	386 ± 8	549 ± 11	10.4 ± 0.3	16.7 ± 0.42	23.7 ± 0.60
Significance	**	**	**	**	**	**
LSD(0.05)	11	25	32	0.5	1	1.3

CP =Crude protein CF= Crude Fibre NFE= Nitrogen-Free extract ** = P<0.01

Digestibility *in vivo*: The mean dry-matter and organic-matter digestibility *in vivo* and the digestible and metabolisable energy of the experimental diets are shown in Table 4.5. Generally digestibility of the diet with 100% PA was low and comparable to results obtained *in vitro* (see section 3.3). As expected DMD, OMD, DE and ME of the total diet were all increased with the increasing proportion of lucerne.

Table 4.5: Apparent digestibility *in vivo* of *Posidonia* (P) mixed with different proportions of lucerne (Luc) (means ± SE).

Diet P : Luc	Dry matter Digestibility (%)	Organic matter digestibility (%)	Digestible energy (MJ/Kg DM)	Metabolisable energy (MJ/Kg DM)
100 : 0	32.9 ± 1.4	22.2 ± 0.9	5.8 ± 0.3	4.7 ± 0.2
75 : 25	39.4 ± 1.6	25.6 ± 1.1	7.1 ± 0.3	5.7 ± 0.3
50 : 50	47.5 ± 1.4	31.4 ± 0.9	8.6 ± 0.3	7.0 ± 0.2
25 : 75	54.2 ± 1.4	43.9 ± 1.2	10.0 ± 0.3	8.1 ± 0.2
0 :.100	63.9 ± 1.5	66.5 ± 1.6	11.9 ± 0.3	9.6 ± 0.2
Significance	**	**	**	**
LSD (5%)	5.0	3.9	1.4	0.8

** = P<0.01

The digestible and metabolisable energy levels of the diet with 100% PA were 5.8 and 4.7 MJ/Kg DM respectively, which is generally within the range reported for low-quality roughages (ARC 1980). Again as expected these values increased significantly with an increasing proportion of lucerne.

Combination of the results for intake (Table 4.3) and digestibility (Table 4.5) gives the figures for intakes of digestible dry matter and digestible organic matter shown in Table 4.6.

Table 4.6: Voluntary digestible dry-matter and organic matter intakes of *Posidonia* (P) mixed with different proportion of lucerne (Luc) (means ± SE).

Diet P : Luc	Digestible dry matter intake (g/Kg W0.75/d)	Digestible organic matter intake (g/Kg W0.75/d)
100 : 0	2.7 ± 0.21	1.5 ± 0.12
75 : 25	10.5 ± 0.31	5.7 ± 0.15
50 : 50	14.1 ± 0.43	8.0 ± 0.25
25 : 75	19.8 ± 0.64	14.2 ± 0.45
0 : 100	36.3 ± 1.7	34.6 ± 1.6
Significance	**	**
LSD (0.05)	2.8	2.5

** = P<0.01

Figure 4.2 shows the dry-matter digestibility of just the *Posidonia* in the mixed diets as calculated by the equation previously mentioned (section 4.2.2). According to this approach there was no significant difference for dry matter-digestibility of *Posidonia* in the different diets.

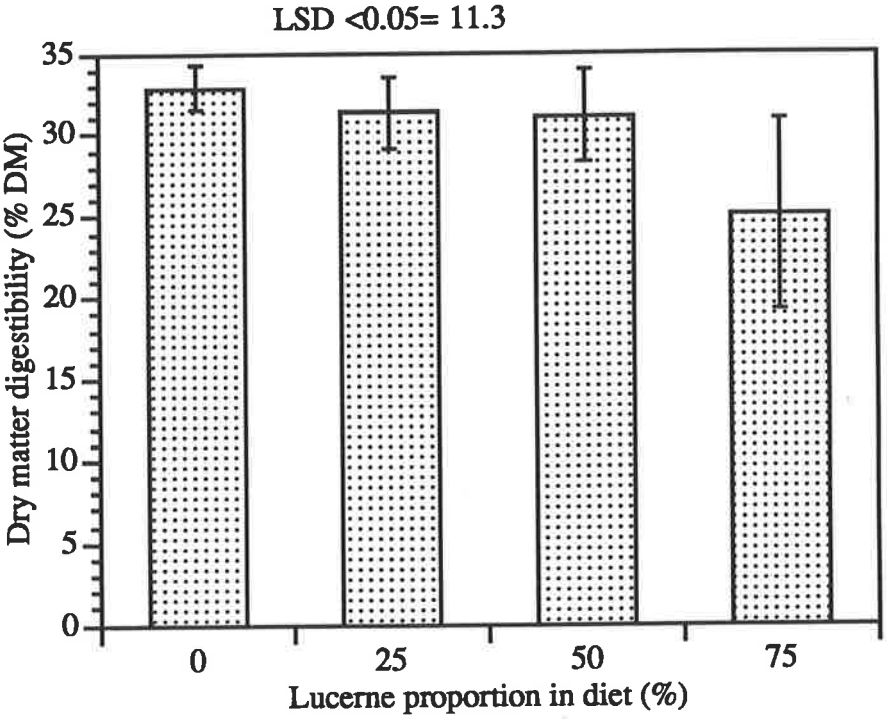


Figure 4.2: Dry-matter digestibility of *Posidonia* in mixed diets

Body weight changes: The effects of the diet treatment with different proportion of *P. australis* on sheep body weight changes has been presented already in Table 4.3. Data show that during the preliminary period (of voluntary intake measurement) only the group of sheep that ate a diet with 100% and 75% diet lost weight. These data indicated the sheep did not eat enough diet (with 100% and 75% PA) to maintain their body weight, i.e. the intake of digestible nutrients was lower than that required for maintenance.

4.2.3 Discussion

Leading on from earlier work (Chapter 3) the purpose of the experiment described in this section was:

(i) determination of the voluntary intake of *Posidonia;*

(ii) measurement of the digestibility of *Posidonia in vivo*, to compare with the digestibility *in vitro* already measured.

In summary, both the voluntary intake and the digestibility of a diet solely of *Posidonia* were lower than that required for maintenance.

Various factors influence the quality of feed and undoubtedly crude fibre is one of the important factors (Van Soest 1981). Although no attempt (except in previous chapter) has been made to clarify the exact chemical and physico-chemical structure of the fibre fraction, the high concentration of fibre (Table 4.2) and low digestibility of organic matter (Table 4.5) supports the suggestion that, like that of other low-quality roughages, the fibre fraction of *P. australis* was made of lignin and other compounds which were poorly available to the ruminant microbes. The crude fibre content of *Posidonia* is similar to or higher than the values reported elsewhere for cereal straw (AFIC 1987). The restricting role of this lignin component however, could probably be reduced by applying some delignification method (Ibrahim 1983). *Posidonia also* contains a low crude-protein content (5.6%) and therefore appears also to have insufficient crude protein to allow rumen micro-organisms to grow and metabolize efficiently (Leng 1982).

The voluntary intake of the 100% *Posidonia* used in this study was generally comparable to the lower range reported for rice straw (1.0-2.7 Kg DM/100Kg LW) in sheep (Doyle *et al.* 1986). Therefore it can mentioned that the voluntary intake of *Posidonia* was at the lowest rate of straw as a conventional lignocellulosic feedstuff. Possibly explanations for this low intake could be:

(i) Low dry-matter digestibility. Minson (1980) has reported that intake can be up to 20% lower for low-digestiblity grasses than for high-digestiblity ones.

(ii) High fibre content. An increase in the proportion of fibre may correlate with a reduction in the non-structural carbohydrates of the cell contents. Van Soest (1965) reported significant correlations between intake and neutral detergent fibre (r= -0.65).

(iii) Low protein content. Generally the intake of a feed will mostly be limited by the level of fibre and its physical composition if protein and other essential nutrients are available in sufficient quality. As Minson and Milford (1967) reported, however, when the crude protein content of a feed falls below approximately 6-8 percent appetite is depressed and therefore intake by the animal will be less than might be expected from simply a consideration of the physical composition of the feed.

(iv) Taste and/or smell. These have been regarded as palatability factors that may affect intake (Arnold 1981; Grovum 1988), but nothing is known of these regarding *Posidonia* and sheep.

(v) Phenolic compounds, tannins, alkaloids and allergenic compounds. If present in any quantity these could possibly affect intake of *Posidonia* (Hegarty 1981; Howarth 1988; Liener 1990).

The apparent dry matter digestibility *in vivo* of 100% *Posidonia* was low (32.9%), though comparable with digestibility *in vitro* reported earlier, 34.7% (section 3.3). Lignin could be the major factor causing decline in the digestibility of *Posidonia* cell wall with maturity (Harkin 1973). It is supposed that the lignin protects the structural polysaccharides, which are combined with it, from microbial breakdown (Boda 1990). Because of both low digestibility and low intake the digestible and metabolizable energy of PA was correspondingly low.

As a consequence of low nitrogen content, low intake and low digestibility *Posidonia* on its own has limited usefulness. Supplementation of such poor quality roughages with green forage material, eg. legumes, however, has been shown clearly to improve the nutritional status of animals fed them (Mosi and Butterworth 1985, Moran *et al.* 1983) and that strategy was applied here.

In this experiment, in addition to measuring voluntary intake and digestibility of *Posidonia* as a whole diet, four others diet treatments including diets with 25, 50, 75 and 100% lucerne were employed. The addition of 25% lucerne to diet caused the highest actual intake of *Posidonia* (Figure 4.1), but the consumption of total DM (Table 4.3) was further improved

with increasing the proportion of lucerne. Depression of intake of diet with 100% *Posidonia* is possibly associated with level of crude protein and the first effect of lucerne addition was perhaps to overcome this deficiency (Mosi and Butterworth 1985).

Although the use of lucerne improved the digestibility of the total diets it did not improve the digestibility of *Posidonia* in itself the mixtures (Figure 4.2). The increase in total digestibility of the mixed diet due to increased proportions of lucerne may have been associated with increased levels of crude protein or reduce level of ADF-ash. The relations between these constituents and digestibility have been discussed by Van Soest (1982).

In conclusion *Posidonia* with its low protein content and low intake and digestibility cannot meet even the maintenance requirement sheep when fed as a sole diet. The use of high quality supplementary forage, e.g. lucerne, can contribute substantially to the utilisation of this novel, non-conventional plant as a feed for ruminants. In addition to direct supplementation, however, there are variety of other methods that can be used for improving the nutritive value of such lignocellulosic materials as *Posidonia* and in the next sections and the next chapter several different categories of processing methods will be employed. These are partial decomposition, biological processing through prior use as bedding matterial for broiler chickens and supplementation with molasses.

4.4 Experiment 4: Voluntary intake and digestibility *in vivo* of decomposed *Posidonia australis* (DP)

4.4.1 Introduction

Results reported in the previous section showed that a diet of *Posidonia* is low in both voluntary intake and digestibility. It was suggested that to improve the nutritive value of *Posidonia* some physical, chemical and biological processing may be effective.

The simple processing of plant material by decomposition has been suggested by various authors to improve the nutritive value of lignocellulosic feedstuffs. Partly-decomposed *Posidonia* is available in the same area at Kingston, South Australia where dried *Posidonia* was collected (see section 4.2). *Posidonia* over many years has been cleared from the beach

and dumped some 150 m back from the water line. Over time and exposure to sun, wind, rain and biological activity this material has changed considerably in physical appearance - it is darker and of much smaller particle size. In this section it is referred to as "Decomposed *Posidonia*" or DP.

Doyle *et al.* (1986) have reported that during composting, organic materials are decomposed through biochemical processing involving micro-organism. The first stage of composting involves a rise in temperature of the composted material, an increase in the number of certain micro-organisms and decomposition of organic compounds. The degree to which the temperature rises, particular micro-organism multiply and the rate of degradation of the composted materials are dependent upon factors such as moisture content, oxygen availability, pH, the nutrient ratios in the composting materials and the prevalence of particular types of microbes.

Han (1978) has reported that aerobic fermentation can increase the percentage of crude protein of straw; Doyle *et al* (1986) by contrast considered that the losses of organic matter, in particular neutral detergent soluble, during composting and consequent increases in the ash and lignin content of the fermented residue indicate that this pre-treatment process is unlikely to improve the feeding value of lignocellulosic feedstuffs.

Seagrass has long been recognised as a major, if local, producer of decaying organic matter and the changes, collectively known as detail processing, have been described in several reviews of coastal marine detritus (Mann 1976; Fenchel and Jorgensen 1977). Living seagrass fronds have a low protein content combined with a high proportion of structural carbohydrates and are thus considered to be of poor nutritional quality (Fenchel and Blackburn 1979). The decomposition of seagrass, however, the biological and chemical dynamics of which have been reasonably well described (Pomeroy 1980), is thought possibly to enhance its nutritional value by the build up of microbially-derived protein and the degradation of refractory components (Newell 1965; Fenchel 1970; Mann 1972; Klumpp and Van Der Valk 1984).

The experiment of this present section aimed to study the effect of partial decomposition on the nutritive value of seagrass, with measurement of chemical composition, voluntary intake and digestibility.

4.4.2 Results

Chemical composition: Table 4.7 shows the chemical composition of the decomposed *Posidonia* (DP) and mixed diet (DP + lucerne). The crude protein, crude fibre and ether extract content of the diet with 100% DP was 5.2, 8.2 and 1% of dry matter respectively. In comparison with *Posidonia* in previous experiment (Table 4.2) the value of all three nutrients showed a decline (from 5.6, 34.0 and 1.2%) with the decrease for crude fibre markedly greater than for the others. Concomitantly the ash content of DP rose considerably, from 20.5 to 40.5% and the nitrogen free extract slightly, from 38.7 to 45.1% .

Table 4.7: Chemical composition of decomposed *Posidonia* (DP), alone and mixed with different proportions of lucerne (Luc)

Diet DP : Luc	Ash (%)	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Nitrogen free extract (%)
100 : 0	40.5	5.2	8.2	1.0	45.1
75 : 25	32.5	8.4	13.6	1.3	44.2
50 : 50	24.5	11.6	19.1	1.6	43.2
25 : 75	16.5	14.8	24.6	1.8	42.3
0 : 100	8.5	18.0	30.1	2.1	41.3

Voluntary intake: The mean dry matter and organic matter voluntary intakes of the various diets and of DP alone and in the mixtures are shown in Table 4.8 and Figure 4.3. On the basis of metabolic weight (g/KgW$^{0.75}$/d) the dry matter intake of the experimental diets varied from 4.1 for the diet with 100% DPA to 66.0 for diet with 0% DP. Statistical analysis of this data showed significant increase in the dry matter intake of the diets with increasing proportions of lucerne (Table 4.8).

Table 4.8: Voluntary dry matter and organic matter intake of decomposed *Posidonia* (DP) mixed with different proportion of lucerne(Luc) (Mean ± SE) and sheep body weight changes (Mean).

Diet	Dry matter		Organic matter		Sheep body weight changes
DP : Luc	g/sheep/d	g/KgW$^{0.75}$/d	g/sheep/d	g/KgW$^{0.75}$/d	g/sheep/d
100 : 0	80 ± 5	4.1 ± 0.2	47 ± 2.8	2.4 ± 0.1	-577
75 : 25	449 ± 35	20.9 ± 1.2	303 ± 24	14.1 ± 0.83	-369
50 : 50	919 ± 50	40.9 ± 1.5	691 ± 38	30.8 ± 1.12	-69
25 : 75	1122 ± 35	47.2 ± 1.1	937 ± 29	39.4 ± 0.91	+177
0 : 100	1694 ± 88	66 ± 2.7	1550 ± 81	60 ± 2.5	+269
Significance	**	**	**	**	
LSD (0.05)	141	4.6	122	3.9	

**P<0.01

Organic matter intake (g/KgW$^{0.75}$) of diets varied from 2.4 (diet with 100% DPA) to 60 (diet with 0% DP), (Table 4.8). Figure 4.3 shows that the highest intake of DP itself occurred in the diet with 50% lucerne.

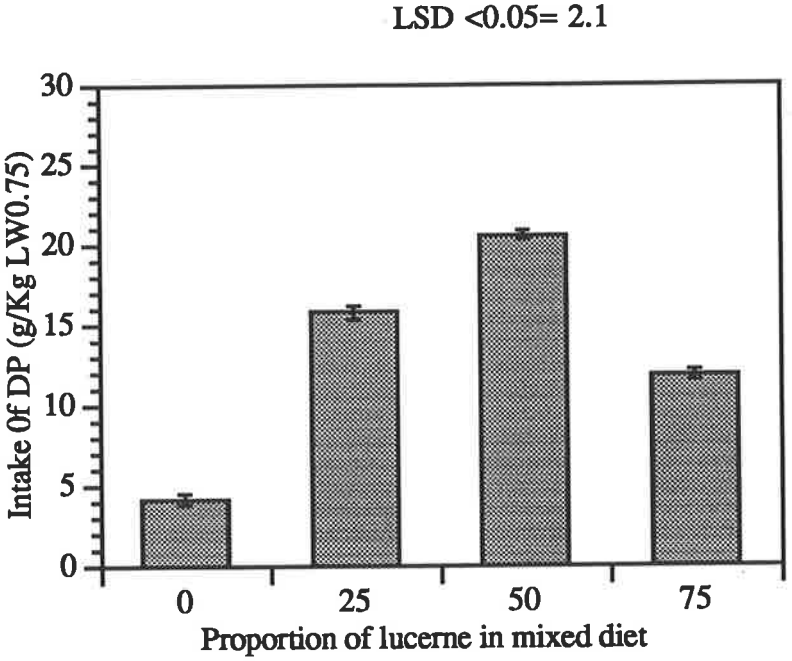


Figure 4.3: Voluntary intake of decomposed *Posidonia* in mixed diets

A comparing of the results in intake of this experiment (Table 4.8) with the results of the previous experiment (Table 4.3) indicates that due to decomposition dry matter intake of diets with 100, 75% and 50% DP markedly decreased (4.1, 20.9 and 40.9 vs 8.0, 26.8 and 29.8) while the intake of 100% lucerne (as control) increased slightly (66 vs 56.7).

Voluntary intakes of individual nutrients of the experimental feeds are shown in Table 4.9. The intake of CP, CF and NFE (g/Kg $W^{0.75}$/d) in diet with 100% DP were 0.2, 0.3, and 1.8 respectively, all lower than in the previous experiment (Table 4.4).

Table 4.9: Voluntary intake by penned sheep of individual nutrients of decomposed *Posidonia* (DP) mixed with different proportions of lucerne (Luc) (Means ± SE)

Diet	g/sheep/day			g/Kg$W^{0.75}$/day		
DP:Luc	CP	CF	NFE	CP	CF	NFE
100:0	4.1 ± 0.24	6.5 ± 0.38	35.9 ± 2.1	0.2 ± 0.0	0.3 ± 0.02	1.8 ±0.1
75:25	37.7 ± 3.0	61 ± 4.8	198 + 4.8	1.8 ± 0.1	2.8 ± 0.17	9.2 ± 0.17
50:50	107 ± 3.8	175 ± 9.5	397 ± 22.3	4.7 ± 0.1	7.8 ± 0.28	17.6 ± 0.66
25:75	166 ± 2.7	276 ± 8.6	474 ± 14.9	3.6 ± 0.1	11.6 ± 0.27	20 ± 0.46
0:100	305 ± 15.8	510 ± 27	700 ± 36	11.9 ± 0.5	19.8 ± 0.83	27.2 ± 1.13
Significance	**	**	**	**	**	**
LSD(0.05)	30.0	38.0	59.0	0.6	1.2	1.9

CP =Crude protein CF= Crude Fibre NFE= Nitrogen-Free extraction ** = P<0.01

Digestibility *In vivo* : The dry matter digestibility (DMD), organic matter digestibility (OMD), digestible and metabolizable energy (DE and ME) of the experimental diets are shown in Table 4.10. Generally the digestibility, and thus also the energy value, of DP increased markedly in comparison with non-decomposed *Posidonia* (Table 4.5). Figure 4.4 shows the DMD of DP itself in the mixed diets, as calculated by the equation already mentioned in section 4.2. This figure shows that the DMD of DP in mixture with 25% and 50% lucerne is the same as for DP alone, but is significantly higher in 75% lucerne mixture. The DE content (MJ/Kg) of the diets ranged from 9.5 (with 100% DP) to 11.9 (with 25% DP). These figures were higher than the range reported earlier for non-composted *Posidonia* (section 4.3.2) and for other low-quality roughages (ARC 1980).

Table 4.10: Apparent digestibility *in vivo* of decomposed *Posidonia* (DP) mixed with different proportions of lucerne (Luc), (means ± SE)

Diet DP : Luc	Dry matter Digestibility (%)	Organic matter digestibility (%)	Digestible energy (MJ/Kg DM)	Metabolisable energy (MJ/Kg DM)
100 : 0	52.1 ± 1.1	38.0 ± 1.4	9.5 ± 0.2	7.7 ± 0.2
75 : 25	55.1 ± 1.2	46.6 ± 1.2	10.1 ± 0.2	8.2 ± 0.2
50 : 50	59.0 ± 1.4	43.3 ± 0.7	10.9 ± 0.3	8.8 ± 0.2
25 : 75	64.3 ± 2.1	52.6 ± 1.2	11.9 ± 0.4	9.7 ± 0.3
0 :100	65.4 ± 1.8	65.6 ± 2.0	12.2 ± 0.4	9.8 ± 0.3
Significance	**	**	**	**
LSD (5%)	4.9	4.0	0.9	0.7

** = P<0.01

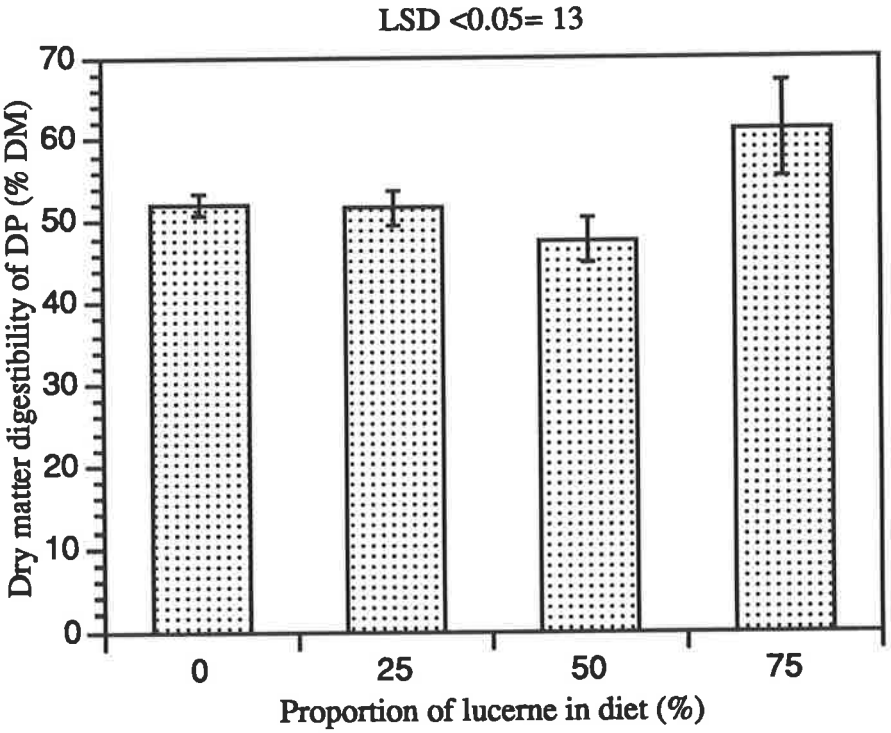


Figure 4.4: Dry matter digestibility of decomposed *Posidonia* in mixed diets

Digestible voluntary intake: Digestible dry matter (DDM) and digestible organic matter (DOM) intakes of the straight DP and the mixed diets are shown in Table 411. DDM and DOM of experimental diets ranged from 2.1 and 0.9 (100% DP) to 30.4 and 20.7 (25% DPA) respectively. A interesting result is that in comparison with last experiment (Table

4.6) the DDM and DOM of all mixed diets with decomposed *Posidonia* increased significantly due to a considerable increase in digestibility and despite a lower voluntary intake.

Table 4.11: Voluntary digestible dry matter and digestible organic matter intake of decomposed *Posidonia* (DP) mixed with different proportion of lucerne (Luc) (Means ± SE)

Diet treatment (DP : Luc)	Digestible dry matter intake (g/Kg $W^{0.75}$/d)	Digestible organic matter intake (g/Kg $W^{0.75}$/d)
100 : 0	2.1 ± 0.10	0.9 ± 0.07
75 : 25	11.5 ± 0.58	6.6 ± 0.48
50 : 50	24.2 ± 1.41	13.3 ± 0.48
25 : 75	30.4 ± 1.32	20.7 ± 0.60
0 : 100	43.0 ± 0.72	39.4 ± 0.64
Significancel	**	**
LSD (0.05)	2.7	1.6

** = P<0.01

Body weight changes: The effects of the diet with 100, 75, 50, 25 and 0% DP on sheep-body weight changes are presented in Table 4.8. All sheep that were fed with diet containing 100, 75 and 50% DPA lost body weight during the preliminary period. Weight losses here were larger than in the previous experiment.

4.4.3 Discussion

As described in the introduction and leading on from the previous section (4.3) this experiment had been aimed to determine the effect of decomposition on the nutritive value of *Posidonia*. Therefore three characteristics of the decomposed *material* (DP), chemical composition, voluntary intake and digestibility *in vivo* , were studied.

In general summary decomposition of *Posidonia* resulted in an increase in ash content and in digestibility, but a decline in organic matter and in voluntary intake.

A comparison of the data on chemical composition of *Posidonia* and decomposed *Posidonia* (Table 4.2 and 4.7) indicates that there were some important changes due to decomposition.

The natural composting of *Posidonia* may be through both physical and biochemical processes. These processes cause losses of organic matter, in particular neutral detergent fibre (from 34 to 8%), and an increase in ash content (from 20.5 to 40.5%). An increased ash content due to decomposition is in agreement with result of Odium (1984), who pointed out that detrital processing of seagrass causes remineralization. Another important possibility for the increased ash content could be just mixing of *Posidonia* with coastal sands and minerals over time. Loss of crude fibre during composting was mentioned by Doyle *et al.* (1986). The crude protein content of decomposed *Posidonia* was 5.2% of dry matter, showing no change due to decomposition. Although it could have been expected that decomposition processes that include mostly biological (microbial) decay might causes increases in protein content this experiment showed a constant proportion of crude protein. The explanation of this could be that the intensity of biological decay on this seagrass was low, and/or the mixing up of coastal sands was high, so that any increase due to biological process did not result in any overall increase in CP content.

Comparison of the data of Tables 4.8 and 4.3 shows that the dry matter and organic matter voluntary intake ($g/KgW^{0.75}$) of decomposed *Posidonia* markedly decreased (from 165 and 8 to 80 and 4.1). This result is in some contrast with the work of Garnsworthy and Cole (1990) who pointed out that in ruminants when a roughage is changed to smaller particles its voluntary intake generally increases, due to the faster rate of passage of material from the rumen. This contrast might be explained by the change in size due to decomposition as being different from that effected by simply chopping or grinding roughage, or more likely that the increase in ash content (up to 40% of dry matter) affected palatability and, as a result, voluntary intake decreased.

On the other hand decomposition of PA greatly increased DMD and OMD, from 32.9 and 22.2% to 52.1 and 38% respectively. Two major factors that could possibly cause an increase in digestibility are degradation of fibre content and decrease in intake. Klumpp and Van der Valk (1984) reported that in the case of *Posidonia* there was a 50% decline in the

amount of hemicellulose relative to stable lignin during the first 10 days of decomposition, and a total fibre loss of 50% after 240 days. On the other hand the low voluntary intake of DP would be subjected to rumen microbial digestion for a longer period of time (Garnsworthy and Cole 1990). Another possibility for high digestibility could be because of a high solubility of the ash content of DPA.

In conclusion, for any practical use of *Posidonia*, whether decomposed or not, three points should be considered: (i) improving its protein content (ii) decreasing its ash content and (iii) increasing its voluntary intake.

For possible achievement of these aims the additional experiments of the next section were planned. In these experiment the possibility for improving the nutritive value of *Posidonia* by processing it as a litter material for raising broiler chickens and treatment with molasses will be examined.

4.5 Experiment 5: Voluntary intake and digestibility *in vivo* of *Possidonia australis* litter (PL)

4.5.1 Introduction

The results of experiment 2 indicated that although natural decomposition of *Posidonia* caused an increase in its digestibility there was a decrease in voluntary intake and no increase in protein content.

The use of poultry litter as a feed for ruminant was reviewed in section 2.6, where it was mentioned that poultry excreta can be as a useful nitrogen source for ruminants. This next experiment aimed to determine the effect of chicken manure on the nutritive value of *Posidonia*, that is after it had been used as a bedding material during the raising of broiler chickens. A minor aim of this work was to check whether or not *Posidonia* could indeed be used as an alternative litter for chickens.

4.5.2 Results

Chemical composition: Table 4.12 shows the chemical composition (%DM) of *Posidonia* litter (PL) and its mixture with different proportions of lucerne. It is evident that there is a comparatively wide variation the between chemical composition of PL and that of both *Posidonia* (P) and decomposed *Posidonia.* (DP).

Table 4.12: Chemical composition of *Posidonia* litter (PL) mixed with different proportions of lucerne (Luc)

Diet PL : Luc	Ash (%)	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Nitrogen free extract (%)
100 : 0	16.9	18.0	19.5	2.5	43.1
75 : 25	14.7	18.2	22.0	2.4	42.7
50 : 50	12.5	18.4	24.7	2.3	42.1
25 : 75	10.3	18.5	27.2	2.1	41.9
0 : 100	8.1	18.7	29.7	2.0	41.5

Crude protein content of PL (18%) was much higher than that of either *Posidonia* (5.6%) or DP (5.2%). Crude fibre content of PL (19.5) was higher than that of DP (8.2%) but much less than that of *Posidonia* (34%). The ash content of PL (16.9%) twas less than both *Posidonia* (20.5%) and DP (40.5%).

Voluntary intake: The mean voluntary intake of dry matter and organic matter of the experimental diets are presented in Table 4.13. There were significant differences between the dry matter intakes (g/Kg $W^{0.75}$) of 100% PL and P or DP (21 vs 8 and 4.1 respectively). The variation between diets with different proportion of lucerne was less than that for P or DP diets (Tables 4.3, 4.8 and 4.13).

Table 4.13: Voluntary dry-matter and organic-matter intakes of *Posidonia* Litter (PL) mixed with different proportion of lucerne(Luc) (Mean±SE) and sheep body weight changes (Mean)

Diet	Dry matter		Organic matter		Body weight changes
PL : Luc	g/sheep/d	g/KgW$^{0.75}$/d	g/sheep/d	g/KgW$^{0.75}$/d	g/sheep/d
100 : 0	454 ± 15	21 ± 0.4	377 ± 13	17.6 ± 0.37	-331
75 : 25	783 ± 19	35 ± 0.9	668 ± 17	30.2 ± 0.75	-38
50 : 50	912 ± 45	41 ± 1.9	798 ± 39	35.4 ± 1.62	+15
25 : 75	985 ± 51	42 ± 2.1	884 ± 46	37.7 ± 1.86	+115
0 : 100	2071 ± 87	86 ± 3.4	1903 ± 80	79 ± 3.1	+131
Significance	**	**	**	**	
LSD(0.05)	132	5.4	120	5.0	

** = P <0.01

Figure 4.5 shows the voluntary intake of PL itself in the different mixtures. This figure shows that the highest intake of PL occured when it was mixed with 25% lucerne.

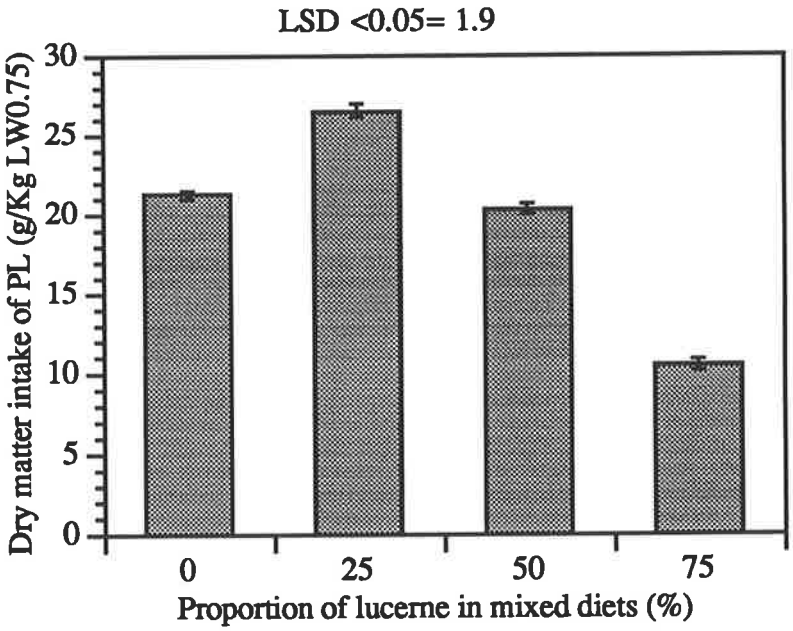


Figure 4.5: Voluntary dry matter intake of *Posidonia* litter (PL) in mixed diets

The higher crude protein content of the PL led to higher intake of this nutrient in all diets(Table 4.14).

Table 4.14: Voluntary intake by penned sheep of individual nutrients of *Posidonia* litter (PL) mixed with different proportion of lucerne (Luc). (Mean ± SE)

Diet	g/sheep/day			g/KgW$^{0.75}$/day)		
PL:Luc	CP	CF	NFE	CP	CF	NFE
100:0	82 ± 2.7	88 ± 2.9	196 ± 6.5	3.8 ± 0.1	4.1 ± 0.09	9.1 ± 0.19
75:25	143 ± 3.5	172 ± 4.0	334 ± 8.2	3.9 ± 0.1	7.8 ± 0.19	15.1 ± 0.38
50:50	168 ± 8.2	225 ± 11	384 ± 19	7.4 ± 0.3	10.0 ± 0.46	17.0 ± 0.78
25:75	182 ± 9.5	268 ± 14	413 ± 22	7.8 ± 0.4	11.4 ± 0.57	17.6 ± 0.89
0:100	387 ± 16	615 ± 26	860 ± 36	16 ± 0.6	24.6 ± 0.97	35.5 ± 1.41
Significance	**	**	**	**	**	**
LSD(0.05)	25.0	38	55	1	1.5	2

CP =Crude protein CF= Crude Fibre NFE= Nitrogen-Free extraction ** = P<0.01

Digestibility *in vivo*: The mean dry matter and organic-matter digestibilities (DMD and OMD) of PL and the mixed diets are shown in Table 4.15. The calculated digestible and metabolisable energy of the diets are shown in the same table.

The DMD of 100% PL diet (50.4%) was higher than for P (32.9%) and approximately equal to that of DP (52.1%). The range of digestible energy (MJ/Kg) values calculated from the DMD of the diet was 9.2 (in 100% PL) to 11.2 (in 25% PL); this range is considerably greater than that for both PA (5.8 to 10.0) and DP (9.5 to 11.9).

The dry matter digestibility of PL in the various mixtures, as calculated, is shown in Figure 4.6. There were no statistical differences between the various diets in this regard.

Digestible dry-matter and digestible organic-matter intakes (DDM and DOM) of the experimental diets (g/KG W$^{0.75}$/d), as shown in Table 4.16, were respectively 10.7 and 8.6 in diet with 100% PL, which were higher than for *Posidonia* and DP.

Table 4.15: Apparent digestibility *in vivo* of *Posidonia* litter (PL) mixed with different proportion of Lucerne (Luc). (Means ± SE)

Diet PL : Luc	Dry matter Digestibility (%)	Organic matter digestibility (%)	Digestible energy (MJ/Kg DM)	Metabolisable energy (MJ/Kg DM)
100 : 0	50.4 ± 1.6	48.5 ± 1.7	9.2 ± 0.3	7.5 ± 0.3
75 : 25	53.4 ± 1.9	48.4 ± 1.7	9.8 ± 0.4	7.9 ± 0.3
50 : 50	57.0 ± 2.0	50.0 ± 1.5	10.54 ± 0.4	8.5 ± 0.3
25 : 75	60.6 ± 1.6	52.7 ± 1.3	11.2 ± 0.3	9.1 ± 0.3
0 : 100	64.8 ± 1.5	56.0 ± 1.3	12.0 ± 0.3	9.7 ± 0.2
Significance	**	**	**	**
LSD (0.05)	3.6	3.7	0.8	0.6

** = P<0.01

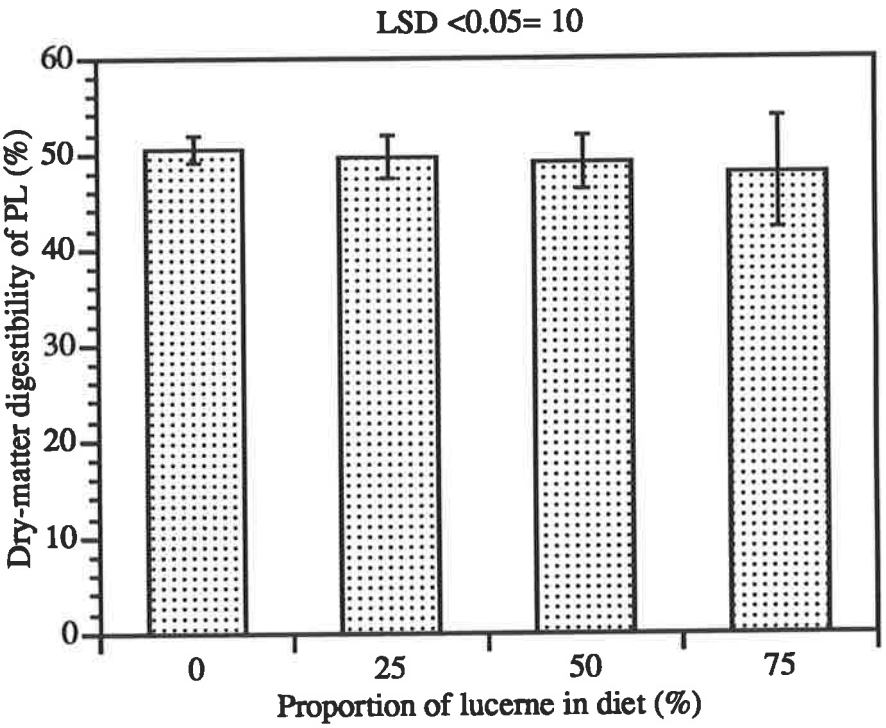


Figure 4.6: Dry-matter digestibility of *Posidonia* litter (PL) in mixed diets

Table 4.16: Voluntary digestible dry-matter and digestible organic-matter intakes of *Posidonia* litter (PL) mixed with different proportions of lucerne (Luc). (Means ± SE)

Diet (PL : Luc)	Digestible dry matter intake (g/Kg $W^{0.75}$/d)	Digestible organic matter intake (g/Kg $W^{0.75}$/d)
100 : 0	10.7 ± 0.58	8.6 ± 0.47
75 : 25	18.9 ± 0.71	14.6 ± 0.63
50 : 50	23.1 ± 1.41	17.7 ± 0.82
25 : 75	25.5 ± 1.47	19.9 ± 1.2
0 : 100	55.4 ± 2.44	44.1 ± 2.0
Significant level	**	**
LSD (0.05)	4.7	2.7

** = P<0.01

Body weight changes: The effects of experimental diets on sheep body weight (metabolic weight) changes are presented in Table 4.13. Only the sheep which ate the diets with 100% and 75% *Posidonia* litter lost weight.

4.5.3 Discussion

The results of the experiment in section 4.4.2 showed that although decomposition caused an increase in digestibility of *Posidonia* it did not improve protein content nor voluntary intake. The concept of using this lignocellulosic material for broiler chickens raised two further experimental possibilities:

(i) improvement to the nutritive and feeding values of *Posidonia* in terms of crude protein content, voluntary intake and digestibility;

(ii) actual testing of dry *Posidonia* as a new bedding material which might be both cheaper and better for chicken meat production.

In summary the results of this experiment shows that the *Posidonia* litter is higher in CP content, voluntary intake and digestibility in comparison with straight *Posidonia*. In addition

the work showed that *Posidonia* was highly effective as broiler bedding. It did not cause any negative effects on the health of the broiler chicken nor on meat production.

A comparison of the data in Tables 4.1 and 4.12 indicates, as expected, a greatly increase, in crude protein content of *Posidonia* (18% vs 5.6%) after its use as a bedding material. Several references, including one report by Krishna Reddy and RAJ Reddy (1989), indicate that poultry excreta could enrich the crude protein content of bedding materials. Several factors like density of birds, age of birds, age of the litter and method of subsequent processing all affect the chemical composition of poultry litter (Jakhmola *et al.* 1988). Muler (1980) reported that about 45-67% of protein content of broiler litter is present as true protein, 18-30% as uric acid and 12-17% as ammonia and a small amount as creatine (2-4%) and other N constituents.

The dry matter digestibility of *Posidonia* after its use as a bedding material increased from 32.9% (Table 4.5) to 50.4% (Table 4.15); and the same proportional increase occurred for organic matter digestibility, DE and ME. Smith (1973) reported, in agreement with these results, that poultry excreta with a high amount of fibre is well digested by ruminants. One explanation for the increase in digestibility and consequently also in DE and ME could be an effect of the NH_3 content of the excreta in causing a break-down of the lignin of the bedding material. A second explanation might be that of biological processing of the *Posidonia* by some species of micro-organism in poultry manure. Due perhaps to both increased digestibility and increased crude protein content the voluntary intake of *Posidonia* increased from 8 to 21 g/Kg $W^{0.75}$/d following its use as broiler litter.

The other aspect of this experiment was to examine the potential of *Posidonia* as a bedding material. The important physical properties of broiler bedding materials include bulk density, particle size and distribution, moisture-retention capacity, compressibility, penetrability, hydroscopicity and biodegradability during the rearing period (Muler 1980). In addition to these properties a potential bedding material must be inexpensive, readily available and easy to transport. *Posidonia* as collected from the Kingston beach can clearly be used as a alternative bedding material on South Australia chicken farms, or whenever it is available, since during this experiment chicken growth proceeded normally. Mortality,

growth rate, feed efficiency and subsequent quality of the chicken meat were all within normal ranges (Sabine *et al.* unpublished).

In conclusion, *Posidonia* can be considered as an alternative inexpensive and readily available bedding material for broiler chickens. After the rearing period the material with high content of CP, high digestibility and intake, can be used as a partial ruminant feedstuff.

Leading on therefore from the conclusion of this experiment in the next experiment the possibility of improving the nutritive and feeding value of decomposed *Posidonia* after use as a bedding material was studied.

4.6 Experiment 6: Voluntary intake and digestibility *in vivo* of decomposed *Posidonia* litter (DPL)

4.6.1: Introduction

The primary aim of this experiment was to examine the possibility of improving the nutritive value of decomposed *Posidonia,* particularly its voluntary intake and digestibility, following its use as broiler litter.

The results will be compared with those derived from experiment 2 of this chapter (section 4.4.2).

In addition, it was possible to examine the potential for using decomposed *Posidonia* as a bedding material for broiler chickens.

4.6.2 Results

Chemical composition: Chemical composition of the experimental diets is shown in Table 4.17. Again it is evident that the chemical composition of decomposed *Posidonia* (DP) changed considerably due to its use as a bedding material.

CP, CF, EE, NFE and ash content of decomposed *Posidonia* litter (DPL) (%DM) was 16.3, 11.9, 2.0, 33.4 and 36.4 respectively. In comparison with just decomposed *Posidonia*, the CP increased 11.2 unit (from 5.2% to 16.3%); the CF and EE content increased and

decreased 3.7 and 1 unit respectively. It seems the manure added to the bedding material caused a decrease in ash content of DP (from 40.5 to 36.4%).

Table 4.17: Chemical composition of decomposed *Posidonia* litter (DPL) mixed with different proportions of lucerne (Luc)

Diet PA : Luc	Ash (%)	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Nitrogen free extract (%)
100 : 0	36.4	16.3	11.9	2.0	33.4
75 : 25	29.5	17.0	16.2	2.1	35.2
50 : 50	22.7	17.7	20.4	2.1	37.1
25 : 75	15.8	18.4	24.7	2.2	38.9
0 : 100	8.9	19.1	28.9	2.2	40.9

Voluntary intake: Mean voluntary intakes of DM and OM and the relevant statistical analysis are presented in Table 4.18.

There was a considerable difference between the intakes (g/Kg $W^{0.75}$/d) of DP and DPL (12.6 vs 4.1), but the intakes of the mixed diets of DP and DPL were not greatly different. The intake of individual DPL in the various mixtures is shown in Figure 4.7. This data shows the highest intake for DPL was when it mixed with 50% lucerne.

The average voluntary organic-matter intake (g/Kg$W^{0.75}$/d) was higher with DPL as well (8), in comparison with DP (2.4).

The higher crude protein and crude fibre content of DPL, in comparison with DP, led to higher intake of these nutrients (Table 4.19). The intake of nutrients in the mixed diets depended on the concentration of these in the DPA and lucerne.

Table 4.18: Voluntary dry-matter and organic-matter intakes of decomposed *Posidonia* litter (DPL) mixed with different proportions of lucerne(Luc) (Means ± SE) and sheep body weight changes (Mean)

Diet	Dry matter		Organic matter		Body weight changes
DPL:Luc	g/sheep/d	g/KgW$^{0.75}$/d	g/sheep/d	g/KgW$^{0.75}$/d	g/sheep/day
100 : 0	272 ± 15	12.6 ± 0.4	173 ± 10	8.0 ± 0.23	-369
75 : 25	398 ± 16	18.2 ± 0.5	280 ± 12	12.8 ± 0.32	-231
50 : 50	805 ± 26	36 ± 0.8	622 + 20	27.8 ± 0.64	-108
25 : 75	857 ± 24	38 ± 0.7	721 ± 21	32.1 ± 0.59	-15
0 : 100	1420 ± 57	62 ± 2.1	1294 ± 52	56.6 ± 1.93	+146
Significance	**	**	**	**	
LSD(0.05)	58	2.4	56	2.3	

** = P <0.01

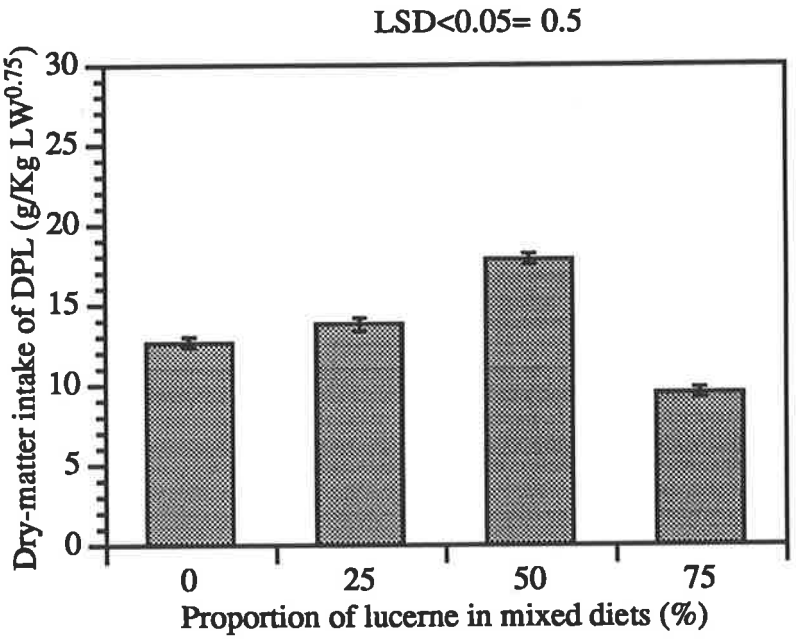


Figure 4.7: Voluntary dry-matter intake of decomposed *Posidonia* litter (DPL) in mixed diets

Table 4.19: Voluntary intake by penned sheep of individual nutrients of decomposed *Posidonia* litter (DPL) mixed with different proportions of Lucerne (Luc). (Means ± SE)

Diet	g/sheep/day			g/KgW$^{0.75}$/day		
DPL:Luc	CP	CF	NFE	CP	CF	NFE
100:0	44 ± 2.4	32 ± 1.78	91 ± 5.01	2.0 ± 0.1	1.5 ± 0.04	4.2 ± 0.12
75:25	67.7 ± 2.62	65 ± 2.7	140 ± 6.0	3.1 ± 0.1	2.9 ± 0.07	6.4 ± 0.17
50:50	142.5 ± 4.7	164 ± 5.4	299 ± 10.0	6.4 ± 0.1	7.3 ± 0.17	13.4 ± 0.31
25:75	158.0± 4.5	212 ± 6.0	333 ± 13	7.0 ± 0.1	9.4 ±0.18	14.8 ± 0.36
0:100	271 ± 11	410 ± 17	581 ± 23	11.9 ± 0.4	17.9 ± 0.61	25.4 ± 0.87
Significance	**	**	**	**	**	**
LSD (0.05)	12	19	25	0.4	0.8	1.6

CP =Crude protein CF= Crude Fibre NFE= Nitrogen-Free extraction ** = P<0.01

Digestibility *in vivo*:: The mean DMD and OMD *in vivo* and DE and ME of the experimental diets are shown in Table 4.20. DMD, OMD of the diet with 100% DPL were 55.3 and 37.0 respectively and the values of DE and ME for that were 10.2 and 8.2 MJ/Kg DM. There was little difference between DPL and DP in these regards.

Table 4.20: Apparent digestibility *in vivo* of decomposed *Posidonia* litter (DPL) mixed with different proportion of lucerne (Luc). (Means ± SE)

Diet DPL : Luc	Dry matter Digestibility (%)	Organic matter digestibility (%)	Digestible energy (MJ/Kg DM)	Metabolisable energy (MJ/Kg DM)
100 : 0	55.3 ± 1.6	37.0 + 1.0	10.2 ± 0.3	8.2 ± 0.2
75 : 25	57.0 ± 1.8	45.7 ± 1.7	10.5 ± 0.4	8.5 ± 0.3
50 : 50	59.4 ± 2.3	52.8 ± 1.5	11.0 ± 0.5	8.9 ± 0.4
25 : 75	62.0 ± 1.7	64.0 ± 1.2	11.5 ± 0.3	9.3 ± 0.3
0 : 100	63.3 ± 1.5	62.8 ± 1.1	11.7 ± 0.3	9.5 ± 0.2
Significance	*	**	*	*
LSD (0.05)	5	2.4	1	0.9

** = P<0.01; *= P<0.05

The DMD of DPL in the various mixtures, as calculated from the formula described in section 4.2, is presented in Figure 4.8. There were no differences between treatments in this regard.

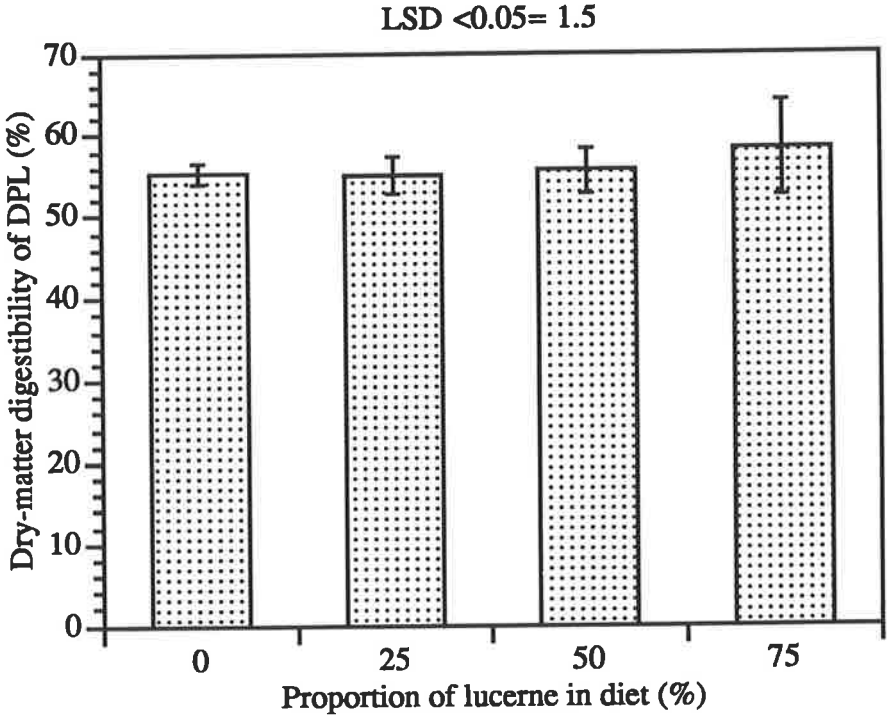


Figure 4.8: Dry-matter digestibility of DPL in mixed diets

Table 4.21: Voluntary digestible dry-matter and digestible organic-matter intake of decomposed *Posidonia* litter (DPL) mixed with different proportion of lucerne (Luc). (Means ± SE)

Diet (DPL : Luc)	Digestible dry-matter intake (g/Kg W0.75/d)	Digestible organic-matter intake (g/Kg W0.75/d)
100 : 0	6.9 ± 0.31	3.0 ± 0.06
75 : 25	10.4 ± 0.42	5.8 ± 0.08
50 : 50	21.4 ± 1.29	14.6 ± 0.10
25 : 75	23.6 ± 0.31	20.6 ± 0.10
0 : 100	39.3 ± 1.79	35.5 ± 1.33
Significance	**	**
LSD (0.05)	2.6	1.8

** = P<0.01

Table 4.21 shows digestible dry-matter and digestible and organic-matter intakes (g/Kg $W^{0.75}$/d) of the experimental diets. In the case of the diet with 100% DPL these two values increased considerably in comparison with DP (6.9 and 3 vs 2.1 and 0.9). But changes in relation to the mixed diets were not significant.

Body weight changes: The effects of the diet treatments on sheep body weight are presented in Table 4.18. Except for the sheep fed with 100% lucerne, they all lost body weight.

4.5.3 Discussion

As already mentioned in the introduction to this chapter the major purpose of this experiment was to examine the possibility of improving the nutritive value of decomposed *Posidonia* by using it as a bedding material for broiler chickens. An assessment of the possibility of employing decomposed *Posidonia* as a bedding material was as a minor aim of the experiment.

As also already noted in the discussion section of the previous experiment, broiler excreta caused a great increase in the CP content of decomposed *Posidonia* (from 5.2 to 16.3%), voluntary intake was improved about three fold. The possible explanations for these changes were also discussed previously.

In spite of improving CP content and intake the use of decomposed *Posidonia* as litter caused no considerable increase in digestibility of this material. An explanation for this phenomenon could be that the intake of decomposed *Posidonia* is more controlled by its physical form, in terms particularly of particle size and ash content, so that using decomposed *Posidonia* as a bedding material did not increase its digestibility.

With regards to the value of decomposed *Posidonia* as a bedding material, all rearing parameters were in the normal range. In comparison with *Posidonia* bedding the bulk density of decomposed *Posidonia* was higher, which is an advantage, but it seemed that moisture-retention capacity of this bedding was lower than that of *Posidonia*, which would be a relative disadvantage.

4.7 Experiment 7: The effect of molasses supplementation on voluntary intake and digestibility *in vivo* of *Posidonia*

4.7.1 Introduction

In the previous experiments of this current chapter different nutritional aspects of two physical form of *Posidonia australis* were studied. In addition the possibility of improving their nutritive value by using them as bedding material for broiler chicken was examined. Results of those experiments showed that broiler excreta in many cases was able to increase the protein content, digestibility and voluntary intake of the *Posidonia* diets.

In this present experiment it was assumed that there may be other traditional supplementary methods that can increase the palatability and intake of *Posidonia.*

Among traditional methods suplementation of fibrous material with molasses have long been recognised. Numbers of research workers. e.g. Ibrahim (1983) and Doyle *et al.* (1986) reported that the voluntary intake of various high-fibre feeds improved by such suplementation. There is no information, however, about the application of these methods for improving the nutritive value of *Posidonia* .

The reason then for undertaking this experiment was to examine the effect of molasses suplementation on the nutritive value of *Posidonia* and the possibility of its overcoming the low-intake problem.

4.7.2 Results

Voluntary intake: Voluntary dry matter and organic matter intakes ranged from 8.2 and 6.7 to 16.8 and 14.1 g per Kg $W^{0.75}$ per day respectively (Table 4.22). There were significant differences ($P<0.01$) between the voluntary dry-matter and organic-matter intakes of treated and untreated *Posidonia.* Increasing the proportion of molasses caused significant increases in voluntary intake. A comparison of the data in this table and that of Tables 4.3, 4.8, 4.13 and 4.18, however shows that suplementation of *Posidonia* with lucerne was much more effective than molasses suplementation in increasing voluntary intake. In

addition, the effect of molasses on the intake of *Posidonia* was less than that caused by either decomposition or the addition of chicken manure.

Table 4.22: Voluntary dry-matter and organic-matter intake of molasses-treated *Posidonia* (means ± SE)

Diet	Dry matter		Organic matter		Body weight changes
%Molasses	g/sheep/d	g/KgW$^{0.75}$/d	g/sheep/d	g/KgW$^{0.75}$/d	g/sheep/d
0%	166 ± 2.2	8.2 ± 0.1	136 ± 2	6.7 ± 0.05	-169
5%	192 ± 3	9.3 ± 0.0	159 ± 2	7.7 ± 0.02	-154
10%	247 + 5	11.6 ± 0.1	206 ± 4	9.6 ± 0.05	-115
15%	287 ± 5	13.1 ± 0.2	239 ± 4	10.9 ± 0.19	-108
20%	372 ± 4.1	16.8 ± 0.1	311 ± 3	14.1 ± 0.06	-57
Significance	**	**	**	**	
LSD (0.05)	5	0.4	4.4	0.31	

** = P <0.01

Digestibility *in vivo*: Apparent dry matter and organic matter digestibilities of untreated and molasses-treated *Posidonia* are shown in Table 4.23. DMD of diets with 0, 5, 10, 15 and 20% molasses were 33.5, 35.0, 36.9, 39.5 and 42.4% respectively. The diet with 5% molasses showed no significant increase in DMD and OMD over the control, but the other treatments (more than 5%) showed increases in this regard.

The digestible energy (apparently digestible energy) and metabolisable energy content (MJ/Kg) values calculated from the DMD of the relevant diets were 5.9 and 4.8, 6.2 and 5.0, 6.6 and 5.3, 7.1 and 5.8, and 7.6 and 6.2 for 0, 5, 10, 15 and 20% molasses treatment, respectively (Table 4.23). These data show that molasses treatment was not able to lift the energy content of PA sufficiently to change a low-quality to high-quality roughage (ARC 1980).

Voluntary intake of digestible dry matter and digestible organic matter of the experimental diets are shown in Table 4.24. Because of the effects of digestibility on the calculation of digestible dry-matter and organic-matter intake of molasses treated *Posidonia*, these values are different from the intakes of untreated *Posidonia* (0%).

Table 4.23: Apparent digestibility *in vivo* of molasses-treated *Posidonia* (Means ± SE)

Diet (% Molasses)	Dry matter digestibility (%)	Organic matter digestibility (%)	Digestible energy (MJ/Kg DM)	Metabolisable energy (MJ/Kg DM)
0%	33.5 ± 1.0	22.7 ± 0.6	5.9 ± 0.2	4.8 ± 0.2
5%	35.0 ± 1.5	23.7 ± 0.7	6.2 ± 0.3	5.0 ± 0.2
10%	36.9 ± 1.2	25.0 ± 1.0	6.6 ± 0.2	5.3 ± 0.2
15%	39.5 ± 1.3	26.8 ± 0.6	7.1 ± 0.3	5.7 ± 0.2
20%	42.4 ± 1.4	28.8 ± 0.6	7.6 ± 0.3	6.2 ± 0.2
Signi. Level	**	**	**	**
LSD (5%)	2.8	1.9	0.54	0.4

** = P<0.01

Table 4.24: Voluntary digestible dry-matter and digestible organic-matter intakes of *Posidonia* mixed with different proportions of molasses . (Means ±SE)

Diet (% Molasses)	Digestible dry matter intake (g/Kg W0.75/d)	Digestible organic matter intake (g/Kg W0.75/d)
0	2.8 ± 0.09	1.5 ± 0.05
5	3.3 ± 0.15	1.8 ± 0.06
10	4.3 ± 0.14	2.4 ± 0.09
15	5.2 ± 0.22	2.9 ± 0.09
20	7.1 ± 0.22	4.1 ± 0.08
Significance	**	**
LSD (0.05)	0.38	0.19

** = P<0.01

Body weight: The effect of the different concentrations of molasses on sheep body weight changes are presented in Table 4.22. All sheep lost weight during the experiment.

4.7.3 Discussion

Following on from the previous experiments (sections 4.3, 4.4, 4.5 and 4.6) the purpose of this experiment was to examine the possibility of improving the nutritive value of *Posidonia* by adding molasses as a supplementary ingredient.

In summary molasses at high enough concentration improved both the voluntary intake of *Posidonia* by sheep and its digestibility.

Voluntary intake of *Posidonia* treated with 20% molasses was more than twice than that for untreated material. It has been known for a long time that molasses is a palatable feed (Morrison 1957; Mc Donald *et al.* 1988) and that inclusion of molasses into animal diets based on low-quality feed increases voluntary intake (Ernst *et al.* 1975; Schiere *et al.* 1988). As previous presented the ash content of *Posidonia*, decomposed *Posidonia* and these two after use as bedding material (PL and DPL) was high, due perhaps to the presence of beach sands and dust, so because of its ability to control dust this could be another reason to add molasses to *Posidonia* as well as to increase platability and to provide energy.

4.8 General discussion

This section was aims to discuss generally some important results of the experiments of this current chapter.

In terms of chemical composition protein and ash are two considerable nutrients. Table 4.1 compares the CP content of *Posidonia*, decomposed *Posidonia* and both these two after using as bedding matterial. This table shows that the CP content of *Posidonia* and decomposed *Posidonia* is low per se, but after being used as bedding material this value increased up to more than 16% of dry matter. In addition, increasing the proportion of lucerne to mixed diets in all experiments caused considerable increase in CP content. In general it can be concluded that in order to use *Posidonia* or its different physical forms as a feed for sheep, it should be supplemented by some protein source.

One of the important, nutrient in the experimental feeds was ash. As Table 4.1 shows the ash content of *Posidonia* and decomposed *Posidonia* was 20.5 and 40.5% respectively.

Although ash as a source of minerals can be an advantage for a feed its high level can also decrease the proportional amounts of some other valuable nutrients, and consequently decrease both palatability and nutritive value of the feed. The suplementation of *Posidonia* and decomposed *Posidonia* with chicken manure and low-ash content forage was able to decrease the proportion of ash in the feed. In addition, adding some supplement such as molasses can decrease the dust problems of these feeds.

Determination of voluntary intake and digestibility *in vivo* of *Posidonia* and decomposed *Posidonia* were two important aims of this experiment. Figure 4.9 compares the voluntary intake of the experimental feeds as a whole and as combined diets. One important result from this chapter is that clearly the voluntary intake of P, DP, PL and DPL is so low that on their own cannot these materials meet the sheep's requirements for a whole diets. Supplementation, however with a protein source or a platable forage, such as lucerne or molasses, could be used to compensate for this deficiency. The different intakes of the diets with solely 100% lucerne in the different experiments here was probably due to different environmental and physiological conditions governing at in different times for the experimental animals.

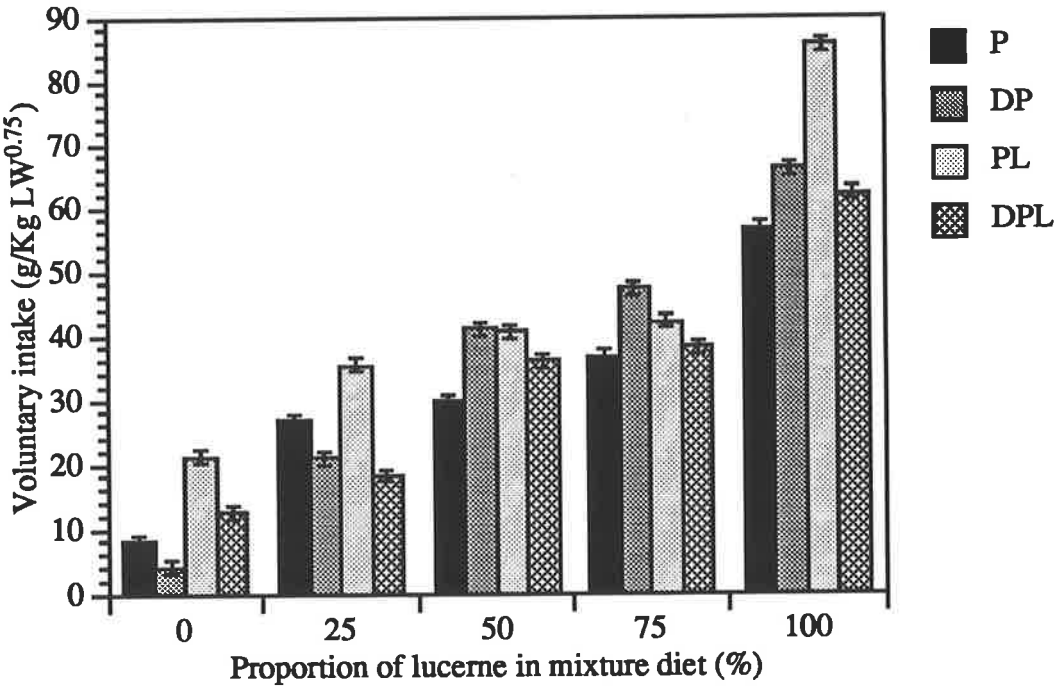


Figure 4.9: Comparison of dry matter voluntary intake of diets in experiments 3, 4, 5 and 6

Figure 4.10 compares dry-matter digestibility of the diets in the first four experiment of this chapter. It is evident that DMD of pure *Posidonia* is very low, so that it can be classified as a poor-quality roughage. However the results show that some physical, biological and supplementary methods can be useful to improve its nutritive value in this regard. Decomposition and supplementation with chicken manure and lucerne can improve *Posidonia* digestibility *in vivo*.

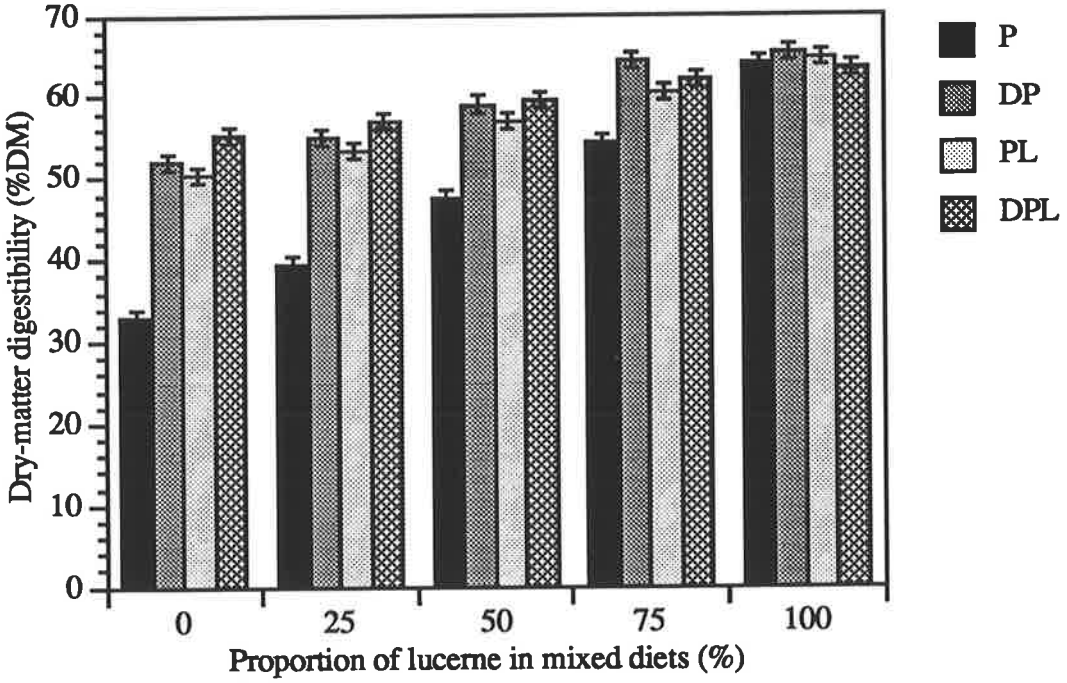


Figure 4.10: Comparison of dry-matter digestibility of diets in experiments 3, 4, 5 and 6

The combination of the effects on both digestibility and voluntary intake is revealed in the form of digestible dry-matter and organic-matter intakes are shown in Figure 4.11 and 4.12. Although there are differences between these two values in these two figures, but the trends of increasing due to increase of lucerne proportion are similar.

Digestible energy of the diets in the different experiments is presented in Figure 4.13. Although this value in pure *Posidonia* is so low that this feed must be classified as a poor quality roughage. Processing, including decomposition and chicken manure could promote the DE value of *Posidonia* up to almost equal to that of good quality feedstuffs.

A comparison of the data of body weight changes in the different experiments shows that body-weight changes apparently did not follow digestible energy intakes of the coresponding diets. The explanation for this could be that, (i) in short-term experiments of this type body weight dose not necessarily change according to the combination of intake and digestibility; (ii) Although the body gain of sheep is related to the energy produced from feed but the energetic efficiency of the ruminal fermentation is not necessarily directly related to the efficiency of production of the ruminant animal, for example, the growth rates of the animal can be improved without a concomitant increase in fibre digestion (Van Nevel and Demeyer 1988; Mc Sweeny *et al.* 1994) and in contrast increase in fibre digestion can be accoured without increase in animal growth . In last case, the existance of unknown anti-nutritive factors could be expected.

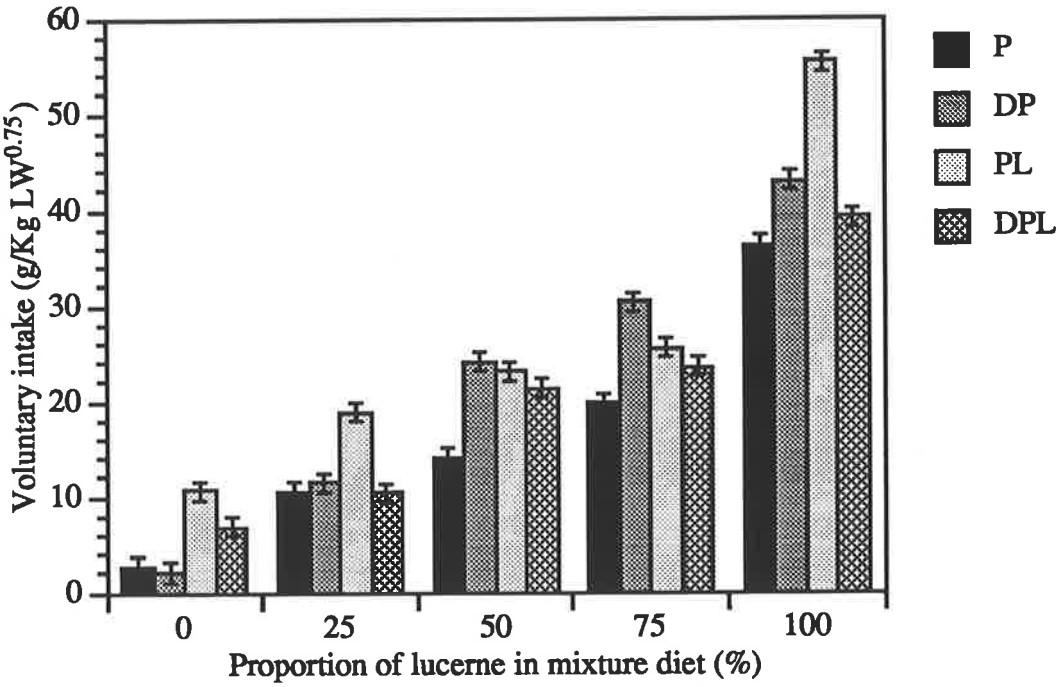


Figure 4.11: Comparison of digestible dry matter intake of diets in experiment 3, 4, 5 and 6

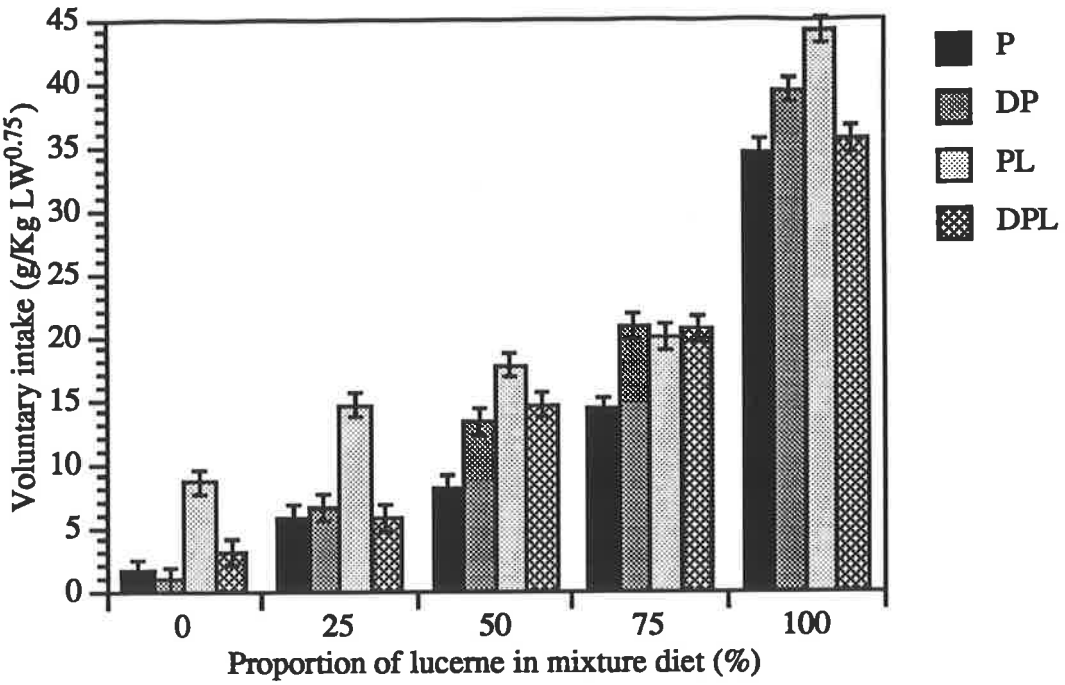


Figure 4.12: Comparison of digestible organic matter intake of diets in experiment 3, 4, 5 and 6

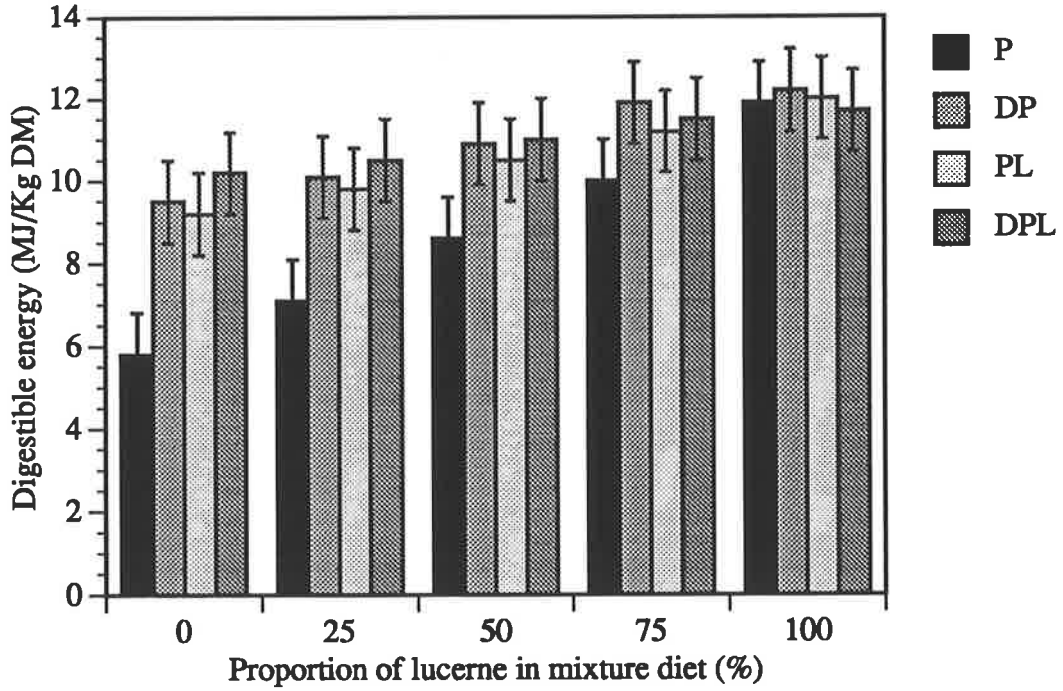


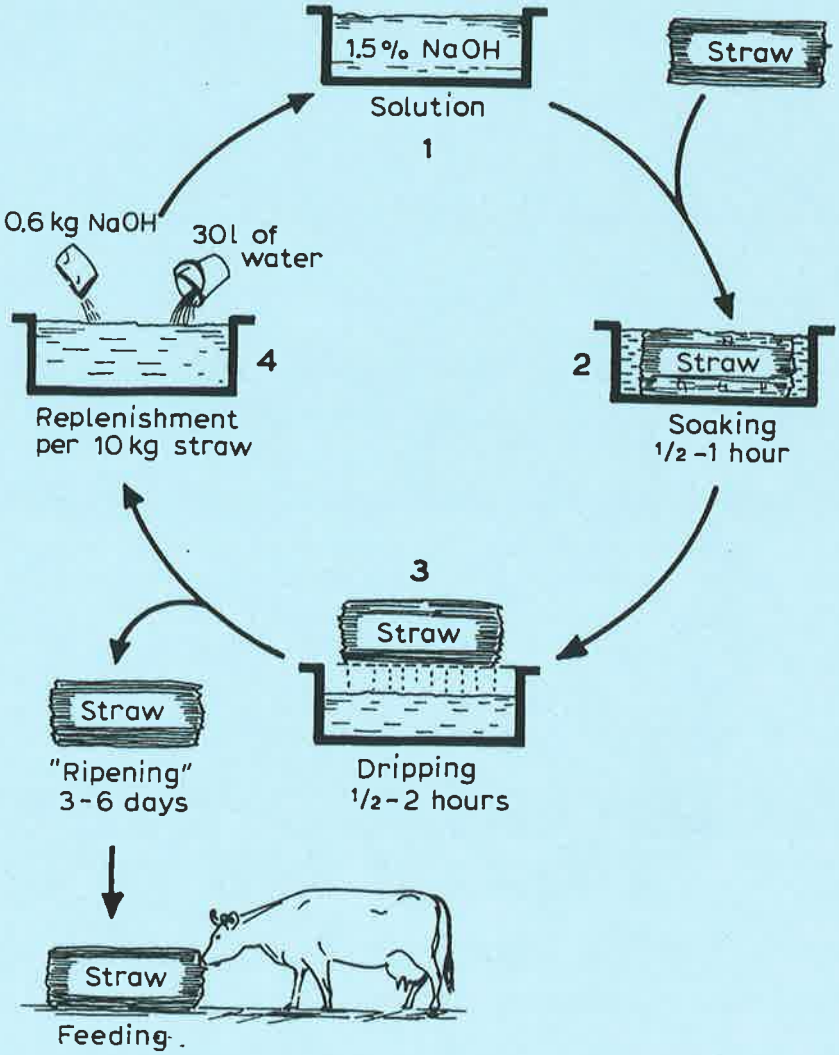
Figure 4.13: Comparison of digestible energy of diets in experiments 3, 4, 5 and 6

In summary it can be concluded that these methods of evaluation of *Posidonia in vivo* confirmed the results of laboratory work *in vitro* of previous chapter. *Posidonia* is low in protein, energy, voluntary intake and digestibility, and high in fibre content. In spite of these characteristics it is potentially susceptible to improvement in nutritive value by physical and biological methods. Meanwhile, the use of both *Posidonia* and decomposed *Posidonia* as bedding material for broiler chickens revealed some intersting results. Not only can improve its nutritive value but also it showed that *Posidonia* can be used as a alternative commercial bedding material.

The next chapter will examine susceptibility of *Posidonia* to be improved by other chemical, physical and biological methods.

CHAPTER 5

Pretreatment of *Posidonia australis* with alkalis and fungi



"A great number of physical, chemical and biological methods have shown positive effects on the nutritive value of roughages". (Sundstøl 1988).

CHAPTER 5

Pretreatment of *Posidonia australis* with alkalis and fungi

5.1 Introduction

The results of Chapter 3 indicated that the fibre content of *Posidonia* is high and that probably the high lignin fraction of this fibre contributes to its low digestibility *in vitro* (34.7%). In order to achieve some practical understanding of the possible nutritive value of *Posidonia* the experiments of chapter 3 were followed by some work *in vivo* (Chapter 4), which determined predominantly voluntary intake and digestibility of both un-processed and processed (physical, biological and supplementary) *Posidonia*. As recorded there it was found that decomposition and/or supplementation with chicken manure or molasses can improve the nutritive value of *Posidonia* in some aspects. It thus seemed useful to determine if some other, perhaps more usual, methods of treatment could also improve the nutritive value of *Posidonia*.

Posidonia, as a lignocellulosic material, could possibly be an abundant source of energy for ruminants. In this type of material the cellulose and other structural carbohydrates might be closely associated with lignin and other similar components that would make them less available to microbial fermentation in the digestive tract (Han 1978). Because of the particular physico-chemical structure of the cell-wall components of such lignocellulosic materials they have generally not been used to their full potential as ruminant feedstuffs (Jung 1989, Latham 1979).

It has been understood for a long time, however, that the feeding value of such fibrous materials can be upgraded by a range of physical, chemical and biological treatments (Ibrahim 1983; Jung *et al.* 1992). For instance, in order to improve the digestibility and accessibility of the structural carbohydrate of highly-lignified materials to microbial enzymes in the rumen various chemical treatments have been used. Most attention has been directed

to use of alkalis, although a wide range of other chemicals have also been tried (Wilkins 1981).

Sodium and calcium hydroxide and ammonia are three important alkalis that have been routinely used in experimentation to increase the digestibility of roughages for livestock. Although alkali treatment of fibrous materials has been researched extensively, the estimation of the level of alkali and of the reaction times that would result in the highest digestibility of *Posidonia* or any other novel materials requires much ongoing research.

In the area of biological treatment the main attempts to treat lignocellulosic materials have been with white-rot fungi, which degrade lignin itself rather than structural carbohydrates (Han 1978; Latham 1979; Jung *et al.* 1992). In the present experiments two species of available white-rot fungi, *Coriolus versicolor* and *Phlebia gigantea*, were used.

There appear to be no reports elsewhere in the literature on the effects of the above-mentioned treatments on *Posidonia*. Therefore, the general purposes of the series of experiments described in this chapter were:

i) determination of the possibly effects of type and levels of alkali in different reaction times and the effect of fungal treatment on the nutritive value of *Posidonia* ;

ii) comparison the relative effectiveness of the above methods;

iii) provision of further useful information for considering cost-effective feedlot feeding of sheep with *Posidonia*.

To achieve these aims two main series of experiments were carried out: i) effect of the above treatments on digestibility *in vitro* of *Posidonia* and ii) their effects on the disappearance of structural carbohydrates of *Posidonia* in the rumen of sheep, as measured *in sacco*.

5.2 Experiment 8: The effect of alkali and fungal treatments on the digestibility *in vitro* of *Posidonia australis*

In this series of experiments the effects of sodium hydroxide, calcium hydroxide, ammonification and two species of fungi on the digestibility of *Posidonia* were separately examined.

5.2.1 Materials and methods

***Posidonia*:** *Posidonia* used in this experiments came from the same sources as that used in the experiment described in section 4.3. Adequate amounts of *Posidonia* were chopped to make a particle size of about 2 cm.

Treatment procedures

a) NaOH spraying method: Sixteen samples, each of 1 Kg (DM basis) were prepared. They were spread on a smooth surface covered with thick plastic sheeting and treated with solutions containing different amounts of NaOH (2%, 4%, 6% and 8% of *Posidonia*, w/w). For each concentration of NaOH 4 samples were prepared in order to provide different reaction times (1, 2, 3 and 4 days). The required amount of NaOH were dissolved in as much water as was necessary to produce a final water content of the feed of about 50%. The NaOH solution was sprinkled over the *Posidonia* from a plastic garden watering can. The *Posidonia* was thoroughly mixed with the solution by hand and then sealed in a plastic bag and held at ambient temperature in a covered polyethylene bucket. After appropriate reaction times the bags were opened, the contents transferred to trays and placed in a forced-draught oven at 60°C for 48 hrs. After drying the treated *Posidonia* samples were ground by hammer-mill (1-mm mesh sieve) and kept in air-tight plastic containers for subsequent digestibility determinations *in vitro* and *in sacco* .

b) $Ca(OH)_2$ soaking method: Treated *Posidonia* was prepared by soaking representative samples for 1, 2, 3 and 4 days in canvas bags with a pore size of 1x1 mm solutions containing different concentrations of calcium hydroxide (0%, 2%, 4%, 6% and 8%) in 30 litre buckets. The liquid-to-*Posidonia* ratio (W/W) was 15:1. The suspensions of

calcium hydroxide containing *Posidonia* were stirred every six hours. At the end of soaking periods the canvas bags containing *Posidonia* were transferred from the buckets to a basket for drainage of free liquid.

The treated *Posidonia* samples were then dried and ground as described above for the NaOH treatment.

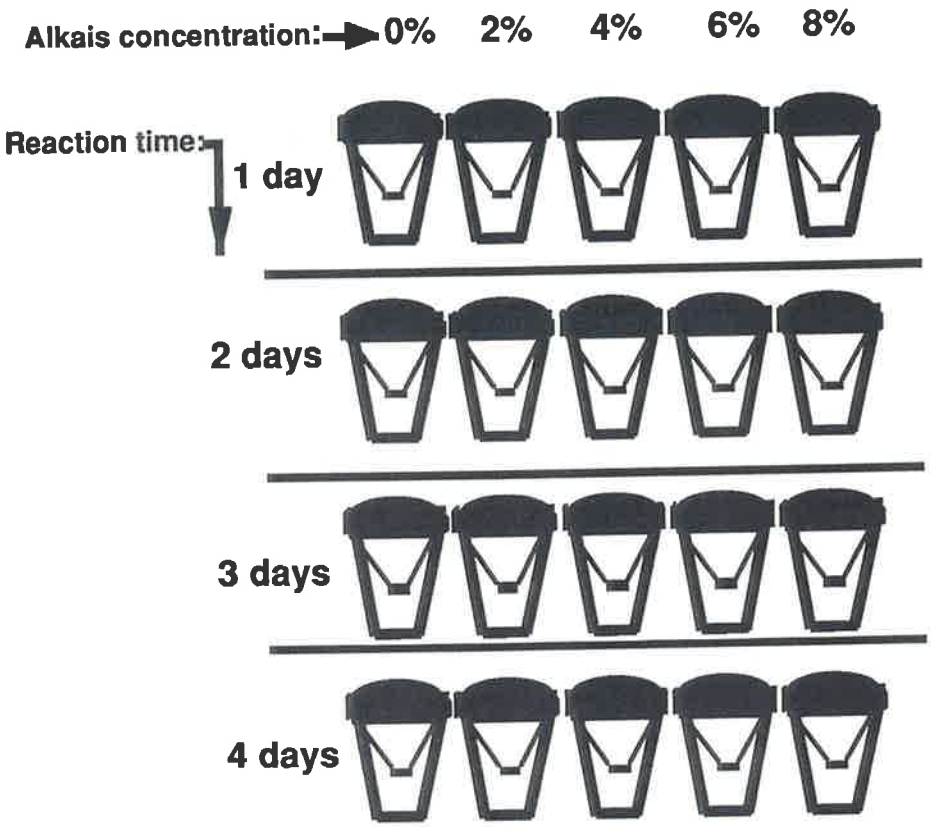
c) NH_3: Ammonification of *Posidonia* was carried out applying a urea solution under anaerobic condition. Sixteen samples of 1 Kg *Posidonia* (DM basis) were prepared. They were sprayed with urea (fertiliser grade, 46% N) solution by the same methods and at the same concentrations as for NaOH treatment. After the samples and urea were mixed they were separately placed in double plastic bags, pressed and then sealed in another plastic bag so that all air was excluded. Bags were held at ambient temperature (minimum 20°C) and for each concentration of urea (2%, 4%, 6%, and 8%) four reaction times (5, 10, 15 and 20 days) were applied. The treated samples were then dried and ground as already described.

For all three different alkali treatments one untreated sample was used as a control. The general view of keeping the treated *Posidonia* in polyethylene buckets, in anaerobic condition is shown in plate 5.1.

d) Fungal treatment: The author is grateful to acknowledge that the fungal species were kindly provided by Dr. Olga Collett, CSIRO Division of Forest Products, Victoria, Australia. He also acknowledges the technical help in fungal culture received from Mr. Reza Balali, Ph.D student, Department of Crop Protection, Waite Institute.

The modified method of Jung *et al.* (1992) was applied to treat *Posidonia* with fungi. Two species of white-rot *Basidiomycetes*, *Coriolus versicolor* (CV) and *Phlebia gigantea* (PG) were selected, based on both their specificity for lignin degradation and their availability. They had been originally isolated from fruiting bodies on a pine log and pine wood respectively. Fungal cultures (obtained from Victoria) were maintained in the Department of Crop Protection, Waite Institute. Fifteen days before *Posidonia* inoculation each fungus was grown in a number of petri dishes containing medium based on 1.25% malt extract, 1.8% agar and tap water and incubated at 25°C to produce an appropriate mycelial mass.

Plate 5.1: General view of keeping alkalis-treated *Posidonia* in buckets during intraction times (top) (incubation times for NH3 treatment were 5, 10, 15 and 20 days); and sample preparation for inoculation with fungi (A1, A2; C1, C2; and B1, B2 are chopped and ground samples respectively for *Coriolus versicolor*, *Phlebia gigantea* treatments and control) (bottom)



For each fungus two physical forms (chopped and ground) of *Posidonia* were tried, of particle lengths approx. 2-5 cm and 1mm respectively. Samples (100 gr) were placed into 2-litre Erlenmeyer flasks and 186 ml of distilled water added (65% moisture). A rubber stopper fitted with a glass port, plugged with glass wool, was used to seal each flask and to provide aeration (Leisola *et al.* 1983). Samples were sterilised by autoclaving for 30 minutes at 121°C and 1.1 Kg/cm^2 pressure. Each flask was aseptically inoculated twenty times with mycelial plugs, consisting of a relatively small mass (50 to 100 mg each time) of actively growing mycelium from one or other fungal species and then incubated at 25-28°C and 90% humidity for 30 days.

As controls two others flasks (one for chopped and another one then for ground sample) were prepared as above but without fungal inoculation.

After relevant reaction times the treated samples were dried and ground as already described. The arrangement of samples for inoculation is shown in Plate 5.1.

Measurements: Dry matter, organic matter, dry-matter digestibility and organic-matter digestibility *in vitro* of samples were determined by the methods already mentioned in Chapter 3.

Experimental design: A factorial-type design was used for DM and OM digestibility measurements using four replications (R). The experimental factors for each alkali treatment were as follows:

Alkali concentration (factor A): 0% (A1), 2% (A2), 4% (A3), 6% (A4), 8% (A5).

Reaction time (factor B): 1 day (B1), 2 days (B2), 3 days (B3), 4 days (B4); (for urea 5, 10, 15, and 20 days respectively).

Thus the total number of measurements were 80 [A (5) x B (4) x R (4))]. All data were subjected to analysis of variance using the Super-ANOVA (Abcus Concepts Inc.) program. Comparison of means was carried out using LSD procedures (Snedecore and Cochran 1971).

The same general design was used for fungal treatment, including two factors:

Fungi (factor A): control (A1), CV (A2), PG (A3)

Physical form (factor B): chopped (B1), ground (B2)

The total number of measurements were thus 24 (A (3) x B (2) x R (4)

5.2.2 Results

Sodium hydroxide: The effects of different concentrations of sodium hydroxide for different reaction times on dry-matter and organic-matter digestibility of *Posidonia* are shown in Figures 5.1 and 5.2. These data are also presented with statistical details in Appendices 5.1 and 5.2 respectively.

Posidonia treated with any concentration of NaOH had higher DMD and OMD values than the control (0%) ($P<0.01$). These values increased with increasing concentration of NaOH ($P<0.01$).

Reaction periods (days) had a significant effect ($P<0.01$) on the DMD and OMD of the *Posidonia* treated with different concentration of NaOH. The interaction between concentration of NaOH and reaction time was not significant ($P>0.05$) (Appendices 5.1 and 5.2).

The OMD of *Posidonia* at any NaOH-concentration and any reaction time was lower than DMD, but the effect of treatment on the these two values was similar.

Calcium hydroxide: The digestibility of dry matter and organic matter of *Posidonia* treated with calcium hydroxide is given in Figures 5.3 and 5.4 respectively. The corresponding statistical details are shown in Appendices 5.3 and 5.4.

At all concentrations of alkali both dry-matter and organic-matter digestibility was increased. In general reaction time had no significant effect ($P>0.01$) on either DM or OM digestibility.

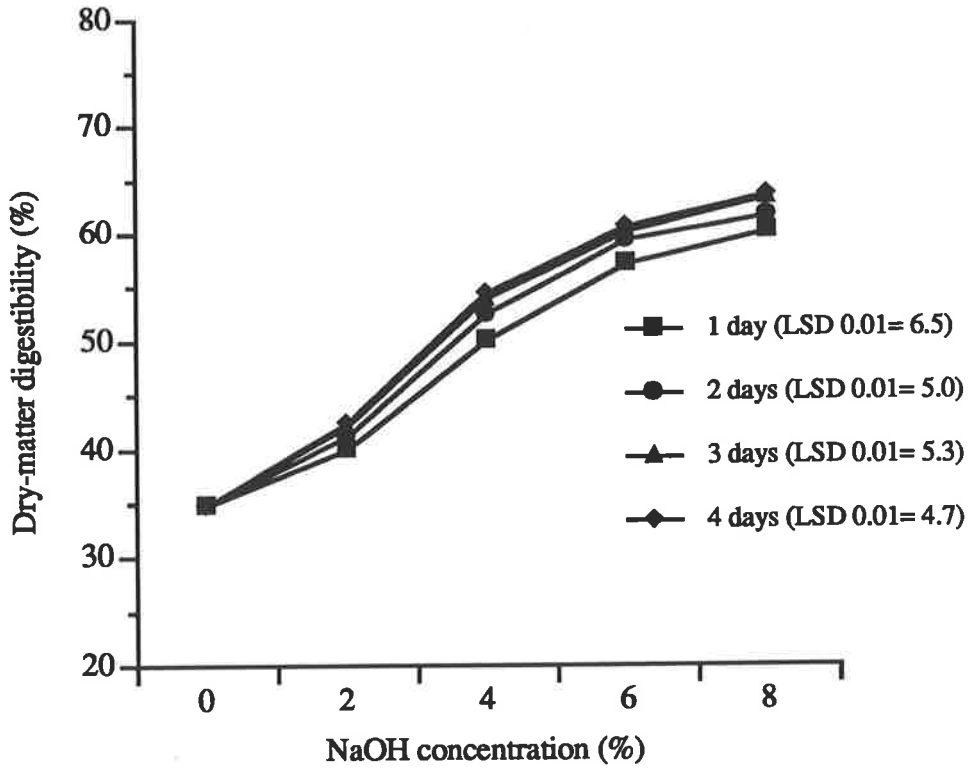


Figure 5.1: The effect of NaOH treatment on dry-matter digestibility *in vitro* of *Posidonia australis*

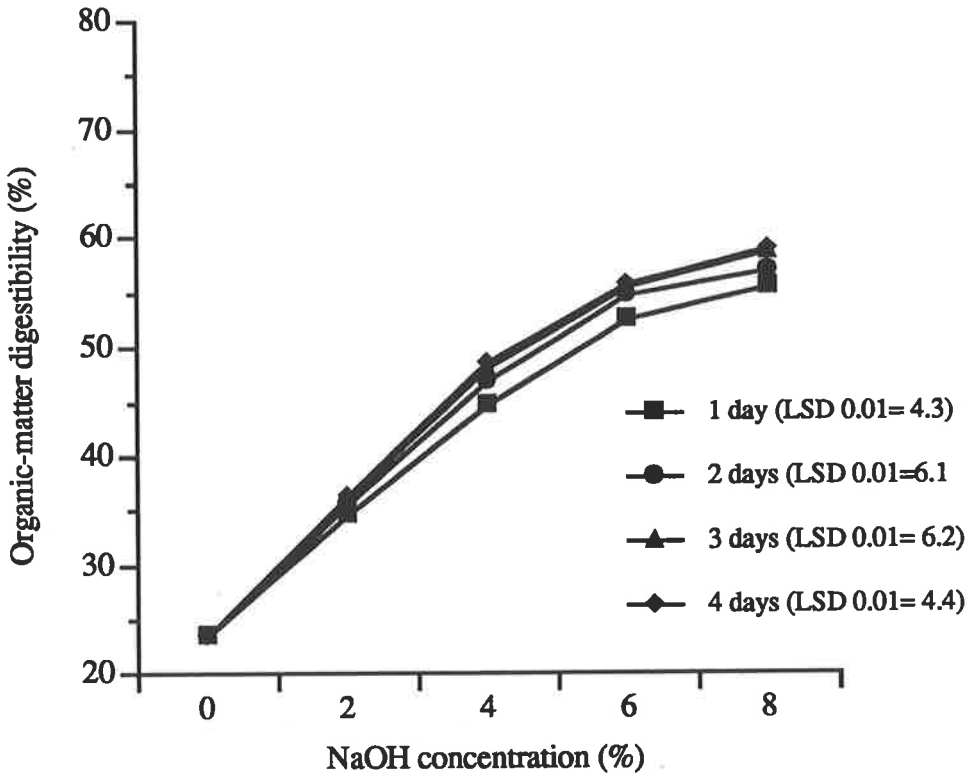


Figure 5.2: The effect of NaOH treatment on organic-matter digestibility *in vitro* of *Posidonia australis*

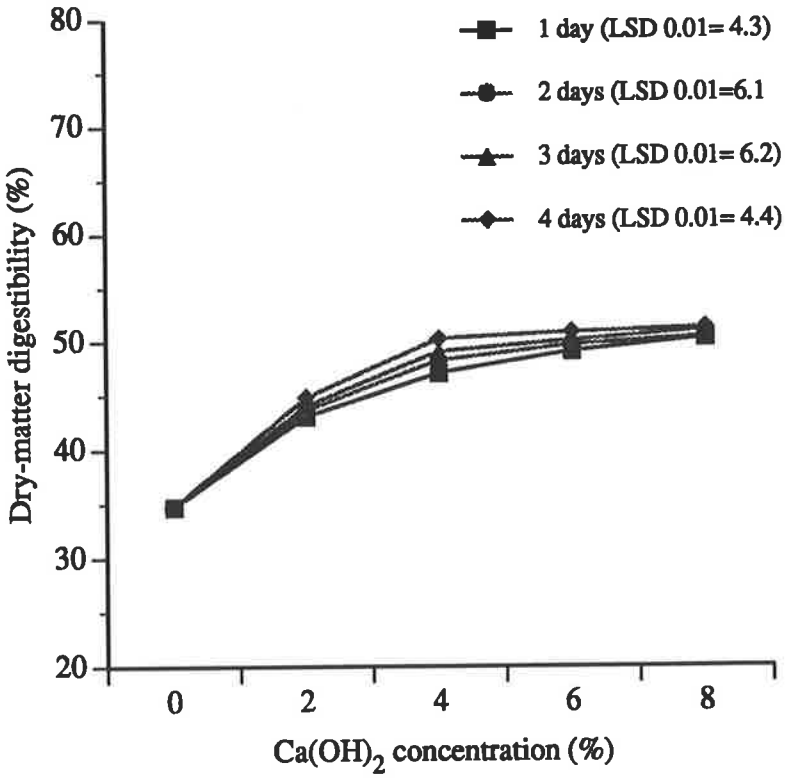


Figure 5.3: The effect of $Ca(OH)_2$ treatment on dry -matter digestibility *in vitro* of *Posidonia australis*

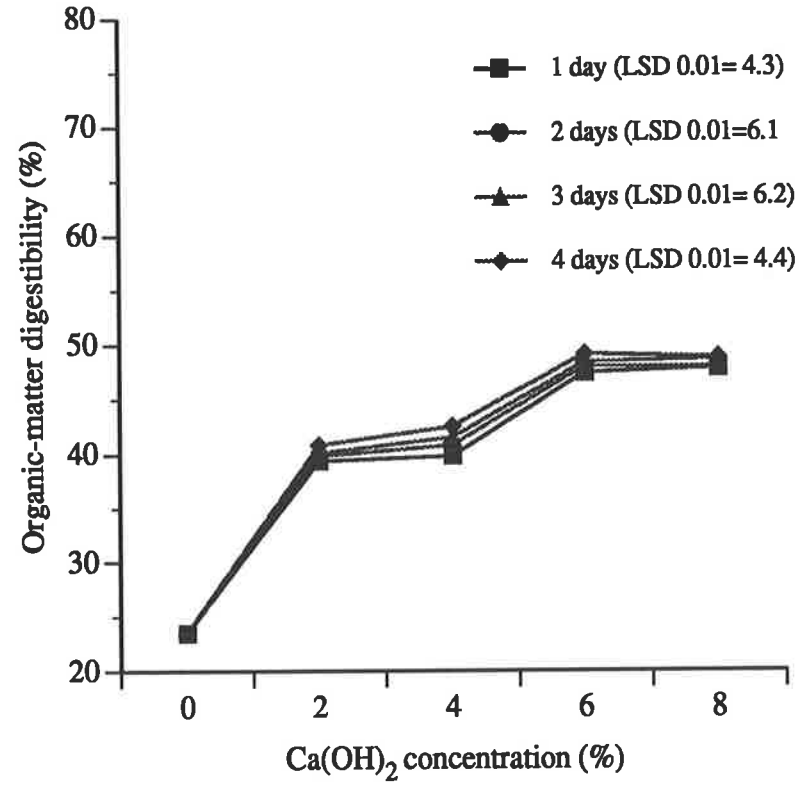


Figure 5.4: The effect of $Ca(OH)_2$ treatment on organic-matter digestibility *in vitro* of *Posidonia australis*

The interaction between $Ca(OH)_2$ concentration and reaction time was not significant.

Ammonification: DMD and OMD measurements *in vitro* of *Posidonia* treated with different concentrations of urea for different times are shown in Figures 5.5 and 5.6 and Appendices 5.5 and 5.6.

These data show that at all concentrations of urea both dry matter and organic matter digestibilities were increased significantly above control.

The influence of 15 and 20 days of incubation was especially significant on dry-matter and organic-matter digestibility, with ammonification over 20 days the most effective.

The reaction between urea concentration and incubation time was not significant.

Regression (linear) equation and regression coefficient of alkali (NaOH, Ca $(OH)_2$ and NH3) concentration and dry matter digestibility of *Posidonia* were calculated and are shown in Figure 5.7 and Table 5.1. These data show that the correlation between these two factors for each alkali is positive. Generally, the regression coefficient (r^2) for $Ca(OH)_2$-treated *Posidonia* over any reaction time is slightly less than that for NaOH and NH3-treated *Posidonia.*

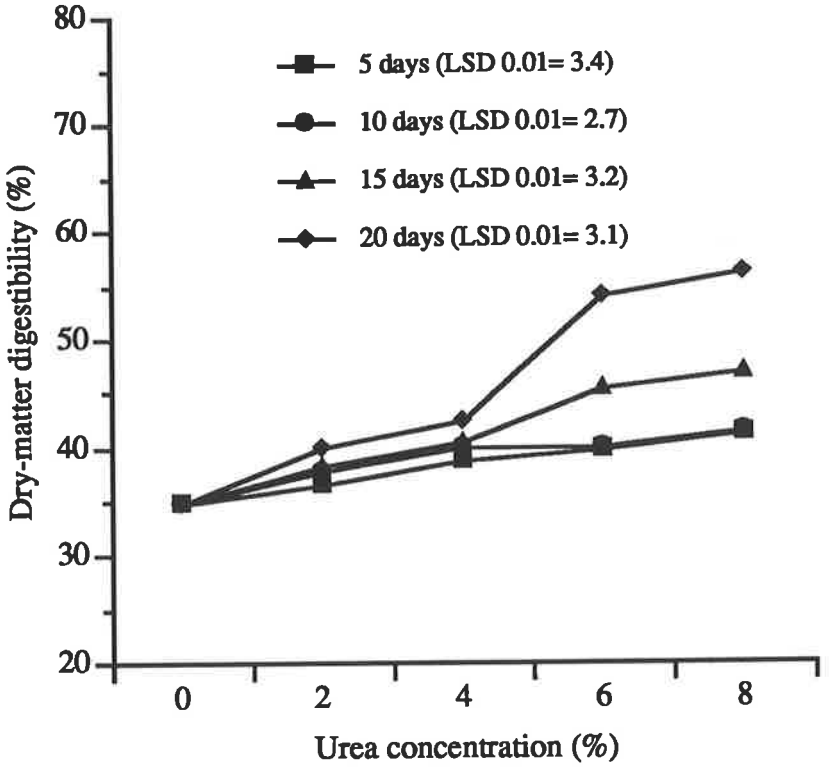


Figure 5.5: The effect of NH_3 treatment on dry-matter digestibility of *Posidonia australis*

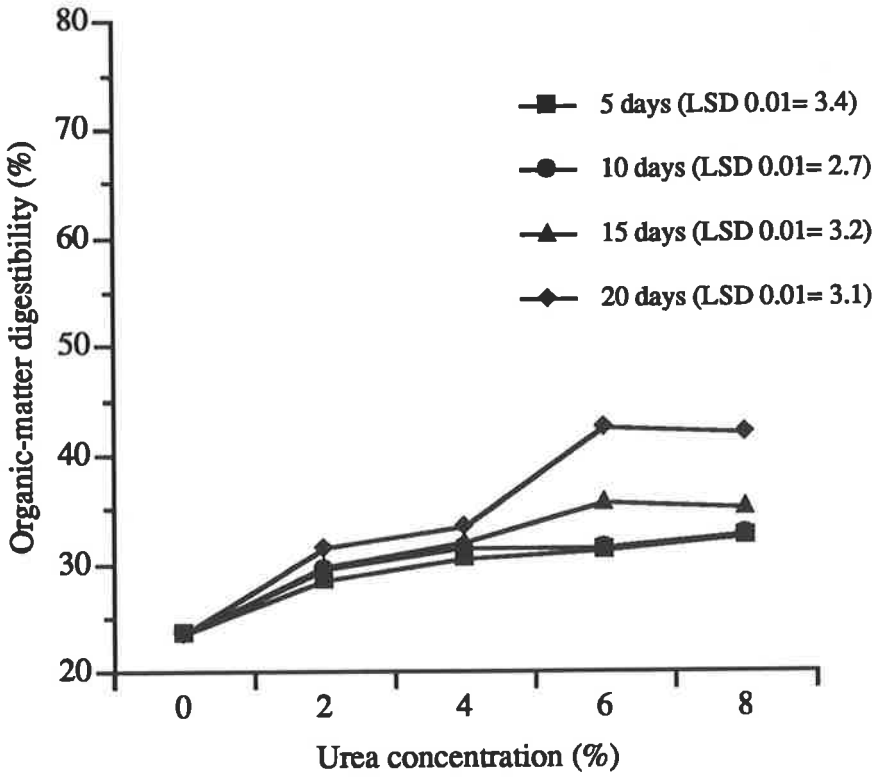


Figure 5.6: The effect of NH_3 treatment on organic-matter digestibility of *Posidonia australis*

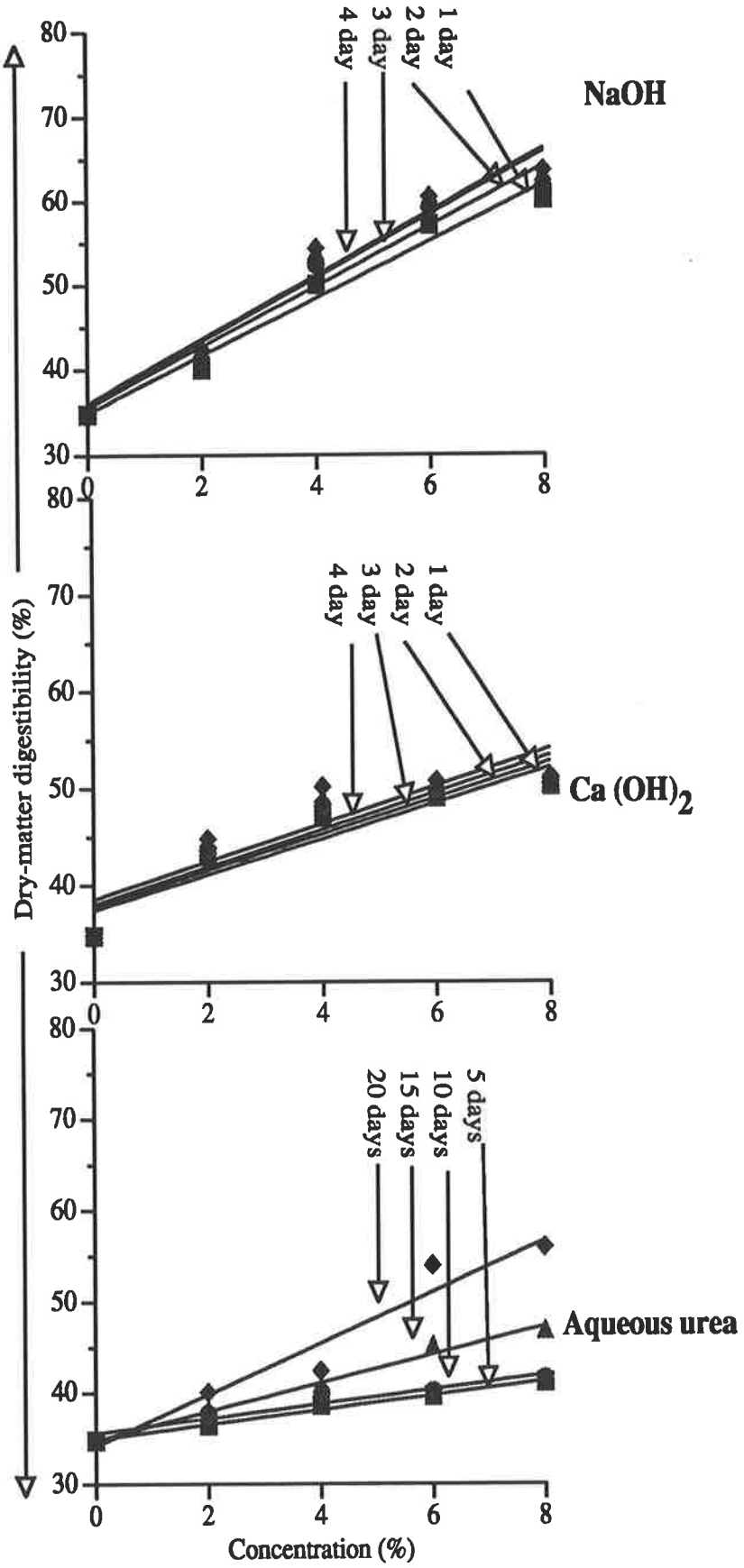


Figure 5.7: Linear regression of dry-matter digestibility of *Posidonia australis* on alkalis and aqueous urea concentrations

Table 5.1: Relationship between alkali concentration and dry matter digestibility of *Posidonia australis* with sodium hydroxide, calcium hydroxide and aqueous urea (NH_3) for different incubation times

Y	X	r^2	Regression equation
Dry-matter digestibility	NaOH concentration (0, 2, 4, 6, 8%)		
		1 day = 0.973	Y = 338X + 34.8
		2 days = 0.959	Y = 357X + 35.5
		3 days = 0.960	Y = 376X + 35.8
		4 days = 0.960	Y = 378X + 35.9
" "	$Ca(OH)_2$ concentration (0, 2, 4, 6, 8%)		
		1 days = 0.873	Y = 185X + 37.3
		2 days = 0.832	Y = 187X + 37.8
		3 days = 0.822	Y = 193X + 38.0
		4 days = 0.775	Y = 195X + 38.5
" "	Aqueous urea (NH_3) concentration		
		5 days = 0.987	Y = 80X + 34.8
		10 days = 0.909	Y = 80X + 35.5
		15 days = 0.983	Y = 158X + 34.8
		20 days = 0.945	Y = 283X + 34.1

Fungi: The influence of fungal treatment on the dry-matter and organic-matter digestibilities of chopped and ground *Posidonia* is shown in Tables 5.2 and 5.3 respectively. The data indicate that after the 30 days incubation both fungal species, *Coriolus versicolor* and *Phelobia gigantea,* caused a significant ($P<0.01$) increase in DMD and OMD of *Posidonia.*

The physical form of *Posidonia* used (chopped and ground) had a slight effect on the digestibility of fungi-treated *Posidonia.* Grinding was more effective than chopping (40.1% *vs* 39.1% for DMD and 26.8% *vs* 26.1% for OMD) in this regard. The interaction between fungal treatment and physical forms was not significant.

Table 5.2: Dry-matter digestibility *in vitro* of chopped and ground *Posidonia australis* treated by fungi

Fungal treatment	Physical form of *Posidonia*		Mean
	Chopped	Ground	
Control	34.1	35.2	34.7[a]
Coriolus versicolor	39.9	41.2	40.6[b]
Phlebia gigantea	43.2	44.0	43.6[c]
Mean	39.1[a]	40.1[b]	

Significance:	LSD*(0.05)
Interaction (Fungi x physical form)	NS**
Fungi	1.1
Physical form	0.9

* LSD= Least significant difference ** Non significant
Different letters indicate significant difference

Table 5.3: Organic-matter digestibility *in vitro* of chopped and ground *Posidonia australis* treated by fungi

Fungi treatment	Physical form		Mean
	Chopped	Ground	
Control	21.0	21.7	21.4[a]
Coriolus versicolor	26.2	27.1	26.7[b]
Phlebia gigantea	31.0	31.6	31.3[c]
Mean	26.1[a]	26.8[b]	

Significance:	LSD*(0.05)
Interaction (Fungi x physical form)	NS**
Fungi	1.1
Physical form	0.6

* LSD= Least significant difference ** NS Non significant
Different letters indicate significant difference

5.3: Experiment 9: Rumen degradability of structural cell-wall constituents of alkali and fungal-treated *Posidonia*.

In order to achieve a better understanding of the role of microbial activities on the rumen degradability of *Posidonia*, some complementary-studies *in sacco* were carried out as follows:

i) examination of the rumen degradability (DM and OM) of untreated *Posidonia* following different incubation times (0, 12, 24, 36, 48 and 72 hrs);

ii) examination of structural cell-wall degradability (NDF, ADF, ADL, cellulose and hemicellulose) following the most effective alkali and fungal treatments determined in the previous experiment.

5.3.1: Materials and methods

***Posidonia*:** Untreated and treated *Posidonia* (NaOH, Ca $(OH)_2$, ammonification and fungi), samples which were prepared for the previous experiments were also used in this experiment.

Structural cell-wall constituents of untreated and treated samples were determined by the methods already mentioned in Chapter 3.

Degradability (DM, OM, NDF, ADF, ADL, cellulose and hemicellulose) of untreated and treated samples were determined *in sacco* as follows:

a) Surgical procedures: The author acknowledges the technical help received from Dr. Neville Yates and Mr. Komang Gede Wiryawan, a former academic member and a Ph.D student respectively of the Department of Animal Science, Waite Institute.

A simple canula was inserted into the rumen of each of 4 experimental sheep by the procedures described by Hynd (1982) in the following manner.

Anaesthesia was induced with sodium pentobarbitone (Nembutal), and maintained via endotracheal tube with cyclopropane. An incision 5-6 cm long was made in the anteroidorsal portion of the flank, the muscles seperated by blunt dissection and the peritoneum cut and secured with mosquito forceps. A part of the dorsal sac of the rumen

was secured with bowel clamps and sutured to the fascia. The rumen was then cut and the exposed rumen walls held with hemostats while a flexible cannula (Hecker 1974) was inserted. An internal flange and rubber stopper completed the preparation. There was very little digesta leakage from the fistula because the incision was made as high as possible on the dorsal flank and a tight seal was maintained between the external and internal flanges.

Routine post-operative care was carried out, including a course of antibiotics for 3 days. Feed intake returned to normal in all four sheep within one week of surgery.

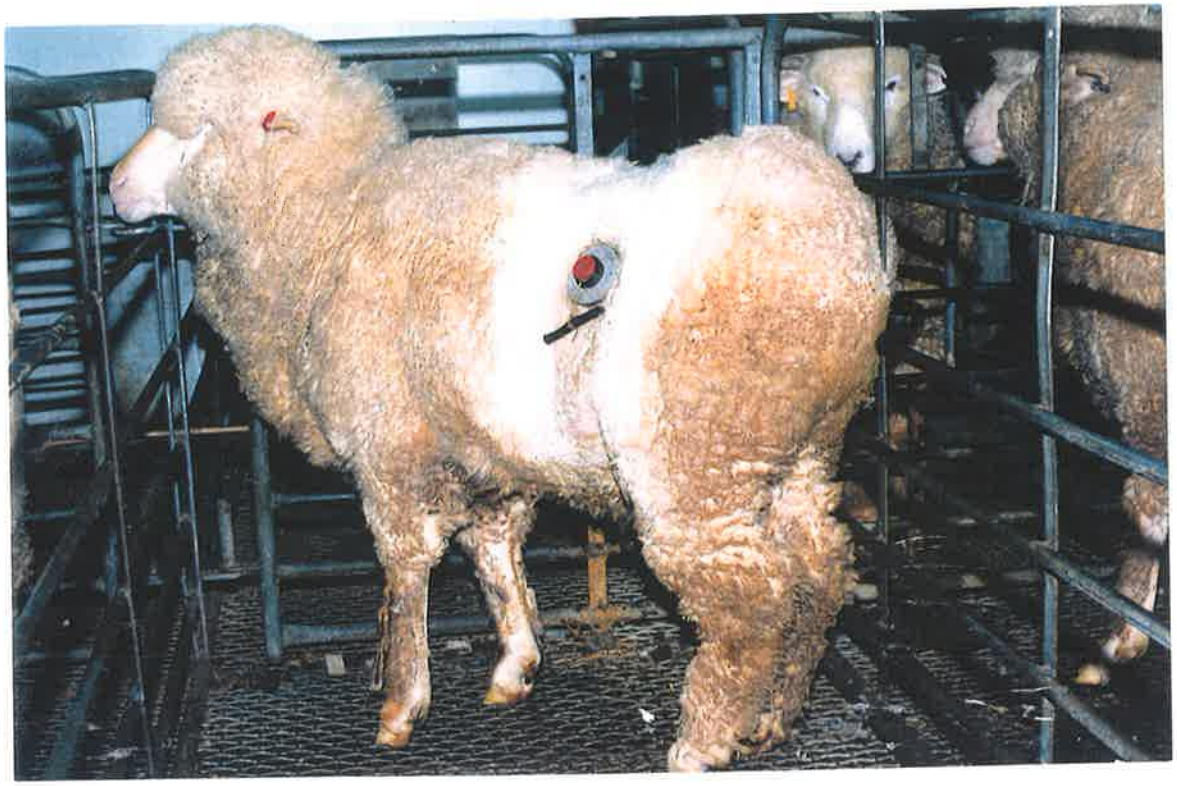
The experiment described here did not commence until 4 weeks post-surgery. During this period the sheep received a ration of lucerne chaff (1000 g/d) and were treated for parasites. Plate 5.2 (top) shows one of the experimental fistulated sheep.

b) Nylon bag characteristics: Bags were made from nylon, normal quality synthetic screen (Swiss Screen Pty. Ltd., Sydney, Australia) with 44 micro pore size. The internal dimensions of these bags were 12 x 8 cm. The bags were numbered with a permanent marker (Plate 5.2, bottom).

c) Incubation procedure: Four bags (replication) were used for each sample in different fistulated sheep. Samples of about 4 grams air dry material were placed in pre-dried and weighed nylon bags to give a sample mass:bag area ratio of 15-25 mg/cm^2. In order to reduce the likelihood of the bag floating a steel ball bearing of about 9g weight was also placed in each bag before tightly closing it with a drawstring (6 bags per string/sheep). The drawstrings were tied and the bags were secured at random at approximately one cm intervals near the end of a weighted 30-cm long nylon line. For securing the bags to the line (Plate 5.2, bottom) antiseptic latex rings (Elastrator Aust. Pty. Ltd.) were used. The line was attached to a wooden handle outside the fistula (Plate 5.2, bottom).

The tied bags containing the samples were placed into the rumen to a depth of 25 cm from the top of the fistula before the morning feeding when the rumen was relatively empty. The bags remained in the rumen for specific times.

Plate 5.2: Close-up view of a fistulated sheep (top) and bag-securing arrangements (bottom) used for experiments *in sacco*



In order to measure the disappearance of readily-soluble and small-particle-size materials four bags containing the same relative amount of sample were moistened and washed under tap water for about five minutes and then dried and treated in a manner similar to other samples. The weight lost due to this washing was regarded as control or zero hour degradation.

Five nylon bags containing *Posidonia* samples from 5 different treatment concentrations (0%, 2%, 4%, 6% and 8%) were suspended at the same time inside the rumen of each fistulated sheep. At the end of the prescribed incubation time all five bags were withdrawn at the same time. This same procedure was undertaken for different incubation times and for different samples.

After incubation the bags were removed and immediately cleaned of adhering digesta by washing under a gentle stream of tap water until the rinsing water was clear. Bags were oven-dried at 65°C for about 4-8 hours to constant weight.

The DM, OM, NDF, ADF, ADL, cellulose and hemicellulose losses were determined by weight differences of these constituents before and after incubation in the rumen.

Experimental design: A completely randomised design (CRD) was used to examine the effects of different treatments on dry-matter disappearance, organic-matter disappearance and the disappearance of structural cell-wall constituents .

5.3.2 Results

Untreated *Posidonia*:

Dry-matter disappearance and organic-matter disappearance of untreated *Posidonia* samples are shown in Figures 5.8 and 5.9. The data indicate that although initial solubility of samples was relatively high (9%) degradability increased significantly with incubation time. An incubation time of 72 hrs was most effective in this regard. Rumen degradability of *Posidonia in sacco* in 72 hrs was similar to the results of digestibility *in vivo* presented earlier in section 4.3.2 (DM: 31.2% vs 32.9%; OM: 21.5% vs 22.2%).

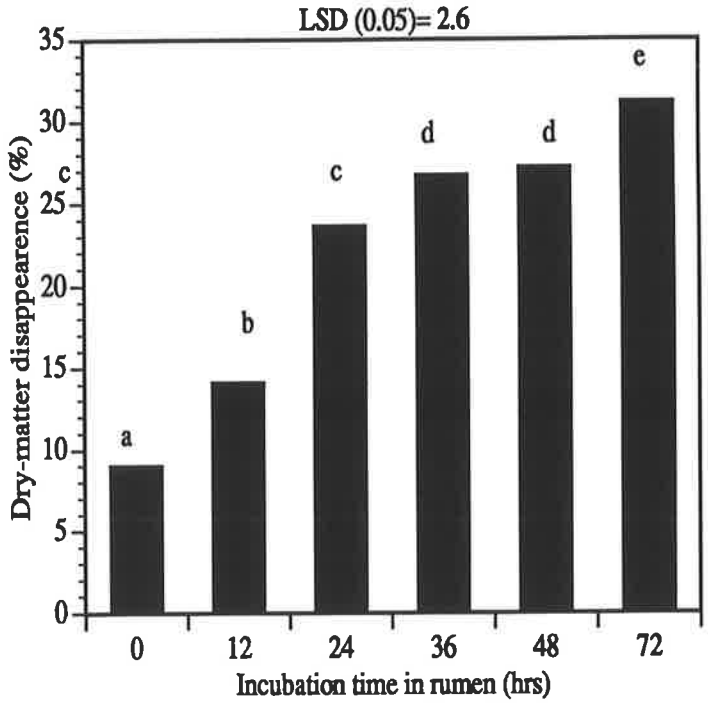


Figure 5.8: Dry-matter disapearance of *Posidonia australis* in the rumen of sheep. Columns with different letters show significant differences

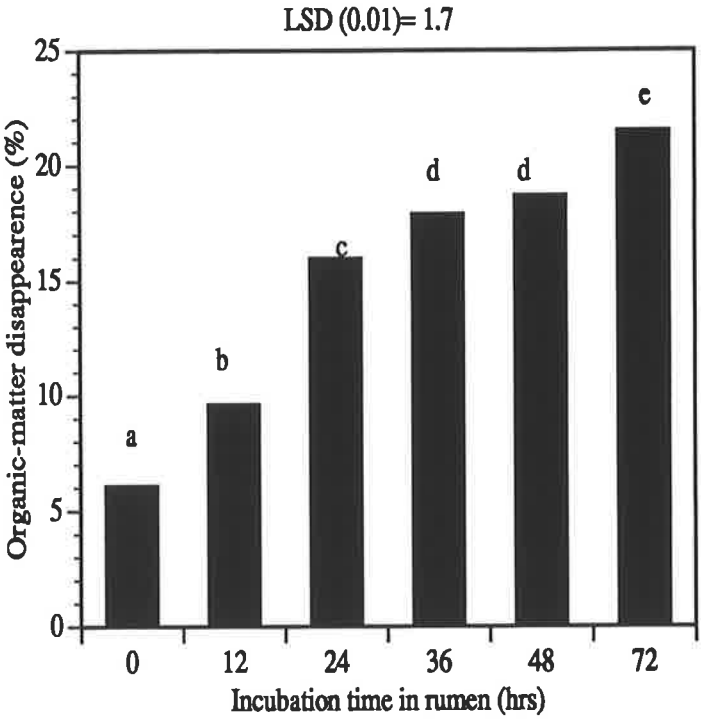


Figure 5.9: Organic-matter disapearance of *Posidonia australis* in the rumen of sheep. Columns with different letters show significant differences

Treated *Posidonia*:

DM and OM disappearances of alkali- and fungal-treated *Posidonia* after 72 hrs incubation in the rumen of sheep are shown in Figures 5.10 and 5.11. The results present the degradability of *Posidonia* treated with 8% NaOH and $Ca(OH)_2$ over 4 days reaction time and 8% urea solution over 20 days.

Posidonia treated with alkalis (NaOH, $Ca(OH)_2$ and NH_3) showed higher rumen degradability for both DM and OM than did that either treated with fungi or untreated. NaOH treatment resulted in significantly higher degradability value than $Ca(OH)_2$ and NH_3 treatments. $Ca(OH)_2$ and NH_3 treatments were the same for DM-degradability but different for OM-degradability.

DM-degradability as the result of fungal treatments were all significantly higher than the untreated control, except for CV (chopped). Organic-matter degradability of fungal-treatment *Posidonia* did not change relative to the untreated control, except that PG (ground) treatment caused a small but significant increase.

The effect of all experimental treatments on the degradability of structural carbohydrates (NDF, ADF, ADL, cellulose and hemicellulose) is presented in Table 5.4. Again the alkali treatments had more effect on degradability than did fungal treatment. Amongst the alkalis the influence of NaOH was greatest.

The fungal treatments, with the exception of PG (Ground), had no effect on the degradability of NDF, ADF, ADL nor cellulose. Amongst the cell-wall constituents hemicellulose was affected more than the others and ADL less.

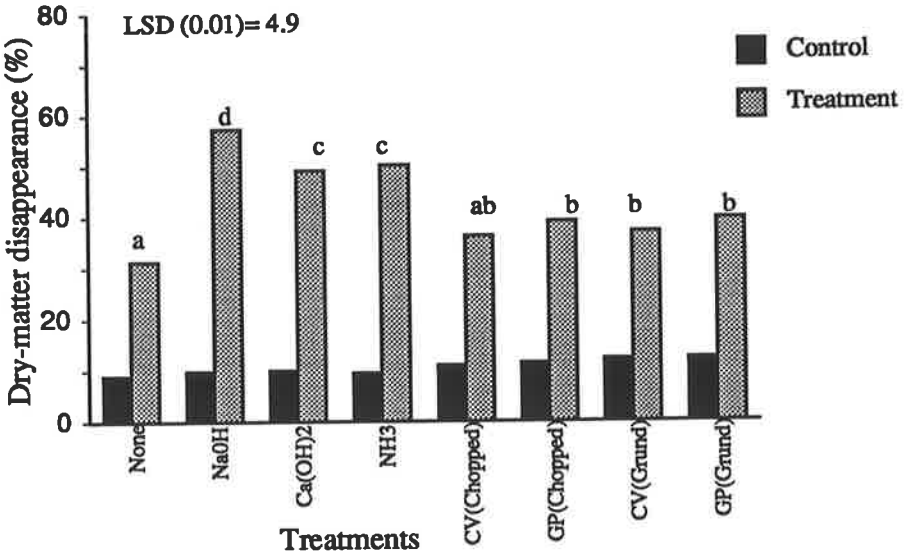


Figure 5.10: Effect of alkali and fungal treatments on the dry-matter disappearence of *Posidonia australis* during 72 hours in the rumen of sheep

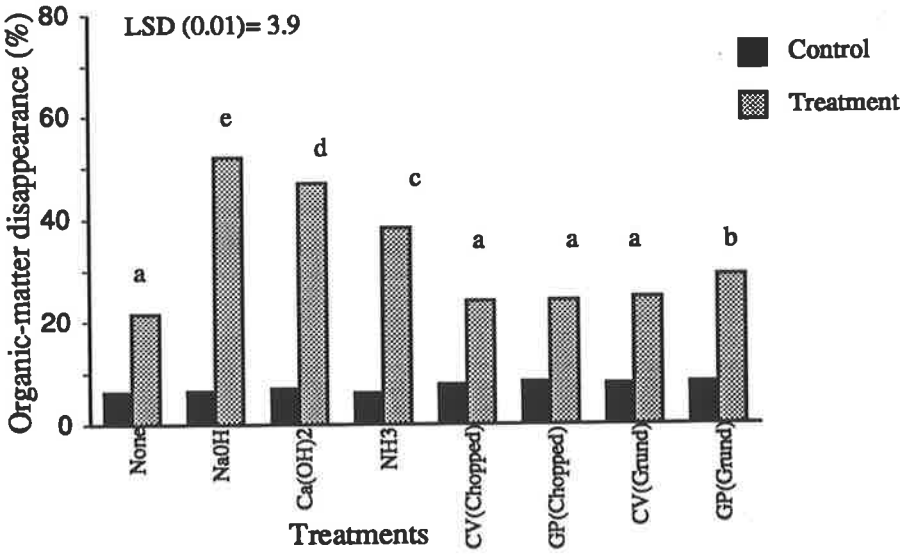


Figure 5.11: Effect of alkali and fungal treatments on the organic-matter disappearence of *Posidonia australis* during 72 hours in the rumen of sheep

Table 5.4: Effect of alkali and fungal treatments on the disappearance of cell-wall constituents of *Posidonia australis* during 72 hours in the rumen of sheep

Treatment			NDF %	ADF %	ADL %	Cellulose %	Hemicellulose %
Control	Untreated		30.0^{a}	27.0^{ab}	13.0^{d}	36.6^{a}	46.5^{ab}
Alkali	NaOH		65.4^{e}	52.4^{e}	14.5^{e}	79.1^{e}	96.0^{f}
	Ca $(OH)_2$		58.7^{d}	45.9^{d}	13.0^{d}	69.1^{d}	88.2^{e}
	NH_3		48.0^{c}	38.4^{c}	11.1^{c}	57.2^{c}	70.9^{d}
Fungi	*C.versicolor*	chopped	30.1^{a}	24.6^{a}	7.1^{a}	35.9^{a}	43b.5^{a}
		ground	31.1^{a}	24.2^{a}	7.5^{a}	37.1^{a}	50.9^{bc}
	P.gigantea	chopped	30.3^{a}	24.2^{a}	7.3^{a}	36.1^{a}	44.7^{ab}
		ground	36.3^{b}	29.5^{b}	8.5^{b}	44.2^{b}	55.1^{c}
LSD (0.01)			4.3	4.2	1.0	5.2	7.2

Means in each column with different superscriptions show significant differences

The effect of fungal treatment on the chemical composition of *Posidonia* is shown in Table 5.5. Incubation of *Posidonia* with both species of fungus in two different physical forms significantly altered OM and total fibre concentrations, but the effects of the two different species were generally the same.

Table 5.5: Effect of fungal treatment on the chemical composition (organic matter and cell-wall constituents) of *Posidonia australis*

Treatment		OM	NDF	ADF	ADL	Cellulose	Hemi-cellulose
		%DM		%NDF			
Chopped	Control	70.6^{b}	44.8^{b}	71.4^{a}	29.1^{b}	42.3^{a}	28.2^{b}
	C.versicolor	58.4^{a}	39.7^{a}	68.8^{a}	27.9^{ab}	42.6^{a}	28.4^{b}
	P.gigantea	58.8^{a}	39.8^{a}	71.3^{a}	27.1^{a}	44.2^{a}	26.2^{a}
LSD (0.01)		2.7	2.0	3.0	1.7	2.4	2.0
Ground	Control	70.4^{b}	44.0^{b}	72.7^{a}	29.6^{b}	43.1^{a}	28.8^{b}
	C.versicolor	57.9^{a}	39.0^{a}	69.4^{a}	26.8^{a}	42.6^{a}	29.1^{b}
	P.gigantea	58.2^{a}	38.9^{a}	72.1^{a}	27.0^{a}	45.1^{a}	26.4^{a}
LSD (0.01)		2.9	2.1	3.8	2.3	2.9	1.7

Mean with different letters within a column of a variable are significantly different

5.4 Discussion

As described in the introduction the first experiment in this chapter was aimed mainly to determine the optimum effects of some commonly-used alkalis and fungi on the digestibility *in vitro* of *Posidonia*.

In summary all alkali and fungal treatments influenced the digestibility of *Posidonia*, but to different extents, as follows:

NaOH: As expected NaOH treatment improved the digestibility of *Posidonia in vitro* and increasing the concentration caused increased digestibility. The optimum reaction time was when *Posidonia* was incubated for 2 days. The effect of reaction time on the digestibility of alkali-sprayed roughages has been discussed by several workers. Agrawal (1975) reported that the digestibility *in vitro* of sprayed roughages was not found to increase with time (up to 20 days). On the other hand Ololade *et al.* (1970) found that sprayed barley straw had a significantly higher digestibility after 24 hrs. The improvement of digestibility due to

reaction time could be related to the specific physico-chemical structure of the cell wall of each roughage.

While the response of low-quality material to sodium hydroxide treatment has been extensively studied both *in vivo* and *in vitro* (Jackson 1977; Kristensen 1982; Castrillo *et al.* 1991) there is no information in the literature on the effects of NaOH treatment on the digestibility of *Posidonia.* As with other lignocellulosic (straw) materials, however, (Rexen and Tomson 1976; Klopfenstein 1978) this present study also showed that a 4% concentration of sodium hydroxide gave an optimum result.

Following an experiment *in vivo* using male calves Singh and Jackson (1971) reported that in terms of digestible organic matter intake of wheat straw spray-treated with NaOH the desirable level of alkali was 3.3g per 100 g straw. In their experiment higher concentrations of the alkali were less effective. Thus the 4% concentration of sodium hydroxide determined here would seem to be a desirable level of alkali for applying to *Posidonia.*

It has also been reported that the performance of animals fed chemically-treated low-quality roughages was higher than the performance of animals fed untreated materials (Jackson 1977; Ibrahim 1983) but any practical application in this regard should be investigated further (Sundstøl 1988).

As previously mentioned *Posidonia* could possibly can be used as an alternative to other roughages a part of the ration for sheep in the Mediterranean-type climate of South Australia. In this particular context chemical treatment of this material should be examine further.

Ca(OH)2: Calcium hydroxide treatment at any concentration used and over time improved the digestibility of *Posidonia.* It seems that an optimum effect was achieved when *Posidonia* was treated with 4% $Ca(OH)_2$ for 4 days. The increase in digestibility of *Posidonia* following treatment with $Ca(OH)_2$ is likely to be related to an increase of mainly soluble carbohydrate liberated by the alkali hydrolysis process (Jackson 1977).

Djajanegara *et al.* (1985) concluded that an increase in digestibility occurs when a soaking method of treatment is used for $Ca(OH)_2$ because it is a weak alkali and is relatively

insoluble. In this present experiment the soaked *Posidonia* was not washed because it was considered that washing of $Ca(OH)_2$-treated materials could lead to greater losses of the digestible organic matter (Djajanegara *et al.* 1985). It has been reported that the high calcium content of straw soaked in $Ca(OH)_2$ suspension has no effect on phosphorus utilisation (Djajanegara *et al.* 1985) nor overall mineral balance (Verma *et al.* 1982).

Owen (1978) reported that the extent of increased digestibility of low-quality roughages following alkali treatment is related to initial levels of digestibility. For example, higher increases in digestibility can be expected following treatment of roughages of low initial digestibility in comparison with roughages of high initial digestibility. The high increase in digestibility of *Posidonia* might be related to this.

Ammonification: Ammonia released from urea on ensiling (anaerobic conditions) appears to have reacted with *Posidonia* to improve its digestibility. The mechanism of reaction is not fully understood, but could be similar to that of sodium hydroxide with fibrous matter generally (Tarkow and Fiest 1969). In this current experiment a urea solution of 6% increased DM digestibility of *Posidonia* by about 10 units, which is generally comparable with the result reported by Jayasuriya (1981) with 4% urea solution. This difference in concentration effect could be due to a different physico-chemical structure of *Posidonia*. Urea solution has attracted considerable attention for the treatment of roughages because in addition to improving digestibility it can also increase the nitrogen content of the treated material. Thus, the consideration of this characteristic of urea-solution treatment might lead to this treatment being more useful and practical than two either of the other alkalis for *Posidonia*, which of course is low in nitrogen content.

Fungi: As the results show both fungal treatments caused significant increases in digestibility of *Posidonia*, but the effect of *Phlebia gigantea* was slightly more than of *Coriolus versicolor* ($P<0.01$). Prior grinding also caused a significant increase in DMD of fungi-treated *Posidonia* ($P<0.05$) over simple chopping.

Many attempts have been made to treat lignocellulosic materials with white-rot fungi, which degrade lignin rather than structural carbohydrates (Han 1978; Latham 1979; Jung *et al.*

(1992). There is no information, however, about treatment of *Posidonia* with these fungi, especially not with the two species applied in this experiment. Jung *et al.* (1992) reported that even though straw treatment with white-rot fungi improved its quality the overall loss of dry matter severely limits the benefit of this method. Some fungi convert lignin and structural carbohydrates into fungal protein (e.g. mushroom) which suitable for animal feed (Han 1978). Some changes may occur in the chemical composition and nutritive value of spent lignocellulosic materials, but this would depend on the type of fungus cultivation for any improvement in the feeding value of fibrous feed.

Additionally it would seem that at present the cost of fungal treatment is too high for the technique to be of commercial use. The results of this present experiment with *Posidonia*, however, can help clarify the nature of cell-wall constituents of other traditional lignocellulosic materials that might be altered by fungal treatment.

As a logical continuation of this wortk the purpose of the second experiment here was to obtain a better understanding of the role of microbial activity on the rumen degradability of both untreated and treated *Posidonia*, concentrating on structural cell-wall constituents and comparing the effect of the different alkalis and fungi used earlier.

In summary, substantial disappearance of the DM and OM of untreated *Posidonia* occurred in 72 hours incubation time, but all treatments increased this. Amongst the alkali and fungal treatment applied in this experiment alkali treatment was more effective than fungal treatment and amongst the alkalis NaOH had the greatest effect on rumen disappearance of *Posidonia*. The degradability achieved in 72 hours was closest in value to the digestibility results *in vitro* and *in vivo* presented earlier in chapters 3 and 4 respectively. Much published data relates to experiments in which the workers tended to incubate bags for only a few different times and then attempted to relate dry-matter losses from the bags to the apparent digestibility of the feedstuff. In this experiment six different incubation times were used to gain a better understanding of the characteristics of *Posidonia* in response to attack by the micro-organisms of the rumen. As a rough guide (Ørskov *et al.* 1980) concentrates require 12-36 hours, good quality forages 24-60, poor quality roughages 48-72 hours incubation time to achieve maximum degradability. Hence the results of this present experiment again confirm

the results *in vitro* and *in vivo* that *Posidonia* can indeed be classified as a poor-quality roughage. The remainder of the study was based on 72 hours incubation time.

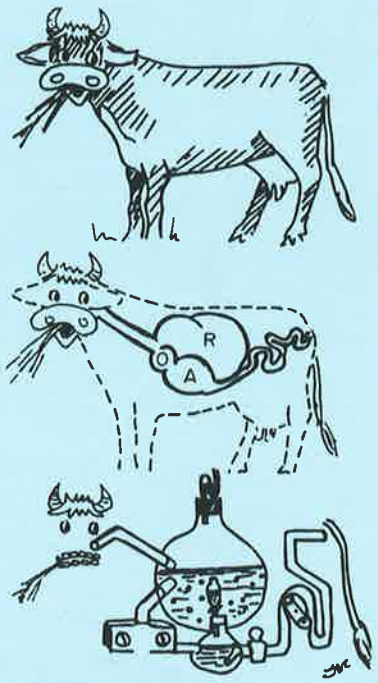
The effects of the different alkalis (in highest concentration and reaction time) and fungi on the DM degradability of *Posidonia* were compared in Figure 5.10. In general the alkalis were more effective than fungi in this regard and amongst alkalis the effect of NaOH was highest. The more intensive effect of NaOH has been reported by several research workers. For example Klopfenstein and Owen (1981) and Paterson *et al.* (1980) reported that NaOH is a stronger base than other alkalis and requires a shorter time for appropriate reaction to occur. It is suggested that although NaOH is a stronger base to improve degradability of *Posidonia*, ammonification also can be usefully applied because of its ability to improve the nitrogen content as well.

The effects of the different treatments on the structural carbohydrates of *Posidonia* were given in Table 5.5. This table indicated that the effect of alkalis on the degradability of NDF and ADF was similar to that on dry-matter degradability, but between fungi only *P. gigantea* treatment was able to increase NDF and ADF degradability, and then only as long as the *Posidonia* was first ground. Generally NDF degradability was substantially increased. The action of the treatments could be explained by solubilization of hemicellulose, increasing the extent and rate of both cellulose and hemicellulose digestion (Wanapat *et al.* 1985).

In conclusion, although all treatments applied to *Posidonia* were able to improve its nutritive value, in practice the extra advantage of ammonification is that it can also increase the nitrogen content of *Posidonia*. It could be preferred. In order to examine next the rumen ecosystem parameters of sheep fed treated *Posidonia*, the further experiments described in the following chapter were then planned.

CHAPTER 6

Ruminal parameters in sheep fed untreated and treated seagrass, *Posidonia australis*



Czerkawski (1986)

"In some respect it can be said that once the ruminant animal has taken in its food the rest of the process is left to the rumen. Microbial fermentation of plant materials produces a number of end products that vary from one diet to another."

CHAPTER 6

Ruminal parameters in sheep fed untreated and treated seagrass, *Posidonia australis*

6.1 Introduction

An important constraint to the utilization of any feed by ruminants is the rate at which metabolically useful products can be derived from rumen fermentation. In some respects it can be said that once the ruminant animal has taken in its food the rest of the process is left to the rumen, essentially to the rumen microbes. Microbial fermentation of plant materials produces a number of end products that vary from one diet to another. A particularly important aspect of rumen fermentation is the degradation of structural carbohydrates including cellulose, hemicellulose and xylan, with the concommitant production of volatile fatty acids, especially acetic (C_2), propionic (C_3) and butyric (C_4) acids. The degradability of these carbohydrate compounds is very different from one plant to another and is generally low in those plants, such as seagrass, with a high lignin content. Such plants, however, can be treated in a variety of ways to increase carbohydrate degradability and thus improve overall digestibility and usefulness to the animal.

The objectives of the experiment described in this chapter were three-fold:

(i) To measure voluntary intake by sheep and digestibility *in vivo* of treated and untreated seagrass.

(ii) To determine the pattern of ruminal parameters in sheep fed seagrass, treated and untreated, in comparison with other more conventional lignocellulosic feedstuffs. This latter objective was undertaken to test the hypothesis that the apparent improvements in dry matter and organic matter digestibility and in cell wall degradability observed in seagrass following chemical treatment (see Chapter 5) are reflected in appropriate changes in rumen metabolism which could be seen as advantageous, i.e. to provide more useful products and metabolites for the sheep's nutritional requirements. For this purpose the levels of total and specific

volatile fatty acids and ammonia, rumen pH and the number of total, viable, cellulolytic and proteolytic bacteria and protozoa before and after feeding were determined.

6.2 Materials and methods

6.2.1 Animals

Twenty South Australian merino wethers, aged 18 months, were selected from a flock grazing at the University's Roseworthy campus, Gawler S.A. The sheep were selected on the basis of uniformity of body weight of between 47.7 and 56.7 Kg. After footparing and treatment for internal parasites, the sheep were allocated at random to individual pens.

Four extra fistulated sheep (mentioned in section 5.2) were used to determine degradability of diets.

6.2.2 Diets

Seagrass, *Posidonia australis,* with a particle length of approximately 3-5 cm was as collected from the beach at Kingston , South Australia. Lucerne chaff with particle length of 2-4 cm was obtained from stocks at the Waite Institute. Urea fertilizer was purchased from Top Australia Ltd, Port Adelaide, S.A., sodium hydroxide from ACE chemical company, S.A., and sugarcane molasses from Stockmol Molasses Pty Ltd, S.A. Tap water was used to make solutions with all of the chemicals mentioned above.

The abbreviations used througout this chapter for the experimental diets and the proportions (DM basis) of their ingredients are shown in Table 6.1.

6.2.2.1 Treatment of Seagrass

With Sodium hydroxide. 200 Kg (DM basis) of seagrass were mixed with an equal amount (w/w) of 10% sodium hydroxide solution. In order to achieve proper mixing a plastic sheet 10 m x 6 m was spread on the ground, the seagrass spread about 30 cm high and the NaOH solution sprayed on top. The seagrass and NaOH solution were mixed into

Table 6.1: The abbreviations used for the experimental diets and the proportions of their ingredients (DM basis)

	Diets	
Abbreviation	75%	25%
Control	Untreated seagrass	Lucerne
NaOH	Sodium-hydroxide-treated seagrass	Lucerne
NH_3	Ammonia-treated seagrass	Lucerne
Mol	Molasses-treated seagrass	Lucerne
NH_3/Mol	Ammonia + Molasses-treated seagrass	Lucerne

each other manually several times until an apparently homogeneous mixture was obtained. This mixture was stored until pelletted.

With Ammonia: 200 Kg seagrass were treated with a 10% urea fertilizer solution as described above for NAOH. The mixture was transferred to metal bins, 150 cm x 150 cm x 75 cm and sealed with an airtight plastic cover. This should have caused a rise in ammonia in the bin according to the following anaerobic reaction:

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

To provide the urease to the bin 0.5 Kg of a mixture of sheep faeces and urine were added to the bins before they were sealed. The mixture was left in an anaerobic condition for 3 weeks, at an average temperatureof 23°C, before pelletting.

With Molasses. 200 Kg seagrass were treated with 20% sugarcane molasses in water solution in the same manner as for NaOH treatment.

With Ammonia / Molasses: To a second 200 Kg of ammonia-treated seagrass (see above) was added 20% solution of sugarcane molasses in water.

The type of bin used to store both treated and untreated seagrass during the 3 weeks before pelleting is shown in Plate 6.1.

Pelleting the diets: Untreated seagrass and all treated seagrasses were each mixed with lucerne chaff (75% : 25%, DM basis), and were pelleted separately (Neutrog Australia Pty. Ltd. , Kanmantoo, S.A.). Pelleted diets are also shown in plate 6.1. Pellet size was approximately 3 cm in length and 1 cm in diameter.

The chemical composition of the experimental diets is shown in Table 6.2.

Table 6.2 : Chemical composition of the experimental diets on dry matter basis (%)

	Diet				
	Control	NaOH	NH_3	Molasses	NH3 / Mol
OM	80.9	78.5	83.6	87.5	80.3
Ash	19.2	19.9	20.1	19.6	19.7
CP	7.7	6.9	10.7	7.7	9.6
NDF	46.6	44.3	42.7	44.3	45.1
ADF	34.0	32.5	32.6	32.5	27.5
ADL	11.6	11.4	11.4	11.3	8.4
Cellulose	23.4	19.7	21.9	22.1	17.0
Hemicellulose	11.2	12.4	8.9	10.3	16.3

6.2.3 Experimental design and management

A Randomized Complete Block Design (RCBD), consisting of 5 treatments diets and four replications, was used. Twenty Merino wethers were randomly assigned to pens. At the end

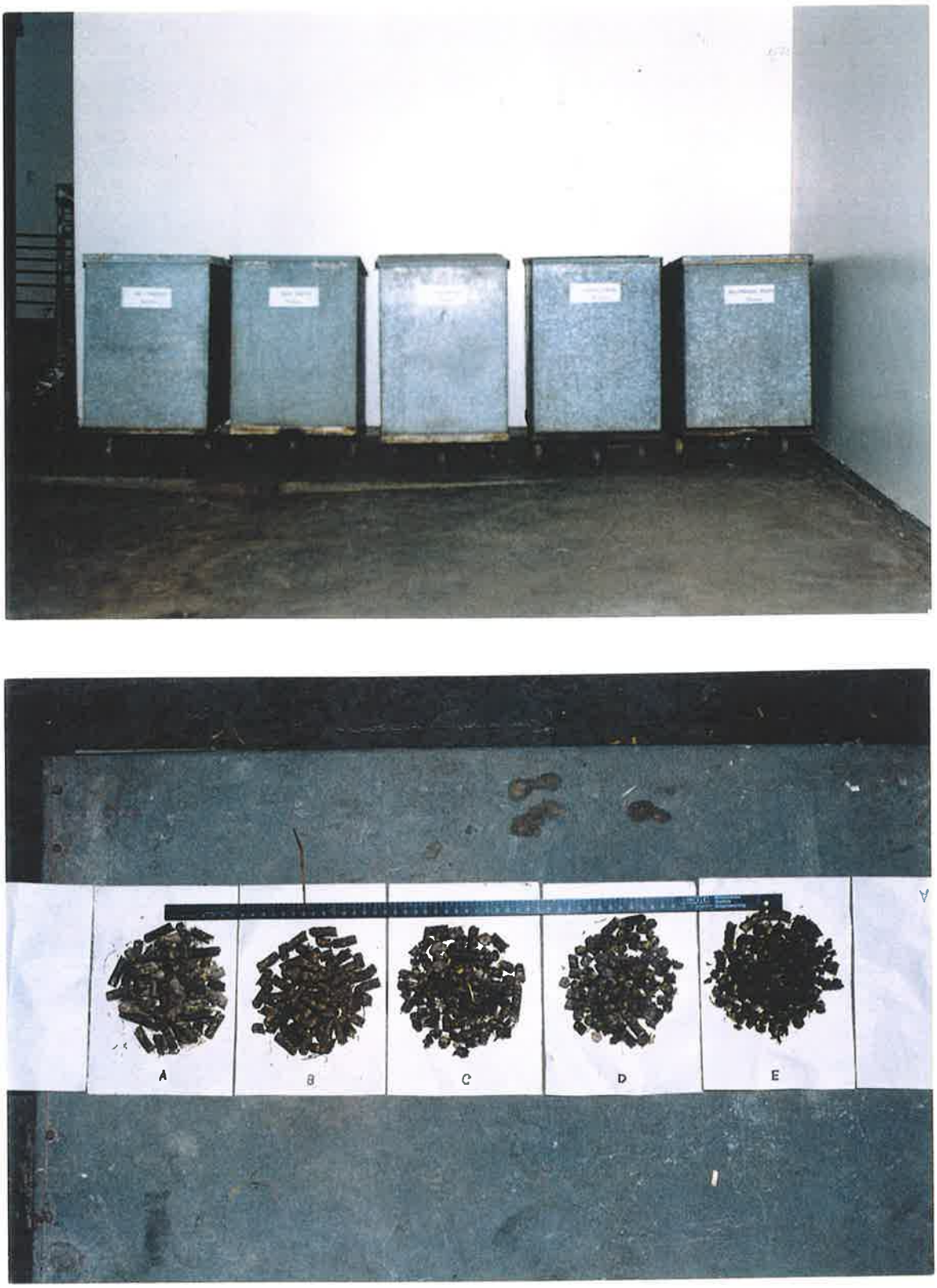


Plate 6.1: Type of bins (above) used to keep treated and untreated seagrass during 3 weeks before pelleting and pelleted diets (below)

of the experiment all data were statistically analyzed by computer using the Super ANOVA program.

The experimental periods consisted of:

(i) Adaptation period of 14 days during which sheep were accustomed to the individual pens;

(ii) Preliminary period of 14 days during which the proportion of experimental feed offered was gradually increased,finally just to exceed voluntary intake, (with 100 to 200 g of refused feed by each sheep per day);

(iii) Collection period of 12 days (Day 29 through day 40).

6.2.4 Quantitative measurements

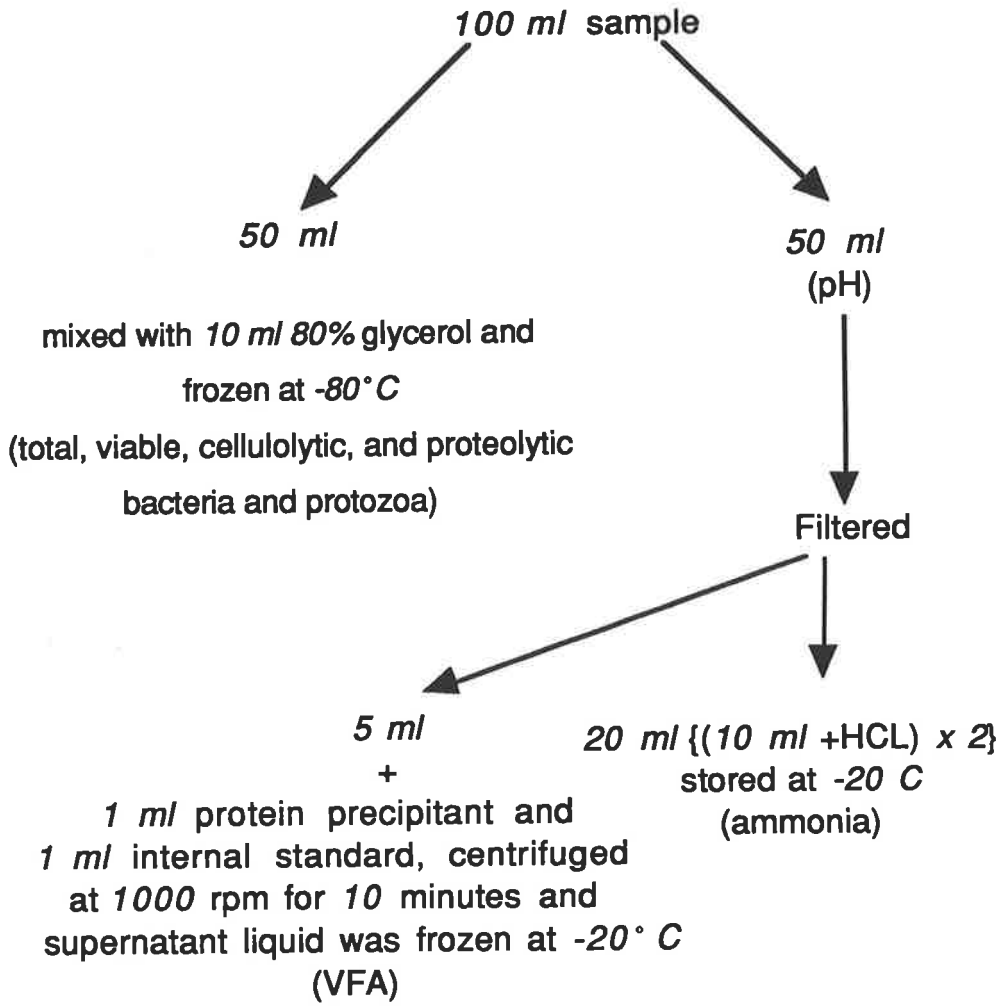
All sheep were weighed, between 0800 and 0900 h, at the beginning and twice a week throughout the experiment.

The feed consumed by all animals was measured daily through the experiment, with feed residues being collected between 0800 and 0900 h each day. Sub-samples of the feed offered and of residues collected between days 29 and 40 were ground and stored for subsequent analysis. Faeces from all sheep were collected and weighed during this same time and-sub samples (10%) taken each day for later chemical analysis. Urine from all sheep was collected into 1.0 M H_2SO_4 daily over 3 days during the collection period. A 10% sub-sample was taken after the total daily output was recorded.

Voluntary intake (VI), dry matter (DM) and organic matter (OM) digestibility, digestible energy (DE) and metabolizable energy (ME) of diets were determined by methods already described (section 4.2); DM, OM and cell-wall content degradability of all diets, plus neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and hemicellulose degradability were also determined *in sacco* by methods already mentioned (section 5.2), using four seperate fistulated sheep.

Plate 6.2: Taking rumen fluid sample from sheep by vaccum pump (above) and subdivision of samples for later measurements (below)

Figure 6.1: Subdivision of rumen fluid samples for measurement of rumen parameters



On the last 2 days of the collection period samples of rumen fluid were taken via stomach tube just before feeding (at 08:00) and at 3, 6, 9 and 12 hrs after feeding. The samples were taken for measurement of VFAs, NH_3, pH and for counting total, viable, cellulolytic and proteolytic bacteria and protozoa. In all measurements of rumen parameters samples of all 4 sheep in each group over two consecutive days were used, except that for VFA determinations only 1 sheep in each group was used.

6.2.5 Analytical methods

The dry matter contents of feed, residues and faeces were determined by drying at 100°C for 24 hrs. Ash contents of these materials were determined by ignition in an electric furnace at 550°C overnight. Nitrogen levels were determined by a Technicon Auto Analyser after micro-Kjedahl digestion (A.O.A.C. 1980). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the procedures of Van Soest and Wine (1967) and Abe and Horri (1978) and lignin was determined as the residue remaining after treatment with 72% H_2SO_4. Cellulose and hemicellulose were estimated by calculation as follow:

Cellulose = ADF - ADL Hemicellulose = NDF - ADF

6.2.6 Measurement of rumen fluid parameters

Rumen fluid (100 ml) was taken from each animal via a throat tube connected to a hand-operated vacuum pump (Plate 6.2). Each sample was divided into two 50 ml portions and transferred to two containers, one for pH, NH3 and VFA determination and the other for microorganism counting. The subdivision of rumen samples for these measurement is shown in figure 6.1.

6.2.6.1 pH

The pH of samples was determined immediately following collection using a Solstar® FET pH/mV meter (model EPM-300: Solstat Scientific Instrumentation, Laboratory Supply Pty. Ltd. , Adelaide, Australia) with a Ross® pH electrode (Orion Research Inc. , Cambridge, U.S.A.).

6.2.6.2 Ammonia

0.005 ml of 1 M HCl was added to each 10 ml rumen fluid in a tightly capped vessel and stored at -20° C. Two replicates were taken for each sample from each animal. After two weeks the ammonia in the samples was determined using a Expandable ion Analyzer (model EA 940; Orion Research Incorporated, Cambridge, MA, U.S.A.) with a Orion® Ammonia

Gas Sensing Electrode (Model 95 - 12; Orion Research Incorporated Laboratory Products Group, Boston, MA. U.S.A).

6.2.6.3 Volatile fatty acids

The technique used was based on a modification of the method described by Erwin *et al.* (1961).

5 ml of rumen fluid, filtered through two layers of gauze, were mixed with 1.0 ml of protein precipitant and 1.0 ml of internal standard and centrifuged at 1000 rpm for 10 minutes. The supernatant liquid was decanted, frozen and stored at -20° C for subsequent analysis. After 4 weeks the samples were thawed, shaken and allowed to stand for at least 1 hr before analysis by gas chromatography (Shimadzu's Model GC- 14 A PFSc, combined with a Delta data system microcomputer and SGE analytical products). GLC conditions were as follows:

Column: Phase BP 21; 0.5 μm film with 25 m x 53 mm ID filled with polyethylene glycol

Initial temperature:	100° C
Temperature increase:	9°C per minute
Maximum temperature :	150° C
Injection Temperature:	240° C
Detector temperature:	280° C
Sample injection size:	0.6 μl

REAGENTS:

Protein precipitant: 375 g metaphosphoric acid were dissolved in 900 ml distilled water and transferred to a 2 litre flask. 500 ml of 100% formic acid were added and the mixture made up to 2 litres with distilled water.

Internal standard: 10.5 ml n-caproate in 2 L H_2O.

Stock VFA solutions: Acetic, propionic and n-butyric acids made to 1.0 M. Valeric and iso-valric acids made to 0.1 M, all using AR grade reagents and RO water.

Stock VFA mixture: 10 ml acetic, 2.5 ml propionic, 2.5 ml n-butyric, 0.25 ml iso-butyric, 5.0 ml n-valeric and 5.0 ml iso-valeric acid stock solutions, diluted to 100 ml with RO water.

Analytical standard: 1.5 ml stock VFA mixture, 1.0 ml water, 0.5 ml protein precipitate and 0.5 ml internal standard. This standard corresponds to an original rumen fluid sample with the following VFA concentrations, expressed as mM: acetic 60; propionic 15; iso-butyric 1.5; butyric 15; iso-valeric 3; and valeric 3.

Following sample injection into the chromatograph the concentrations of the individual acids were determined by comparing the ratio of of peak heights of the rumen acids to the corresponding internal standard peak heights. Figures 6.2 and 6.3 show examples of the volatile fatty acid standard and rumen peaks respectively.

6.2.6.4 Bacterial and protozoal numbers:

Ruminal microbial populations of the experimental animals were characterised by counting total, viable, cellulolytic and proteolytic bacteria and protozoa.

Total direct bacteria count: 0.1 ml of rumen fluid sample taken from each animal was immediately transferred into a vial containing 0.9 ml of 10% formalin (Ogimoto et al., 1981) to be fixed. This diluted sample (10^{-1}) was further diluted up to 10^{-5} with 10% formalin and was counted by light microscope and haemocytometer. For each animal triplicate samples were used.

Figure 6.2: Peak separation of volatile fatty acid standards was achieved using a Shimadzu Gas Chromatography, combined with a Delta Data System microcomputer.

```
**********************************************************************************
* Time                                                                    Date   *
* 11:07:38    DELTA CHROMATOGRAPHY DATA SYSTEM - AREA PERCENT REPORT   04/03/94 *
**********************************************************************************
* METHOD    :VFA-ANAL                                                            *
* STD1      :VFA                          INJECTION            : 0 OF  1         *
* WEIGHT    :  1.000                      CHROMATOGRAM FILE    :C07000           *
* DILUTION  :  1.000                      CHROMATOGRAM SOURCE  :ACQUIRE          *
*                                                                                *
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Acquired by Method :VFA-ANAL    on 04/03/94
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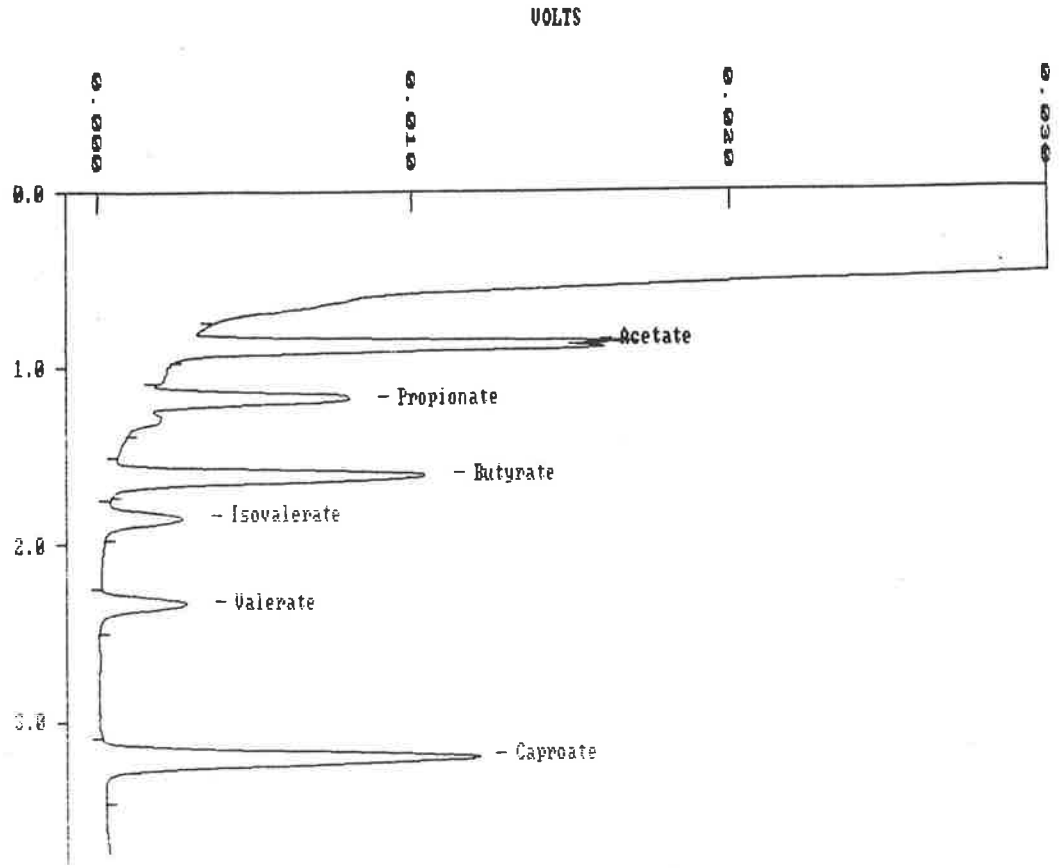

PEAK #	COMPT #	COMPOUND	RETENTION TIME (MIN)	WIDTH (sec)	HEIGHT (mV)	AREA (mV.Sec)	AREA PERCENT
1	1	Acetate	0.8	4.3	14.05	59.92	26.4
2	2	Propionate	1.2	4.5	6.42	32.64	14.4
3	3	Butyrate	1.6	4.7	9.86	47.73	21.0
4	4	Isovalerate	1.8	4.7	2.40	11.52	5.1
5	5	Valerate	2.3	4.7	2.67	13.07	5.8
6	6	Caproate	3.2	5.0	11.96	62.31	27.4
					47.36	227.20	100.0

Figure 6.3: Ruminal volatile fatty acid levels in a sheep fed experimental feed

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*********************************************************************************
* Time                                                                  Date    *
* 11:22:22    DELTA CHROMATOGRAPHY DATA SYSTEM - AREA PERCENT REPORT  04/03/94 *
*********************************************************************************
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* WEIGHT    :  1.000            CHROMATOGRAM FILE   :C07002                   *
* DILUTION  :  1.000            CHROMATOGRAM SOURCE :ACQUIRE                  *
*                                                                               *
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Acquired by Method :VFA-ANAL   on 04/03/94
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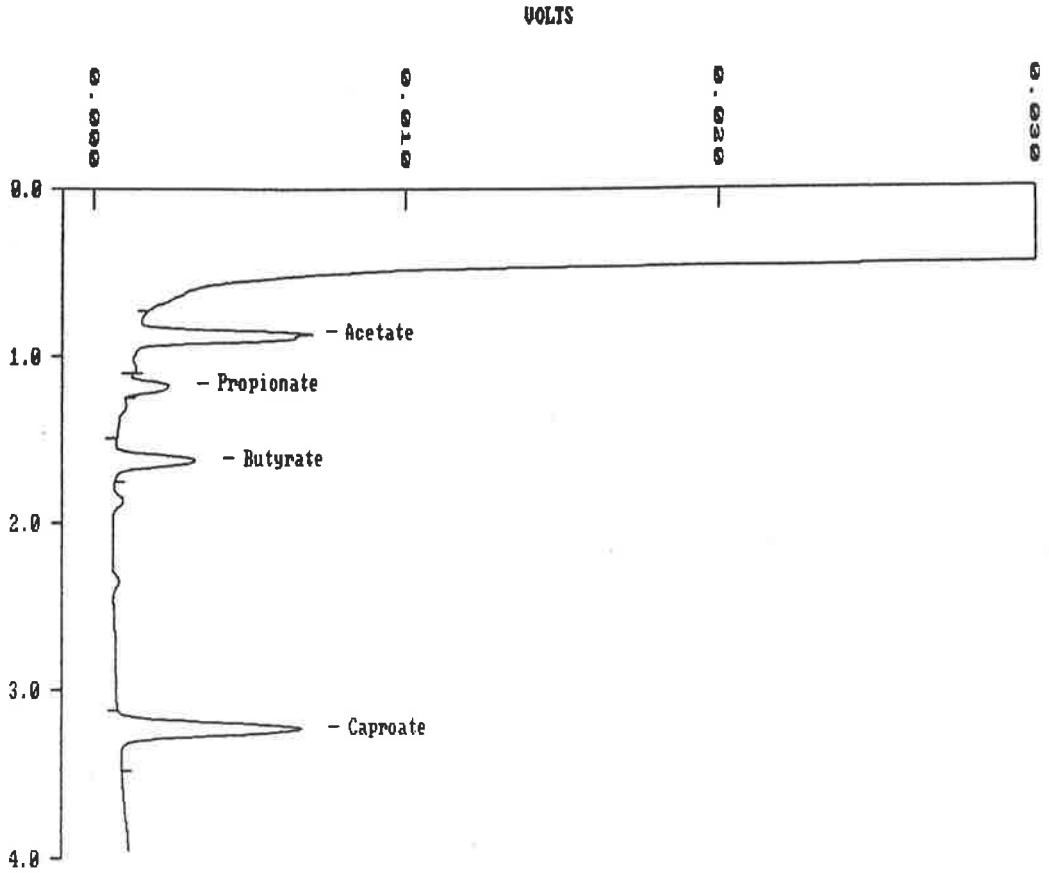


PEAK #	COMPT #	COMPOUND	RETENTION TIME (MIN)	WIDTH (sec)	HEIGHT (mV)	AREA (mV.Sec)	AREA PERCENT
1	1	Acetate	0.9	4.3	5.40	22.57	32.0
2	2	Propionate	1.2	4.3	1.28	5.52	7.8
3	3	Butyrate	1.6	4.7	2.53	12.37	17.5
4	6	Caproate	3.2	5.0	5.81	30.05	42.6
					15.03	70.51	100.0

Rumen protozoal count: Ciliate protozoa were counted by the method of Ogimoto and Imai (1981). 1 ml of rumen fluid was mixed with 5 ml methylgreen-formaldehyde-saline (MFS) solution (Table 6.3) and shaken gently. The protozoa were again counted in a haemocytometer.

Table 6.3: Composition of MFS solution (Ogimoto and Imai 1981)*

Component	Amount per 1000 ml
35% formaldehyde solution	100 ml
Methyl green	0.6 g
Sodium chloride	8.0 g
Distilled water	900 ml

*This solution was prepared in a glass bottle, stoppered tightly and stored in the dark.

Bacterial culture counts: This part included three steps, as follows:

(i) **Dilution procedure:** 40 ml of each rumen sample were mixed with 10 ml of an 80% glycerol solution, as described in section 6.2.6, and stored in a freezer at -80° C for future culture. The samples were mixed with glycerol in order to prevent the bacteria from dying at the time of freezing and thawing. Samples were frozen for one week before culturing.

To culture viable total, cellulolytic and proteolytic bacteria a few pieces of frozen sample were transferred to 1.5 ml vials by scalpel and left for 30 minutes in order to thaw under anaerobic condition. 0.1 ml of each sample was added to 0.9 ml of Bryant dilution solution (Bryant *et al.* 1953 - see Table 6.4). Further serial dilutions were carried out until 10^{-5}, 10^{-6} and 10^{-7} dilutions were obtained. 0.1 ml of each of the last three dilutions was plated out (Medium 98 - 5, Bryant *et al.* 1961 - see Table 6.5) under anaerobic conditions (95% CO_2, 5% H_2) and the inoculum dispersed with a sterile glass spreading rod. Plates were incubated at 39° C in an anaerobic hood for 72 hours and the numbers of growing colonies

counted. Only plates containing between 10 and 100 colonies were used to calculate the number of bacteria in the original fluid (Leedle and Hespell 1980).

(ii) Growth media: The composition of the dilution solution and the growth media for viable total, cellulolytic and proteolytic bacteria are listed in Tables 6.4, 6.5, 6.6 and 6.7 respectively.

Table 6.4: Composition of dilution solution (Bryant and Burkey 1953)

Component	Amount
Mineral solution I[a]	7.5 ml
Mineral solution II[b]	7.5 ml
Cysteine-HCl.H_20	0.05 g
Na_2CO_3	0.3 g
Resazurin, 0.1 % solution	0.1 ml
Distilled water	100 ml

a Mineral solution I: 0.6 g K_2HPO_4, 100ml distilled water

b Mineral solution II: 1.2 g NaCl, 1.2 g (NH_4) 2 SO_4, 0.6 g KH_2PO_4, 0.12 g $CaCl_2$, 0.25 g $MgSO_4$. $7H_2O$, 100 ml distilled water.

To prepare the solution the above mixture was placed in dilution bottles and sterilized in an autoclave at 15 1b (121°C) for 20 min. When the autoclave was exhausted the bottles were immediately closed with sterile butyl rubber stoppers and after cooling to 45-50° C were placed in an anaerobic glove box containing CO_2 gas until reasazurin indicator changed from pink to colorless.

To prepare the medium the mineral solutions resazurin solution, distilled water, glucose, cellobiose, starch, cysteine, rumen fluid were placed in a 500 ml glass bottle and agar was added and autoclaved at 15 lbs (121° C) for 20 min. After sterilization the medium was cooled to 50 C° and before pouring in the petri dish 8% Na_2CO_3 (sterilized) was added.

Table 6.5: Composition of Medium 98-5 (Bryant and Robinson 1961)

Component	Amount
Mineral solution I[a]	7.5 ml
Mineral solution II[b]	7.5 ml
Resazurin, 0.1% solution	0.1 ml
Distilled water	50.0 ml
Agar (Bacto-agar, Difco)	2.0 ml
Rumen fluid[c]	40.0 ml
Glucose	0.05 g
Cellobiose	0.05 g
Soluble starch	0.05 g
Cystein-HCl.H_2O-Na_2S. $9H_2O$[d]	0.5 ml
Na_2CO_3, 8% solution	5.0 ml

a,b See footnotes to Table 6.5.

c Rumen fluid was obtained by filtering rumen contents, obtained from cattle feeding on lucerne hay, through two layers of gauze to remove the large particles and was centrifuged at 25000 xg for 10 minutes and was stored under carbon dioxide in plastic bottles in the refrigerator at -20° C. It was melted just 10 min before media were prepared.

d 2.5 g l-cystein+HCl was dissolved in 50 ml distilled water; adjusted to pH 10 with water. 0.5 ml of this solution was added to 100 ml of medium

Table 6.6: Composition of modified medium M2 (Ronald and Peter 1982)

Component	Amount per 100 ml
Casitol	1 g
Yeast extract	0.25 g
Cellobiose	0.5 g
Glucose	0.1 g
CMC (Carboxymethyl cellulose) 1%	10 ml
Cysteine	0.1 g
Mineral solution I[a]	7.5 ml
Mineral solution II[b]	7.5 ml
Rumen fluid[c]	40 ml
Resazurin 1%	0.1 ml
Agar	1.5 g
Distilled water	30 ml
Na_2CO_3 8%	5 ml

a, b, c: See footnotes to Table 6.4

Table 6.7: Composition of casein medium[c] (Holdeman *et al.* 1977)

Component	Amount	
Brian heart infusion broth (dehydrated)	3.7	g
Yeast extract	0.5	g
Casein	2.0	g
Hemin solution[a]	1	ml
Vitamin K1[b]	0.02	ml
Agar	2	g
Distilled water	100	ml

a Hemin solution: 50 mg hemin was dissolved in 1 ml 1N NaOH; and made to 100 ml with distilled water.

b Vitamin K1: 0.15 ml of vitamin K1 was dissolved in 30 ml of 95% ethanol and was kept in a brown bottle under refrigeration for a maximum for 1 month.

c Preparation preceedure: Brian heart infusion broth, yeast extract, casein, distilled water and agar were placed in a 500 ml glass bottle, boiled and cooled and 1 ml hemin solution and 0.02 ml vitamin K1 were added. The pH was adjusted to 6.8 - 7.0 and 2 g agar was added and autoclaved at 15 lbs (121 C°) for 20 min. After sterilization the medium was cooled to 45-50° C and poured into petri dishes.

(iii) Counting procedures

Viable bacteria: The total number of growing colonies were counted immediately plates were taken from the incubator.

Cellulolytic bacteria: Growing colonies were counted after taking the plates from the anaerobic incubator (72 hrs) and adding Congo Red solution (Ronald *et al.* 1982). The plates were filled up with Congo Red solution and left for about 1 hr, after which they were emptied and destained with 1m NaCl. The cleared zones, as representative of total cellulolytic bacteria, were counted. Table 6.5 shows the modified M2 medium (Ronald *et al.* 1982) which was used to grow cellulolytic bacteria.

Proteolytic bacteria: Growing colonies were counted after taking out the plates (casein agar medium, Holdeman *et al.* 1977) from the anaerobic incubator (72 hrs). Plates were flooded with 10% (v/v) HCl and clear areas - an indicator of casein digestion (proteolytic bacteria) - were counted (Holdeman. *et al.* 1977). The medium components for the proteolytic bacteria culture (casein agar medium) are shown in Table 6.7.

Typical plates of growing colonies of viable, proteolytic and cellulolytic bacteria are shown in Plates 6.3 and 6.4 respectively.

6.3 Results

Voluntary intake, digestibility and body weight changes: Table 6.8 shows voluntary intake (VI), digestibility coefficients and body-weight changes with the experimental diets. The VI of all treated seagrasses increased relative to untreated seagrass, but these increases were not statistically significant. There were also no significant differences in VI between any of the treatments. Of the treated diets, Mol and NH_3 / Mol treatments had lowest and highest VI respectively (39.1 and 41.0).

Dry-matter digestibility of all treated seagrass diets was higher than that for untreated seagrass. Of the chemicals used NaOH caused the greatest increase in dry matter digestibility of seagrass (59.1 vs 49.0) and molasses caused the least (51.5). Organic matter digestibility

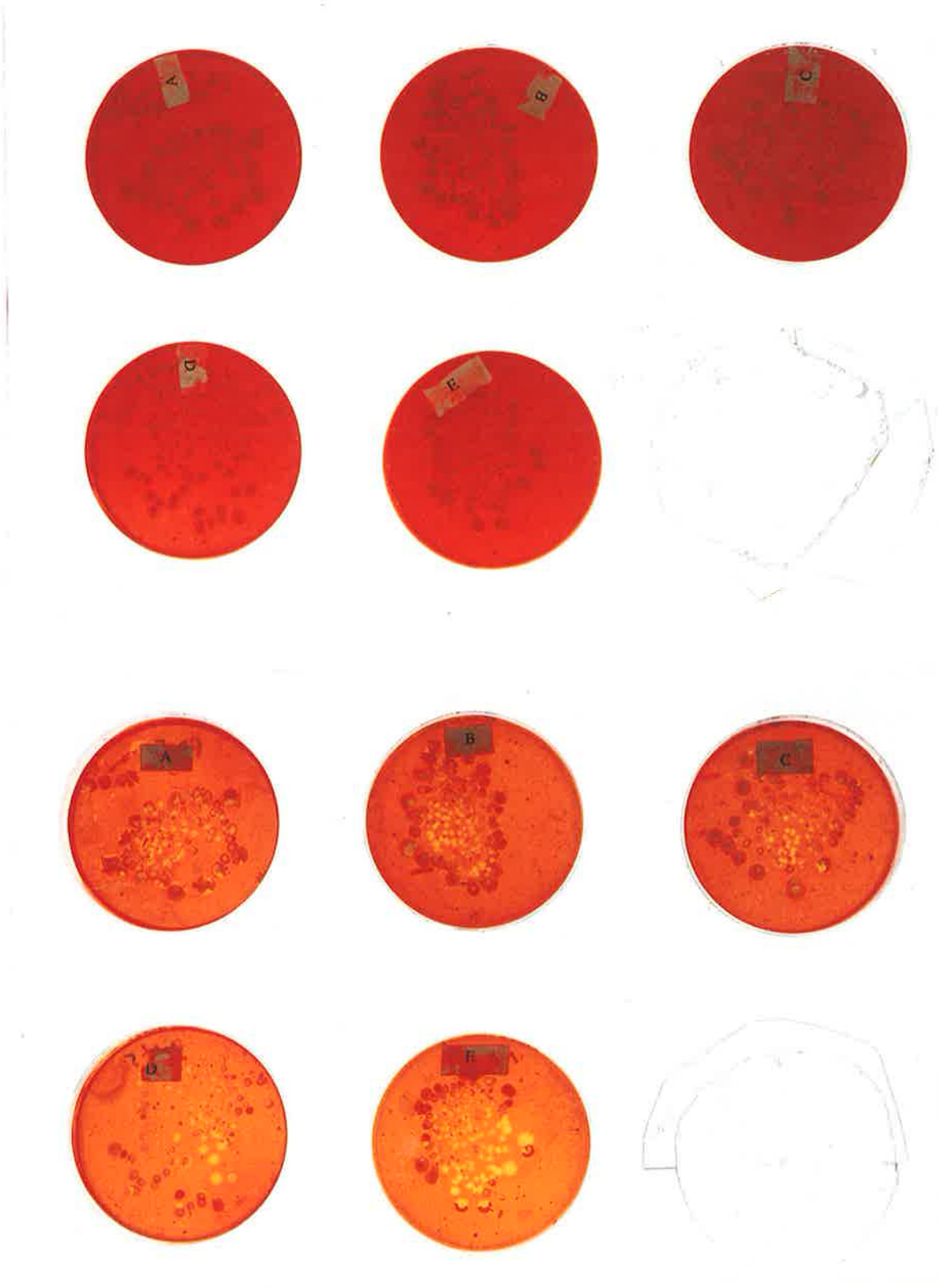


Plate 6.3: Plates showing growing colonies of cellulolytic bacteria after staining (above) and destaining (below). A, B, C, D and E are representative of rumen samples from sheep on the five experimental diets.

Plate 6.4: Plates showing colonies of proteolytic (above) and total viable (below) bacteria. A, B, C, D and E are representative of rumen samples from sheep on the five different experimental diets.

(OMD), digestible energy (DE) and metabolizable energy (ME) of all treated seagrass diets (except OMD of molasses-treated diet) were significantly higher than for the control diet. There were also significant differences ($P < 0.05$) among the treated seagrasses in OMD, DE and ME. Amongst the chemical treatments, NaOH and molasses caused the greatest and least improvements respectively in OMD, DE and ME of seagrass.

Although the body weight gains of all experimental sheep were significantly higher than for the control group these changes were not great. There were no differences between treatments.

Rumen degradability. Dry-matter (DM), organic matter (OM) and cell-wall contents (CWC) degradability of treated seagrass, as measured *in sacco* are shown in Table 6.9. Following chemical treatment degradability of both DM and OM of seagrass was increased ($P < 0.05$). Amongst the chemicals used NaOH and molasses had respectively the greatest and least effect on improving DM and OM degradability.

Table 6.8 : Voluntary intake (VI), digestibility and body weight changes

Diets (Treated Posidonia)	VI (g/Kg LW$^{.75}$/d)	DMD* (%)	OMD* (%)	DE (MJ/Kg DM)	ME (MJ/Kg DM)	Body wt change (g/sh/d)
Control	37.3^{a}	49.0^{a}	41.7^{a}	9.6^{a}	7.8^{a}	+ 16^{a}
NaoH	40.1^{a}	59.1^{d}	51.8^{d}	11.6^{d}	9.4^{d}	+ 25^{b}
NH_3	39.3^{a}	56.5^{c}	45.0^{b}	11.1^{c}	10^{c}	+ 22.5^{b}
Molasses	39.1^{a}	51.5^{b}	43.0^{a}	10.1^{b}	8.2^{b}	+ 25^{b}
NH_3 / Mol	41.0^{a}	56.3^{c}	46.6^{c}	11.1^{c}	9.0^{c}	+ 24^{b}
LSD (P<0.05)	4.7	2.2	1.4	0.45	0.36	+ 6.4

1:Means within each column that share no common superscript differ significantly (P<0.05).
DMD= Dry matter digestibility; OMD= Organic matter digestibility

Table 6.9 : Degradability of dry matter (DM), organic matter (OM) and cell-wall contents of experimental diets[1] (% DM basis)

Diets (Treated *Posidonia*)	DM	OM	Cell-Wall Contents[2]				
			NDF	ADF	ADL	Cellulose	Hemi-cellulose
control	40.4^{a}	33.8^{a}	36.5^{a}	29.6^{a}	10.1^{c}	37.1^{a}	46.9^{a}
NaOH	49.3^{d}	43.3^{d}	47.5^{d}	37.4^{c}	10.5^{d}	50.4^{d}	66.1^{c}
NH_3	46.9^{c}	39.3^{c}	42.4^{c}	33.2^{b}	9.3^{b}	43.5^{c}	54.6^{b}
Molasses	42.8^{b}	34.8^{b}	36.7^{ab}	28.8^{a}	7.9^{a}	37.2^{a}	46.5^{a}
NH_3 / Mol	46.4^{c}	38.5^{c}	38.7^{b}	37.4^{c}	10.0^{c}	41.0^{b}	53.3^{b}
LSD (P<0.05)	1.2	1.0	1.4	1.1	0.2	1.7	2.7

1 Means within each column that share no common superscript differ signficantly (P<0.05).
2 NDF= neutral detergent fibre; ADF= acid detergent fibre; ADL= acid detergent lignin

Degradability of the neutral detergent fiber (NDF) fraction of seagrass was significantly enhanced by NaOH, NH_3 and NH_3/Mol treatments but not by molasses treatment.

There were significant effects on the degradability of cellulose, hemicellulose and lignin of the seagrass by all treatments except molasses. The degradability of hemicellulose was always more than that for cellulose and was increased further still by all treatments, again except by molasses.

Protein balance: Changes in crude protein (CP = N x 6.25) balance in sheep fed the various experimental diets are shown in Table 6.10. Chemical treatment of seagrass did not increase its CP% digestibility. Throughout the experiment, however, sheep fed treated seagrass retained significantly more CP than did controls. In terms of CP retention NH_3 and molasses treatments resulted in highest and lowest increases respectively.

Table 6.10: Protein balance (CP = N x 6.25) in sheep fed experimental diets[1]

Diets (Treated *Posidonia*)	CP				
	Intake	Lose into faeces	Lose into urine	Digested	Retained
	g/sheep/d	% CPintake	% CPintake	% CP intake	% CP intake
control	53.7^{a}	30.8^{b}	53.9^{b}	69.2^{a}	15.3^{a}
NaOH	51.4^{a}	29.9^{ab}	51.8^{a}	70.0^{a}	18.3^{bc}
NH_3	76.5^{b}	29.5^{a}	51.0^{a}	70.5^{a}	19.5^{c}
Molasses	56.6^{a}	30.0^{ab}	52.5^{ab}	70.0^{a}	17.5^{b}
NH_3 + Molasses	72.0^{b}	30.9^{b}	52.0^{a}	69.1^{a}	18.4^{b}
LSD (P<0.05)	6.7	1.1	1.6	1.4	1.9

1 Means within each column that share no common superscript differ signficantly (P<0.05).

pH: Both time of sampling and different diets (interaction of time x diet) showed significant ($P < 0.05$) effects on rumen pH of sheep fed the experimental diets (Table 6.11). Treatment of seagrass resulted in significant increases in both initial and mean rumen pH values. The mean value for rumen pH was highest (7.27) on NaOH-treated and lowest (6.97) on control. Peak values of pH in all 5 groups of sheep were observed just before morning feeding (0 hr) and lowest values, except for NH_3/Mol treatment, were observed 3 hrs after feeding.

Ammonia. Table 6.12 shows daily variation of ammonia level in rumen of sheep fed the experimental diets. As expected, rumen ammonia levels in all groups increased substantially immediately following feeding - peaking at 3 hours and returning to pre-feeding values by 9-12 hours after feeding. NH_3 and NH_3+Molasses treated seagrass diets caused an increase but NaOH caused a decrease in ammonia levels (vs control); molasses-treated seagrass had no effect on rumen ammonia level.

Table 6.11: Changes in rumen pH of sheep fed experimental diets[1] (average of two days)

Diets	Collection (hr after morning feeding)					
(Treated *Posidonia*)	0	3	6	9	12	Mean
Control	7.11^{a}	6.76^{a}	6.91^{a}	6.99^{a}	7.10^{b}	6.97
NaOH	7.49^{d}	7.14^{d}	7.17^{b}	7.22^{d}	7.35^{c}	7.27
NH3	7.25^{b}	6.97^{b}	7.13^{b}	7.21^{b}	7.19^{c}	7.15
Molasses	7.46^{d}	7.16^{e}	7.26^{c}	7.36^{d}	7.40^{d}	7.25
NH3 + Molasses	7.39^{c}	7.10^{c}	6.97^{a}	6.90^{c}	6.81^{a}	7.04
Mean	7.34	7.03	7.09	7.14	7.17	7.15

Significance:		Least Significnt Difference
Interaction (diet x Time)	P<0.05	0.08
Diet	P<0.05	0.05
Time	P<0.05	0.04

[1] Means within each column that share no common superscript differ signficantly (P<0.05).

Volatile fatty acids. Values for the concentration of total volatile fatty acids (tVFA) and for the relative proportions of the main individual acids (acetic, A; propionic, P and butyric, B) and their molar ratios are shown in Table 6.13, 6.14, 6.15, 6.16 and Figures 6.3a and 6.3b.

Table 6.13 shows that clearly the most obvious and most dramatic effect is the substantial fall in mean tVFA levels on all treatments. In all groups, except NH_3-treatment, tVFA levels increased substantially and rapidly following feeding. Peak values of rumen tVFA concentration occurred at 6 hr in control group, in NaOH group at 9 hr, in NH_3/Mol group at 12 hr and in molasses group at 3 hr. The average rumen tVFA concentration of sheep with the NaOH-treated diet was considerably higher than those on other treatments, with molasses treatment the lowest of all.

Table 6.12: Changes in rumen NH_3 (mM) of sheep fed experimental diets[1]

Diets	Collection time (hr after morning feeding)					
(Treated *Posidonia*)	0	3	6	9	12	Mean
control	6.5^{b}	17.3^{b}	9.4^{c}	7.7^{b}	6.3^{b}	9.5
NaOH	5.8^{a}	14.9^{a}	8.3^{a}	7.0^{a}	5.6^{a}	8.3
NH3	9.0^{d}	25.5^{d}	13.3^{e}	10.7^{c}	8.7^{c}	13.4
Molasses	6.1^{ab}	17.3^{b}	8.8^{b}	7.5^{b}	6.5^{b}	9.3
NH_3 + Molasses	8.5^{c}	20.9^{c}	11.9^{d}	11.0^{c}	7.7^{c}	12.0
Mean	7.2	19.2	10.3	8.8	7.0	10.5

Significance:		Least Significnt Difference
Intraction (diet x Time)	$P<0.05$	0.58
Diet	$P<0.05$	0.33
Time	$P<0.05$	0.35

[1] Means within each column that share no common superscript differ signficantly ($P<0.05$).

In comparing data presented in Tables 6.14, 6.15, 6.16 and Figure 6.3a and 6.3b it is observed that, as expected, in all groups the molar ratio of A was considerably higher than that of P + B. There were no striking differences between any of the groups.

Table 6.13: Changes in sheep rumen total volatile fatty acid concentration (mM) of sheep fed experimental diets. Data show mean and standard error.

Diets (Treated *Posidonia*)	Collection periods (hr after morning feeding) 0	3	6	9	12	Mean
Control	67.2 ± 7.0	89.4 ± 1.1	95.0 ± 1.4	93.7 ± 3.9	87.7 ± 5.4	86.6 ± 2.2
NaOH	32.8 ± 1.6	48.7 ± 0.4	62.2 ± 1.7	65.9 ± 2.5	61.7 ± 3.9	54.2 ± 0.5
NH_3	43.0 ± 1.4	42.0 ± 0.8	39.9 ± 0.6	36.3 ± 0.8	39.9 ± 1.4	40.2 ± 0.5
Molasses	23.8 ± 0.4	25.7 ± 2.0	24.3 ± 1.3	20.8 ± 2.0	19.4 ± 0.7	22.8 ± 0.8
NH3 / Mol	17.7 ± 0.1	29.6 ± 0.8	37.3 ± 1.4	39.4 ± 0.6	43.3 ± 0.9	33.5 ± 0.2

Table 6.14: Changes in rumen acetic acid concentration (mM) of sheep fed experimental diets. Data show mean and standard errors.

Diets (Treated *Posidonia*)	Collection periods (hr after morning feeding) 0	3	6	9	12	Mean
Control	51.9 ± 5.1	63.0 ± 0.3	64.8 ± 0.3	62.7 ±3.2	58.9 ± 4.3	60.3 ± 1.3
NaOH	25.9 ±1.5	39.8 ± 0.3	51.5 ± 1.6	53.8 ±2.1	50.4 ±3.7	44.3 ±0.5
NH_3	35.4 ± 1.8	34.1 ± 0.6	32.6 ± 0.9	28.8 ±1.1	32.1 ± 1.3	32.6 ± 0.4
Molasses	18.9 ± 0.0	19.9 ± 1.3	18.9 ±0.7	16.2 ±1.3	15.4 ± 0.4	17.9 ± 0.6
NH_3 /Mol	12.9 ± 0.4	22.0 ± 0.8	27.6 ±1.2	29.6 ± 0.8	32.1 ± 0.3	24.8 ± 0.1

Table 6.15: Changes in rumen propionic acid concentration (mM) of sheep fed experimental diets. Data show means and standard error.

Diets (Treated *Posidonia*)	Collection periods (hr after morning feeding) 0	3	6	9	12	Mean
Control	6.4 ±1.1	17.9 ± 0.5	19.5 ± 0.8	20.0 ± 0.5	18.0 ± 0.3	16.4 ± 0.6
NaOH	4.4 ± 0.2	6.2 + 0.1	7.6 ± 0.0	8.7 ± 0.4	8.2 ± 0.2	7.0 ± 0.1
NH3	5.9 ± 0.5	5.8 + 0.2	5.3 ±0.3	5.2 ± 0.2	5.9 ± 0.1	5.6 ± 0.1
Molasses	3.3 ± 0.5	3.6 ± 0.7	3.9 ±0.0	3.0 ± 0.7	2.6 ± 0.4	3.2 ± 0.1
NH3 / Mol	3.0 ± 0.3	5.9 ± 0.0	7.8 ± 0.2	7.8 ± 0.2	9.2 ± 0.6	6.7 ± 0.2

Table 6.16: Changes in rumen butyric acid concentration (mM) of sheep fed experimental diets. Data show mean and standard error.

Diets (Treated *Posidonia*)	Collection periods (hr after morning feeding) 0	3	6	9	12	Mean
Control	8.9 ± 0.8	8.4 ± 0.3	10.8 ± 0.3	11.1 ± 0.3	10.8 ± 0.3	26.9 ± 0.5
NaOH	2.6 ± 0.1	2.7 ± 0.0	3.3 ± 0.1	3.5 ± 0.0	3.2 ±0.0	14.8 ± 0.3
NH3	1.8 ± 0.0	2.1 ± 0.0	2.1 ± 0.1	2.3 ± 0.0	2.0 ± 0.0	9.6 ± 0.1
Molasses	1.7 ± 0.1	2.2 ± 0.0	1.6 ± 0.6	1.7 ± 0.1	1.5 ± 0.0	6.3 ± 0.3
NH3 / Mol	1.8 ± 0.0	1.7 ± 0.1	2.0 ± 0.0	2.1 ± 0.1	2.1 ±0.1	9.0 ± 0.3

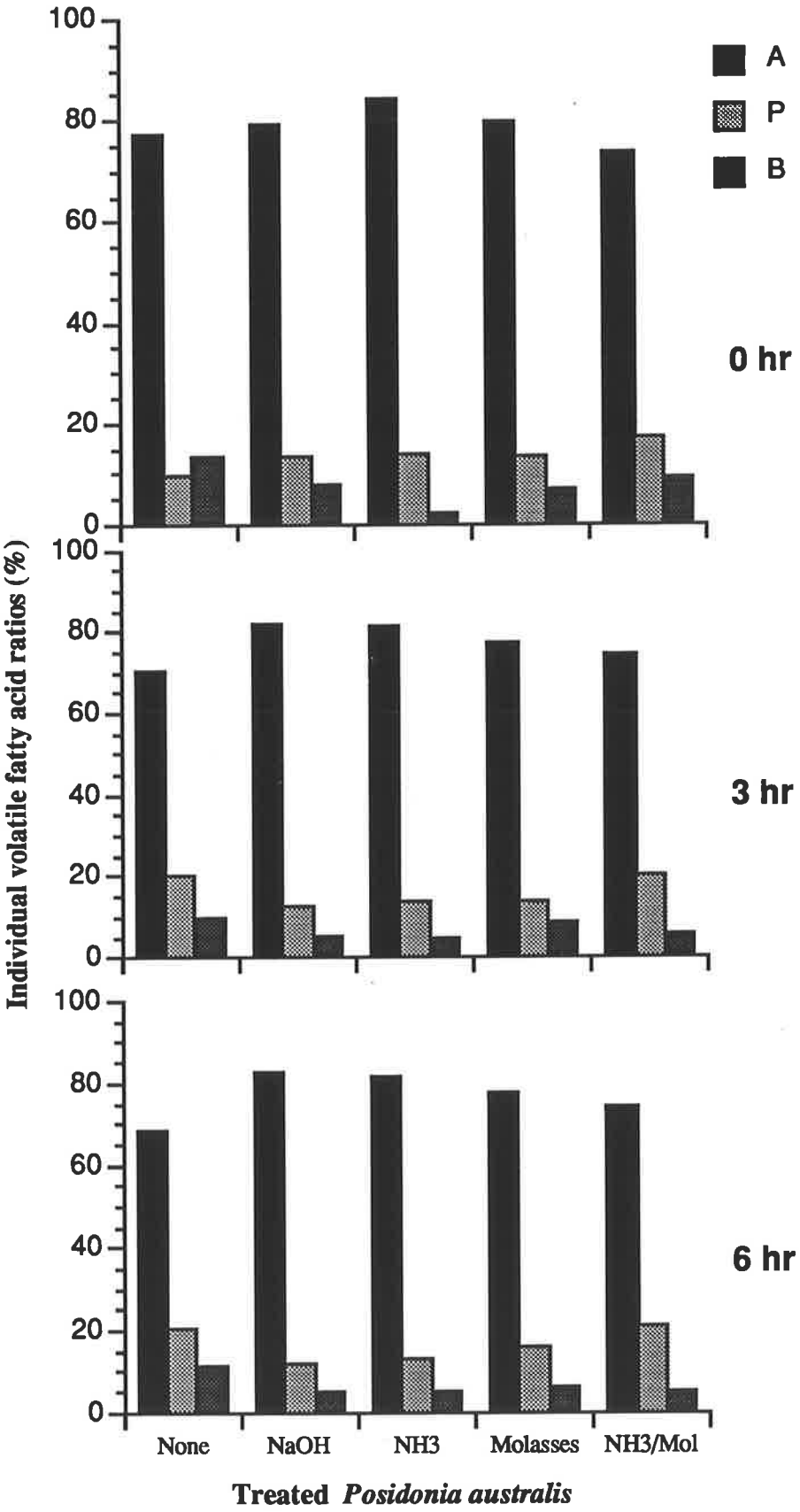


Figure 6.4a: Changes in rumen molar ratios of volatile fatty acids (acetic acid, A; propionic acid, P; butyric acid, B)

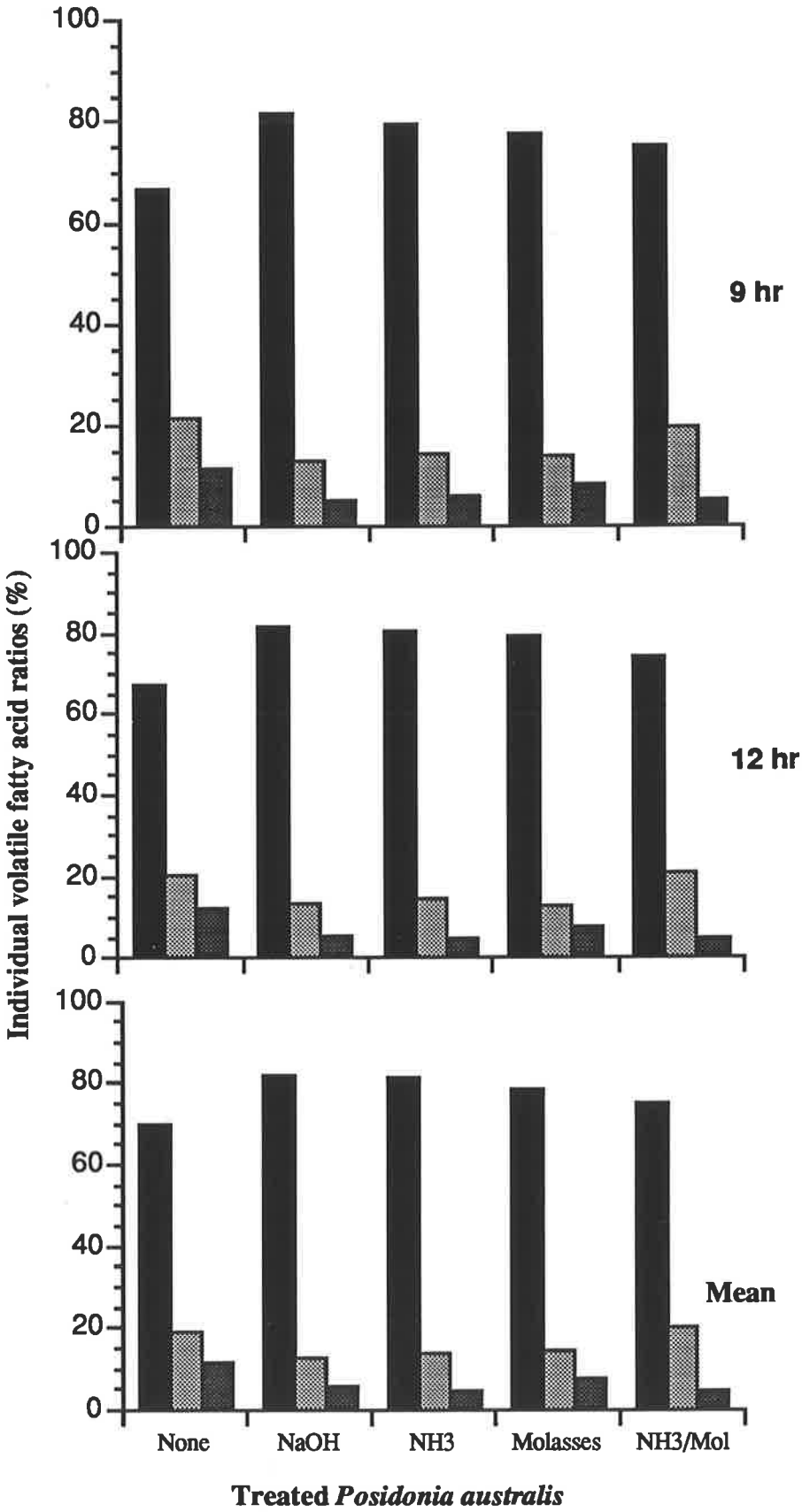


Figure 6.4b: Changes in sheep rumen molar ratios of volatile fatty acids (acetic acid, A; propionic acid, P; butyric acid, B)

Rumen microorganisms. The effect of feeding treated seagrass on rumen total and viable bacteria is shown in Table 6.17. It was observed: (i) that both total and viable bacterial numbers 12 hours after morning feed were higher than before feeding; (ii) that percent viability of bacteria increased after feeding; (iii) that rumen total and viable bacteria numbers of sheep fed treated seagrass, before and after feeding, were significantly higher than those for untreated seagrass, except in sheep fed molasses-treated seagrass (before feeding) in which there was no significant difference.

Ruminal cellulolytic, proteolytic bacteria and protozoal numbers of sheep fed the experimental diets are shown in Table 6.18. The number of ruminal cellulolytic bacteria in sheep fed treated seagrass was significantly higher ($P < 0.05$) than that for controls, both before and after feeding, except for molasses-treatment pre-feeding and NH_3/molasses at 12 hours.

Ruminal proteolytic bacterial numbers in sheep fed treated seagrass, before and after feeding and on average, were significantly higher than in controls, except in the case of molasses treatment before feeding. After feeding the number of proteolytic bacteria increased considerably in all cases (Table 6.18).

Ruminal protozoal numbers in sheep fed treated seagrass were also significantly higher than in controls, before, after and on average (except for NaOH treatment pre-feeding). Again numbers increased dramatically - two-fold or more - after feeding (Table 6.18).

6.4 Discussion

Leading on from earlier work the general purpose of this experiment generally was:

(i) to examine by methods *in vivo* the results of apparent improved nutritive value seen in previous laboratory-scale experiments of chemically-treated seagrass;

(ii) to relate increased nutritive value to altered nutrient production and nutrient balance in the rumen;

(iii) to relate these to changes in the number and function of rumen micro-organism.

Table 6.17: Rumen total and viable bacteria numbers in sheep fed experimental diets before (0) and 12 hr after morning feeding (x 10^9/ml).

Diet	0 hr			12 hr			Average		
(Treated *Posidonia)*	Total	Viabe	Viable (%)	Total	Viable	Viable (%)	Total	Viable	Viable (%)
Control	25.0^{a}	8.8^{a}	34.8^{bc}	51.2^{b}	20.3^{a}	39.7^{b}	38.1^{a}	14.6^{a}	38.3^{c}
NaOH	33.8^{e}	11.3^{c}	33.5^{ab}	63.5^{e}	24.6^{d}	38.7^{a}	48.7^{e}	18.0^{e}	37.0^{a}
NH3	30.0^{c}	10.0^{b}	33.3^{ab}	57.0^{d}	23.2^{b}	40.7^{c}	43.5^{d}	16.6^{d}	38.2^{b}
Molasses	27.5^{b}	8.8^{a}	32.0^{a}	54.9^{c}	23.7^{c}	43.2^{d}	41.2^{c}	16.3^{c}	39.5^{c}
NH3 + Molasses	31.3^{d}	11.2^{c}	35.8^{c}	46.0^{a}	20.1^{a}	43.7^{d}	38.7^{b}	15.7^{b}	40.5^{d}
LSD (P<0.05)	0.88	0.27	1.6	0.42	0.28	0.55	0.45	0.21	0.21

Means within each column that share no common superscript differ signficantly (P<0.05).

Table 6.18: Rumen cellulolytic and proteolytic bacteria and protozoa numbers in sheep fed experimental diets before (0 hr) and after (12 hr) morning feeding

Diet (Treated *posidonia*)	Cellulolytic Bacteria (x 10^9 / ml)			Proteolytic Bacteria (x 10^9 / ml)			Protozoa (x 10^4 / ml)		
	0 hr	12 hr	Mean	0 hr	12 hr	Mean	0 hr	12 hr	Mean
None	4.5^{a}	9.9^{b}	7.2^{a}	2.5^{b}	5.1^{a}	3.8^{a}	16.0^{b}	29.7^{a}	22.9^{a}
NaOH	6.3^{d}	13.1^{e}	9.7^{d}	2.3^{a}	7.2^{c}	4.8^{c}	12.5^{a}	36.6^{c}	24.6^{b}
NH3	5.2^{c}	12.1^{d}	8.7^{c}	3.3^{d}	9.6^{e}	6.5^{e}	22.5^{e}	47.0^{d}	34.8^{d}
Molasses	4.5^{b}	10.9^{c}	7.9^{b}	2.6^{b}	5.9^{b}	4.3^{b}	17.5^{c}	33.0^{b}	25.3^{c}
NH3 + Molasses	5.0^{bc}	9.1^{a}	7.1^{a}	3.1^{c}	7.9^{d}	5.5^{d}	20.0^{d}	53.0^{e}	36.5^{e}
LSD (0.05)	0.22	0.20	0.20	0.13	0.20	0.13	0.57	0.40	0.35

Means within each column that share no common superscript differ signficantly (P<0.05).

Chemical treatments included NaOH, NH_3, molasses and a combination of NH_3 and molasses (NH_3/Mol). In summary all treatments increased the nutritive value of seagrass in terms of digestibility measured *in vivo* and in weight gain by experimental sheep.

Digestibility and voluntary intake: Chemical treatment was effective in increasing dry matter and organic-matter digestibility of seagrass. All chemically-treated diets had significantly higher DMD and OMD values than those of the untreated seagrass diet. Several workers have observed greater feed digestibility in sheep when roughages are treated by alkali chemicals (Gadden 1920; Beckmann 1922; Siebert 1974; Klopfenstein 1978; Sundstøl et al. 1979; Lesoing and Klopfenstein 1981; Djajnegara et al.1985). Sheep fed NaOH-and NH3-treated seagrass digested much more feed than sheep fed untreated seagrass. It appears that ammonia released from urea on ensiling reacted with seagrass to improve its feed value and the effect of this reaction was comparable to that with anhydrous ammonia or ammonia in a solution of water. The mechanism of reaction of NaOH with seagrass is not fully understood, but could be similar to that of NH_3 with a fibrous material. Alkali treating of seagrass resulted in significant ($P < 0.05$) improvement in cell wall content degradability. Such large increases have been reported in other studies when the method was used to treat maize stover (Gadden 1920; Randele 1972; Fernandez and Greenhalgh 1972).

Although the voluntary intake of seagrass was increased due to all chemical treatments, these changes were not significant. This result is in contrast to that of other research workers who have reported an increase in VI of some fibrous feedstuffs due to pre-treatment with chemicals. This different result here may have been due to the higher concentration of chemicals than were used by others, with a possible decrease in palatability of the diets.

Nitrogen balance: Nitrogen balance is the most common parameter used to measure the influence of feed on protein deposition. The results of N balance studies in this experiment indicated that the feeding of chemically-treated seagrass significantly improved the proportion of crude protein (N x 6.25) retained by sheep. The improvement was mostly the result of decreased N loss in the urine (Table 6.10). The improvement in nitrogen balance for treated seagrass may be a result of the presence of more readily fermentable carbohydrate derived from available cellulose, improving microbial nitrogen capture of the soluble and cell

wall nitrogen in the treated seagrass. The same interpretation could be used to explain N balance differences between the treated seagrasses. This would be in agreement with Moss *et al.* (1992). Sheep fed molasses-treated seagrass retained N more than did controls; it seems reasonable to infer that this resulted from lower losses of ammonia from the rumen (Thomas and Thomas 1985), which may also be a result of giving more readily-fermentable carbohydrate.

pH: One important rumen parameter which is expected to be affected by a change of diet is pH. In this experiment considerable diurnal and dietary variation of ruminal pH was observed (Table 6.11). The pH of rumen digesta was higher when sheep were fed the alkali-treated seagrass diets than when fed the control diet. This is in contrast with the results of Kerley *et al.* (1986) who found a decrease in ruminal pH when alkali-treated straw diets were fed. The possibility exists that more of the alkaline solution was retained on the seagrass in the present study because of the low levels of moisture used.

NH3: Ammonia is the most important source of N for protein synthesis in the rumen. Its concentration in the rumen fluctuates markedly, from less than 1 mM observed in some animals on extremely low protein roughages to perhaps 40 mM, transiently after feeding, in animals receiving rapidly degraded protein or urea. The ammonia concentration in the rumen is an important criteria to evaluate feedstuffs. In this study considerable diurnal variation in ruminal NH_3 concentration was also observed in sheep fed both untreated and treated seagrasses. The peak in durnal variation in all groups of sheep was observed 3 hrs after feeding. An interval of 3 hrs between sampling is obviously too long to detect transient shifts in rumen parameters and thus high or low values for rumen NH_3 as presented in Table 6.12, don't necessarily reflect the absolute highest or lowest values, but merely reflect the values at the time of sampling. As expected NH_3, and NH_3-molasses treated seagrass increased the average ruminal concentration of NH_3, although NaOH and molasses alone did not. These results are generally in agreement with those of Drori and Loosli (1961), Leng and Nolan (1984) and de Waal and Biel (1989).

Volatile fatty acids: In this experiment the average ruminal total VFA concentration in sheep fed untreated seagrass was 86.6 mM (Table 6.13) which is approximately comparable

to that reported by Jayasuriya (1981) for sheep fed untreated rice straw, namely 101.6 mM. Among the diets all treated seagrasses resulted in lower levels of mean tVFA, in spite of increased digestibility of cell-wall contents such as cellulose. In considering this result it must be remembered that the level of any substances in the rumen at any one time is determined not only by its rate of production but also by its rates of metabolism, absorption and passage from the rumen, as well as by any dilution factors. In this experiment the significantly-lower concentrations of total VFA in the rumen of sheep receiving treated seagrass could be due both to the effect of increased rumen volume resulting from higher level of intake and higher consumption of water due to alkali treatments and to increased absorption and metabolism.

The average relative proportions of the main individual VFAs (acetic:propionic:butyric) in rumen of sheep fed untreated seagrass was 60 : 16 : 30. On NaOH, molasses and NH_3/molasses treated seagrass diets the proportion of acetate increased considerably in first 3 hours after feeding, which could also be a result of the presence of more available or fermentable carbohydrate, such as in the molasses itself and as cellulose and hemicellulose, due to the break down of lignin by chemical reatment (Moss *et al.* 1992). This increase did not happened with NH_3-treated seagrass. The propionate : acetate ratio for all diets was as expected for any high-roughage diet (McDonald *et al.* 1988).

Microorganisms: Rumen microorganism are either highly specialized, intermediate or very broad in relation to the type of nutrients that they will use. In some respects it can be said that when the ruminant animal has consumed its food the rest of the process is left essentially to the rumen microbes. Therefore the study of rumen microorganisms can give some basic estimation of relative nutritive value of different feeds. Both total and viable bacteria numbers in all samples taken 12 hrs after feeding were higher than in those taken before feeding, and percent viability also increased after 12 hrs feeding (Table 6.17). The increases in the number of both total and viable bacteria after 12 hrs are generally in agreement with report from both Warner (1966a) and Bryant and Robinson (1961), who concluded that these concentration patterns reflect an initial dilution by feed, water and saliva, then an increase in growth rate in response to incoming nutrients which exceeds the

dilution rate, and finally a depletion of nutrients with a corresponding decrease in growth rate until this is again less than the dilution rate. The increase in viability probably reflects the increasing concentration of nutrients available. Treatment of the seagrass also caused significant increase in average number of total and viable bacteria, relative to untreated material. These data indicate that bacterial numbers do tend to increase with an increased intake of available energy, due in this instance to increased cellulose and hemicellulose availability following alkali treatment (see Table 6.8). This interpretation is in agreement with the work of Grubb and Dehority (1975) who examined the effects on rumen microbial numbers of changing the ration from roughage to high concentrate.

Numbers of each of the three microbial types measured, i.e cellulolytic and proteolytic bacteria and protozoa, increased after 12 hrs feeding in all groups of sheep fed the experimental diets. In addition, the numbers of these three on treated seagrass were higher than on untreated seagrass (Table 6.18). This result is in agreement with Warner (1966a), Bryant and Robinson (1961) and Grubb and Dehority (1975). Higher proportions of cellulolytic bacteria to proteolytic (Table 6.18) may be due to the low content of crude protein in the diets. Lower number of protozoa relative to bacteria are in agreement with many other research worker (eg. Williams and Coleman 1992).

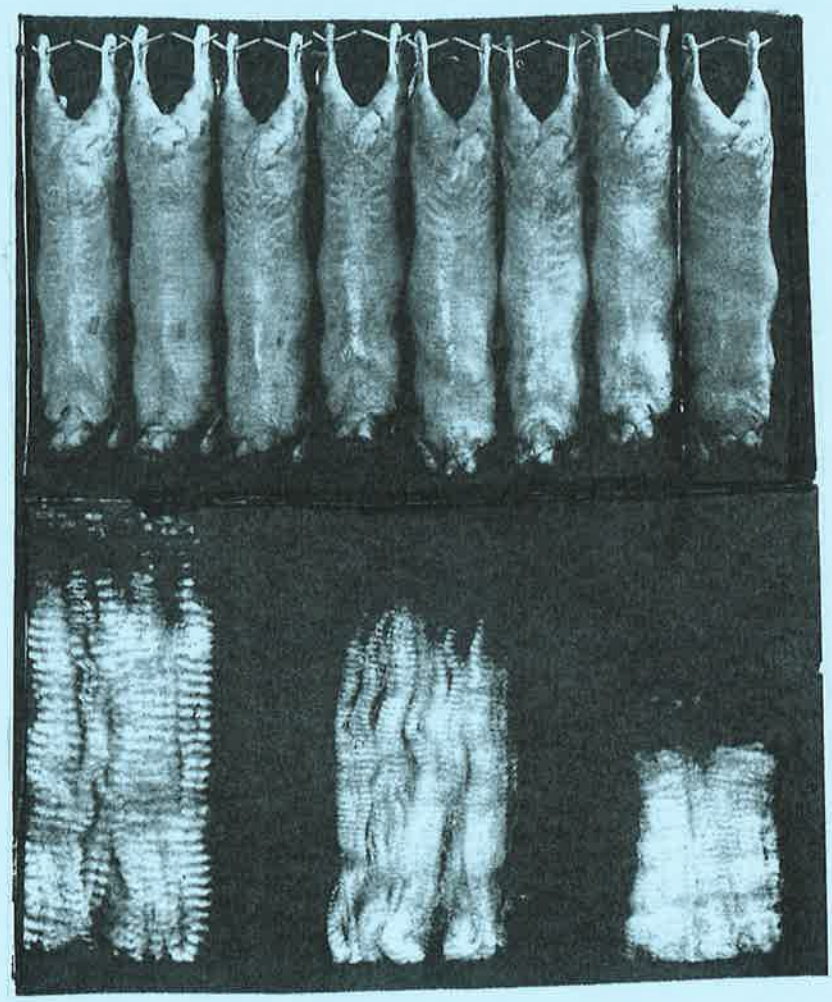
It is important to mention that in this experiment a stomach tube was used for sampling rather than via a fistula. This may have contributed some variability to results for pH and bacteria numbers, due to position effects and possible problems with saliva contamination.

In conclusion the results of this experiment support results found previously both in laboratory tests and short-term experiments *in vivo*. Over all the nutritive value of seagrass can be improved by chemical tratment. By this means the digestibility of seagrass, especially its cell-wall contents and predominantly cellulose can be increased and this increase in turn causes a considerable rise in digestible energy and metabolisable energy. An examination of some important rumen ecosystem parameters showed that the effects of both untreated and treated seagrass on rumen conditions were similar to those caused by other, more-conventional, lignocellulosic feedstuffs. Thus it seems both possible and logical that in order to relate the results of this and the previous experiments to practical livestock

husbandry one should carry out another experiment to clarify whether or not treated seagrass can be used as a substantial portion of ruminant rations in terms of maintenance and production. To achieve this aim a final experiment, simulating lot-feeding parameters, was designed.

CHAPTER 7

Potential of treated-supplemented *Posidonia australis* for sheep maintenance and production in lot feeding



"Animal feed evaluation is undertaken for different purposes. The main reason could be to direct the performance of farm animals through nutrition". (Van der Honing and Steg 1990).

CHAPTER 7

Potential of treated-supplemented *Posidonia australis* for sheep maintenance and production in lot feeding

7.1. Introduction

The potential for *P. australis* to be used as a sheep feed has been already demonstrated in different *in vitro* and short-term *in vivo* experiments (Chapters 4, 5 and 6). It has been shown that the low digestibility of *P. australis* is its primary nutritional limitation. In general the digestibility of *P. australis* for sheep can be increased when the material is treated with alkali. Alkali treatment and supplementation of *P. australis* was found also to increase dry matter intake (Chapter 4, 5 and 6). It was found that amongst various treatments and supplementation ammonia, molasses, chicken manure and lucerne were effective and practical in terms of increasing digestibility and voluntary intake and consequently the nutritive value of *P. australis*. Therefore further study of the potential of treated-supplemented *P. australis* can be useful to clarify whether or not it can be used more widely, as during drought and in times of pasture limitation.

In areas of southern Australia with a Mediterranean-type climate pasture availability is often a major limitation to sheep production during late summer and early autumn (Doyle et al, 1989). A short-fall in feed supplies during drought periods is also a common feature of the Australian sheep industry (Cottle 1991). Continous grazing, especially with high stocking rates, during this period makes paddocks bare, causing soil erosion and pasture deterioration, mainly through the loss of annual legumes.

Drought is part of the environment of South Australia (Jefferies and Nash 1989). Appropriate feed management must therefore be chosen by farmers to cope with recurring droughts and to reducing sheep damage to annual pasture seed reserves and to the soil. Various management strategies such as culling can be adopted by farmers and sheep

producers during droughts and summer feed shortages, but an alternative strategy that has been proposed by research workers (Carter et al 1993) and livestock advisers and has been used successfully by some farmers (Morbey and Ashton 1990; Rodda 1992) is lot-feeding, that is, hand feeding of sheep in a confined area during these periods.

As mentioned in Chapter 2 lot feeding can also be practised with sheep in different physiological states, such as for maintenance, during pregnancy etc. At production levels there are important economical considerations in applying this management strategy, but lot-feeding at maintenance level or drought feeding of sheep during the dry months is often a last resort for farmers in southern Australia and conventional economics is generally replaced by consideration of least cost.

Lot-feeding of sheep for most farmers is a new enterprise (Fels 1980) and its basic requirements, such as suitable facilities, available feedstuffs, biological and behavioural factors, intensive feeding management and formulation of the appropriate low-cost rations must all be understood for this practice to be effective.

This present study was undertaken to provide much-needed information in the following main areas:

- potential seagrass as feed
- useful results both for Australia and particularly for the sheep industry in the author's home country, namely Iran.

The specific objectives of the experiment were:

(i) To measure the short-term digestibility *in vivo* and the voluntary intake of treated and supplemented *P. australis* on a scale such as would be applicable to lot-feeding of sheep.

(ii) To compare the effect of feeding *P. australis* as a non-conventional feedstuff with cereal straw as a conventional feedstuff for sheep maintenance, and to compare seagrass/chicken litter with lucerne hay for sheep production.

(iii) To provide some substantial form of financial or economic analysis to farmers in order to use *P. australis* as a ruminant, particulary sheep, feed in drought and summer feed shortage in a lot-feeding system.

7.2 Materials and methods

7.2.1 Sheep and location of experiment

Fifty South Australian adult Merino wethers, plus 5 spares, averaging 47.8 kg body weight, were selected from the wether flock of the Faculty of Agriculture and Natural Resource Science, Roseworthy Campus, the University of Adelaide, South Australia. These were vaccinated against enterotoxaemia and drenched for internal parasites. The fifty experimental animals were divided into five groups by Randomised Completely Block Design (RCBD), as based on body weight, and the five spares were released on to pasture. The experiment was conducted at the Faculty of Agricultural and Natural Resource Science, Waite Campus, the University of Adelaide, Lat 34° 58' S, Long. 138° 38' E. at an altitude of 122.5 m above sea-level. Sheep were housed individually with continous access to water and feed. The selected location was away from sources of frequent disturbance. The pens allowed an average c. 2.5 m^2 per sheep.

Initially every ten sheep were allocated to each of five experiment diet treatments and the sheep were first given shed ration (see Appendix 5.1) *ad libitum* and were then gradually fed increasing amounts of the experimental feed during an 11 days adjustment period. At the end of the adjustment period ten sheep were allocated to each of the five experimental diets.

7.3 Statistical design and diets

A Randomised Completely Block Design (RCBD) with five treatments and ten sheep replicates for each treatment was used.

The five experimental diets were:

(A) 75% treated seagrass + 25% lucerne;

(B) 75% treated wheat straw + 25% lucerne;

(C) 75% treated wheat straw + 25% chicken litter (seagrass bed + chicken manure from a 7-week broiler raising period);

(D) 50% treated wheat straw + 50% treated seagrass;

(E) 100% treated straw.

These diets were formulated in the purpose of the following comparisons:

Growth: - seagrass vs straw (A vs B)
- chicken litter vs lucerne (B vs C)

Maintenance - seagrass vs straw (D vs E)

The physical characteristics of the *Posidonia* and the lucerne chaff used in this experiment were similar to that already discussed in Chapter 6. The wheat straw, with a particle length of approximately 3-5 cm, was obtained from stocks at the Waite institute. The chicken litter used was similar to that described in Chapter 4.

Seagrass treatment and supplementation: Treatment procedures were carried out at the SAFCULA dairy farm, Virginia, North Adelaide by courtesy of the farm manager, Mr. Bill Fischer. A solution containing 8% fertiliser grade urea (46% N), 15% sugarcane molasses (Chapter 6) and 1% calcium-di phosphate was mixed with either seagrass or straw (1Kg/Kg DM) using a Keenan mixer with digital balance for about 20 minutes. (Plate 7.1). This time allowed for complete mixing. The mixture was then transferred into a plastic lined pit which was dug into the ground (plate 7.2 top), pressed by loader, covered by plastic (Plate 7.2 bottom) and stored under anaerobic conditions for 3 weeks. After this time the silage pits were uncovered and the same mixer used to prepare the experimental rations (A) to (E) (Plate 7.3). These were bagged and delivered to the location of the experiment.

The same procedure was done to treat wheat straw. Treated straw was mixed with lucerne chaff (75% : 25%) and chicken litter (75% : 25%), w/w, to make diets B and C respectively. 100% of this treated straw was used as a diet E in experiment.

The summary of the ingredient proportions of the experimental rations is shown in Table 7.1

Plate 7.1: Machine mixing of treatment solution with seagrass/straw

Plate 7.2: General view of pit (Top) and anaerobic conditions (Bottom) for treatment of seagrass/straw with ammonia and molasses

Plate 7.3: General view of final preparation of experimental feeds (Top) and experimental sheep location (Bottom).

Table 7.1: Ingredient proportions of experimental rations (% DM basis)

Rations	Seagrass (treated)	Straw (treated)	Chicken litter (seagrass)	Lucerne chaff
A	75	-	-	25
B	-	75	-	25
C	-	75	25	-
D	50	50	-	-
E	-	100	-	-

7.4. Feeding technique

The total experimental period of 109 days was divided into three sub-periods of 11, 77 and 21 days respectively. During the first, adjustment period (11 days) the sheep were gradually adapted to the new location and experimental feeds. During the second period (77 days) the main experimental period, the sheep were fed the experimental diets *ad libitum* and residues were collected periodically in order to calculate weekly voluntary feed intake. During this main period a mineral blend salt block containing major and trace elements (Olsson Industries Pty. Ltd.) was provided to the sheep. During the third period (21 days) the sheep were again fed shed ration; this last period allowed for wool growth to be enough for ease of shearing under the last dye-band (See section 7.6.2).

All feed was weighed prior to feeding for each sheep every day and was offered between 0700 and 0730 hr. Feed residues were collected and weighed periodically during each week for determining weekly voluntary dry matter intake. Feed was sampled twice weekly throughout the experiment and was mixed at the end, when sub-samples were used in order to determine chemical composition and *in vitro* digestibility. Rumen degradable protein (RDP) were determined *in sacco* (These methods have already been described in chapter 3). The results of chemical composition and digestibility determinations are shown in Table 7.2.

Table 7.2: Chemical composition of experimental rations

Nutrients*	Rations A	B	C	D	E
DM (% DM)	63.3	65.4	65.8	53.7	54.0
OM	80.3	91.6	88.9	84.2	91.4
Ash	19.7	11.0	11.1	16.0	8.6
CP	9.6	8.8	8.8	6.3	5.7
CF	36	45	41.6	42.4	48.3
Ca	3.4	2.5	2.5	2.1	2.2
P	0.80	0.11	0.7	0.06	0.05
IVDMD	52.1	58.0	59.0	44.0	49.0
RDP	6.7	5.7	5.9	4.2	3.6
DE (MJ/Kg DM)	9.5	10.7	10.9	8.0	8.9
ME	7.7	8.6	8.8	6.5	7.2

*: DM=Dry Matter, OM=Organic Matter, CP=Crude Protein, CF=Crude Fibre, Ca=Calcium, P=Phosphorous, IVDMD=*In vitro* Dry Matter Digestibility, RDP=Rumen Degeradable Protein, DE=Digestible Energy, ME=Metabolisable Energy

Many methods have been introduced for ration formulation for farm animals. Linear programming techniques are the most accurate and there is world-wide use of such programs for formulation of least-cost rations for both ruminant and non-ruminant animals (Shaw and Thornton 1974). Numerous publications are available on computer-based, least-cost ration formulation for farm animals (e.g. Hughes *et al.* 1983; Savvant *et al.* 1983). The South

Australia Department of Primary Industries has developed a computer program for formulation of least-cost rations for sheep and cattle. This program, which is known as TAKE AWAY, calculates least-cost rations on the basis of metabolizable energy (ME), rumen degradable protein (RDP), undegraded protein (UDP) and several macro minerals such as calcium (Ca) and phosphorous (P). A complementary program (RUMNUT) provides data on the minimum daily nutrient requirements for both sheep and cattle, as based on data from ruminant nutrition research provided by the United Kingdom Ministry of Agriculture, Fisheries and Food in 1980 and 1984. The energy system in this program has been adopted by the Australian Standing Committe on Agriculture (SCA 1990).

In this experiment TAKE AWAY and RUMNUT programs were employed to estimate the minimum daily nutrient requirements at maintenance and growth levels for the experimental sheep (Table 7.3), nutrients supplied by the different rations in relation to daily intake (Table 7.4) and daily experimental feed costs (Table 7.10). Indication of prices for all of the ingredients is shown in Table 7.5.

Table 7.3: Minimum daily nutrient requirements of South Australian adult Merino wethers for maintenance and growth, as derived from the RUMNUT computer program (Feed Energy Density= 8.8 Mj/Kg DM)

Animal status	Body weight (Kg)	DMI (Kg)	ME (MJ)	RDP (g)	Ca (g)	P (g)
Maintenance	50	0.72	6.4	49.7	1.17	1.17
Growth (100 g/d)	50	1.30	11.5	89.4	2.78	2.17

Table 7.4: Nutrients supplied by different rations (from TAKE AWAY computer program)*

Rations	Daily intake (Kg/d DM)	ME (MJ/d)	RDP (g/d)	Ca (g/d)	P (g/d)
A	1.4	10.78	93.8	47.6	1.2
B	1.4	12.04	79.8	35	1.54
C	1.4	12.3	82.6	35	9.8
D	1.4	9.1	58.8	29.4	0.84
E	1.4	10.1	50.40	30.8	0.70

7.5 Measurements and daily management

7.5.1 Chemical composition

The experimental feeds were analysed to determine nutrients presented (Table 7.2) by methods which have already been described in Chapters 3, 4 and 5.

7.5.2 Sheep weighing

All sheep were weighed when they entered the experiment, weekly at the same time of day during the main period of the experiment, and then after 21 days feeding with shed rations (the end of third period).

Fat scoring technique: Every week before weighing a fat score for all experimental sheep was determined by the method described by Jamieson 1984; Jefferies 1961; Russle *et al.* 1969. Total tissue depth of the three indicator areas on the sheep body was felt by balls of thumbs and fingers as follows:

(A) Short ribs: both sides of the backbone between the end of the rib cage and the start of the hind legs;

(B) Top of the backbone: top of the backbone between the end of the rib cage and the start of the hind legs;

(C) Tail: fat coverage of the backbone in the tail area.

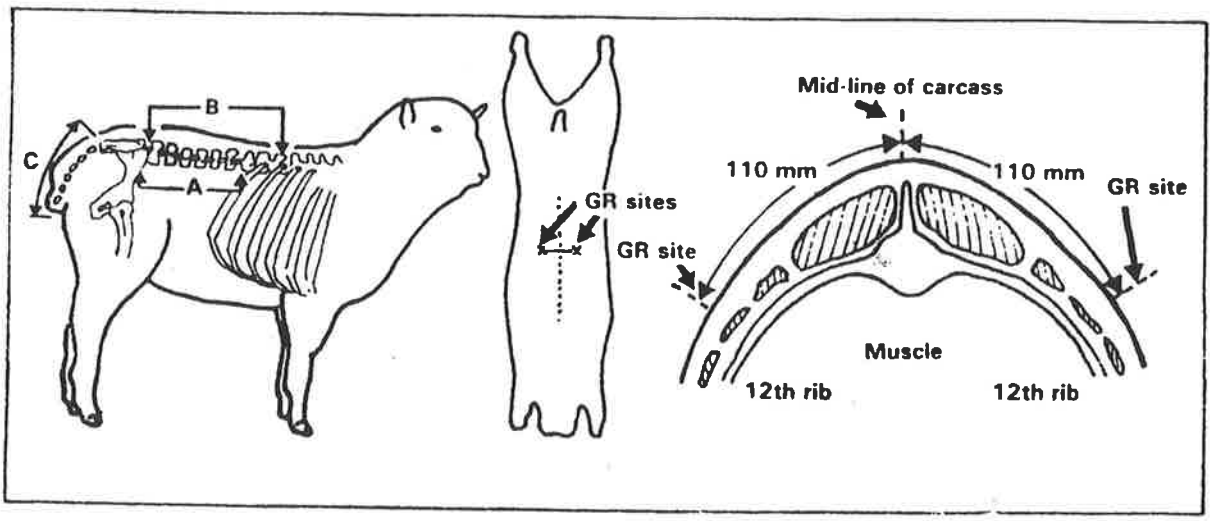
The sites to measure for fat score are shown in Figure 7.1. The sheep were scored on a scale of 1 to 5 according to the categories outlined in Table 7.6.

Table 7.5: The ingredients price of experimental diets based on 1994 market in South Australia

Diet	ingredient*	Kg Amount per Tonne feed (Before treating)	Unit price ($/Tonne)	Total price ($)
A:	Seagrass	750	200	150
	Urea	60	472	28.3
	Molasses	112.5	250	28.1
	Lucerne	250	340	85
Total (per Tonne)				291.4
B:	Straw	750	275	206
	Urea	60	472	28.3
	Molasses	112.5	250	28.1
	Lucerne	250	340	85
Total (per Tonne)				347.4
C:	Straw	750	275	206
	Urea	60	472	28.3
	Molasses	112.5	250	28.1
	Chicken litter	250	200	62.5
Total (per Tonne)				324.9
D:	Seagrass	500	200	100
	Straw	500	275	137.5
	Urea	80	472	37.8
	Molasses	150	250	37.5
Total (per Tonne)				312.8
E	Straw	1000	275	275
	Urea	80	472	37.8
	Molasses	150	250	37.5
Total (per Tonne)				350.3

* In each diet equal amount of seagrass/straw/both,Tap water was added.

Figure 7.1: The sheep body areas to feel when assessing fat score (Jamieson 1984).



7.5.3 Wool growth measurements

Wool growth over short periods of time can be estimated by dye-banding the wool staples at intervals of not less than three weeks (Chapman and Wheeler 1963). Fleece dye-banding may be employed at regular intervals throughout different seasons (Williams and Chapman 1966). The total amount of wool production in the dye-banded intervals is estimated by measurement of the dye-band intervals in the staples in combination with total fleece weights (Langlands and Wheeler 1968).

Dye-band solution preparation: An aqueous solution of 1% (w/v) Durafur Black R was prepared. Firstly, a paste of Durafur black R (1g) and water was made and then during stirring the rest of the water (total 100 ml) was added. The solution was periodically stirred until the flakes of the chemical completely dissolved. Finally 1 ml of concentrated hydrogen peroxide (H_2O_2) was added.

Application of dye solution: The left mid-side of the sheep was chosen to dye-band. The fleece was gently parted and along a line of approximately 20 cm, adequate dye solution

was applied at the skin level by a 10 ml syringe with needle to moisten the emergent part of the fibres. The excess solution of Durafur Black R on the skin surface was removed with the syringe and discarded (Chapman and Wheeler 1963; Williams and Chapman 1966). Dye solution was applied twice, with a 62 days interval, i.e. at the beginning and close to the end of the main experimental period (Plate 7.4 top).

Dye-banded wool removal: At the end of experiment, before whole body shearing, the mid-side dye-banded part of the fleece was separately shorn close to the skin surface. The dye-banded wool from each sheep was used to measure growth in Staple Length (SL) and the weight of wool grown during the main experimental period.

After removal of the dye-banded wool each sheep was shorn and total weights of all greasy wool, including the dye-banded samples, were recorded. A sample of about 100 g was taken from adjacent to the dye-banded area for determination of clean scoured yield (CSY), mean fibre diameter (MFD) and staple strength (SS).

Greasy fleece growth during experimental period: Five samples of approximately 5 gr each were taken from wool shorn from the dye-banded area of each sheep. The wool grown between the two dye bands (experimental period) was cut with a sharp scissors and the proportion by weight of the experimental wool relative to the whole sample was calculated. Experimental wool growth for each animal was then calculated as follows:

Growth in experimental period = Whole greasy fleece weight x mean % of sample weight between two dye bands

Wool scouring: To measure clean scoured yield (CSY) the weight of washed, dried wool needs to be obtained. In this experiment to scour the wool the following procedure was applied:

(i) Weighed samples of approximately 5 gr of fleece wool of each sheep were placed into numbered muslin bags, each approximately 12 x 7 cm and previously dried at 70°C for 1 hr, placed into a desiccator for 20 minutes and weighed.

(ii) Five row of five 500 ml plastic beakers were arranged to contain in sequence 2 rows of used hexane, 1 row of new hexane, and 2 rows of 50°C tap water which facilate to wash 5 sample the same time.

(iii) The bags containing greazy wool samples were agitated and washed for 10 minutes in each beaker, then squeezed well before going into next solution.

(iv) All samples were dried in an oven at 70°C over approximetaly 60 hrs, were placed in a desiccator for 20 minutes and then weighed

Table 7.6: Description of fat-score assessment.

Fat Score	Fat tissue depth (mm)	Fat indicator points		
		Short ribs (A)	**Backbone** (B)	**Tail** (C)
1	0-5	Ends of short ribs feel square. It is easy to feel between them.	Bones are raised and sharp. It is easy to feel between them.	Bones are sharp with little or no cover.
2	6-10	Ends of short ribs are round. It is still possible to feel between them.	Bones are raised and the ends are rounded. It is still possible to feel between them.	Bones barely covered but rounded on edges.
3	11-15	Ends of short ribs are well rounded. It is not possible to press between them	Bones slightly raised. It is still possible to feel them, but not between them.	Bones well covered and just detectable.
4	16-20	Only one or two of the bone ends may be felt, and those will be located nearest the rib cage	Some bones ends may still be felt. Skin begins to float as on fluid.	Very difficult to feel any bones.
5	21-25	It is impossible to feel the bone ends	Backbone is recessed in the fat and difficult to feel.	It is impossible to feel any bones.

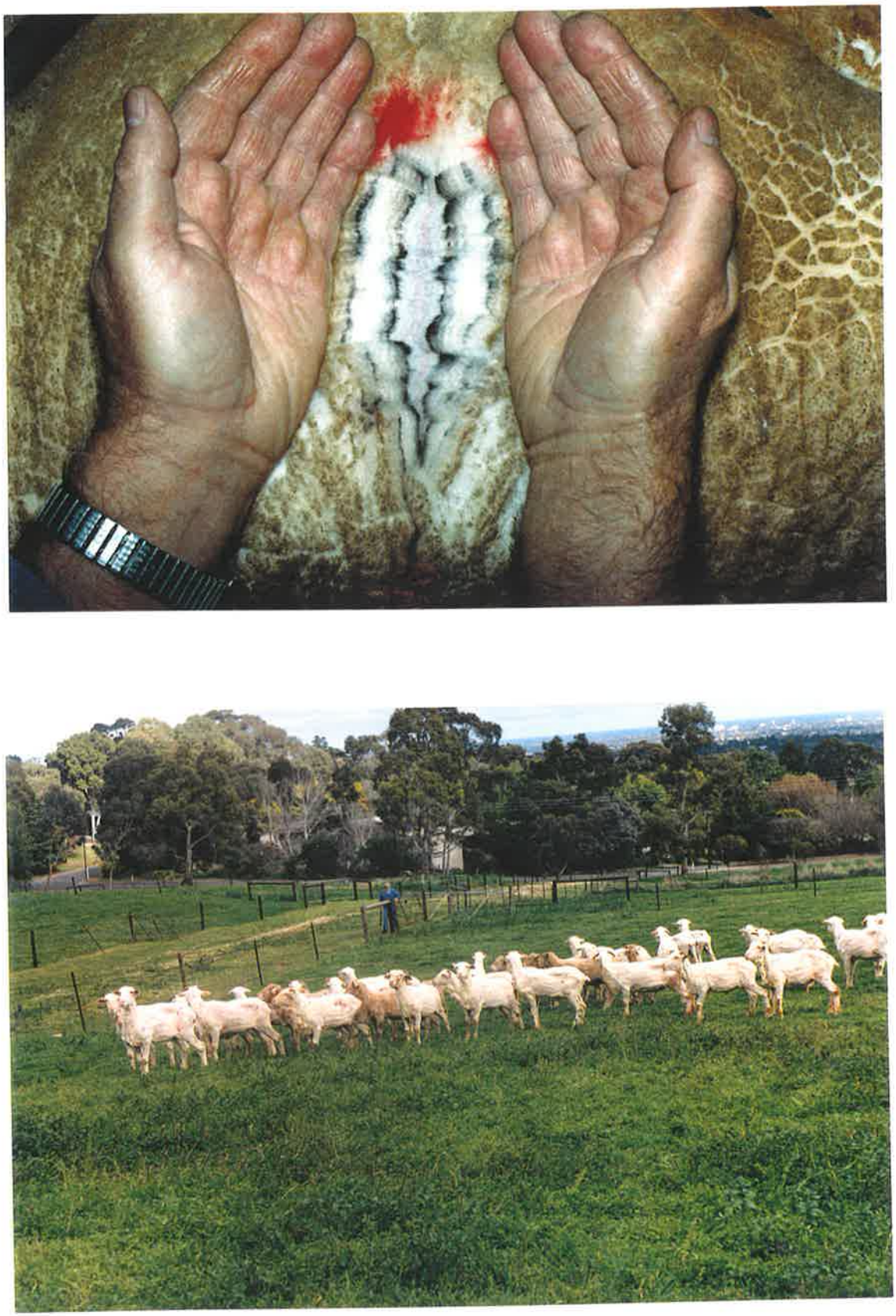


Plate 7.4: General view of dye-banding applied to measure wool growth (Top) and general condition of the sheep in the end of experiment (Bottom)

(v) To calculate clean scoured yield (CSY) the following equation was applied:

$$CSY(\%) = \frac{\text{(Wt. of washed sample in bag) - Wt. of bag}}{\text{Greasy sample wgt}} \times 100$$

The CSY were adjusted using a standard moisture regain of 16 % (by determination of the moisture content (%) of greasy sample (16%) and adding 16% of CSY Wt. to above result.

Staple Length (SL): The SL between dye bands was measured by ruler on five dye-banded staples per sheep.

Fibre Diameter (FD): The mean and variation in FD of wool between the dye bands were determined by a Fibre Finess Distribution Analyser (Lynch and Mitchie 1976) by workers at Turretfield Research Centre, South Australia (Figure 7.2).

Staple Strength (SS): SS was measured on 10 staples per sheep, taken from the whole fleece using a CSIRO measurement system (ATLAS), based on the standard Australian test for the determination of mean staple strength and staple length (AS 2810, 1985). The staple strength calculated by the ATLAS was corrected according to an adaptation of the regression equation of Kavanagh and Bow (1985).

Daily management: Experimental conditions were checked daily. Feed and water troughs were cleaned on a regular basis. General sheep health and behaviour were observed daily.

At the end of the experiment (109 days) all sheep were shorn and released to pasture (Plate 7.4 bottom).

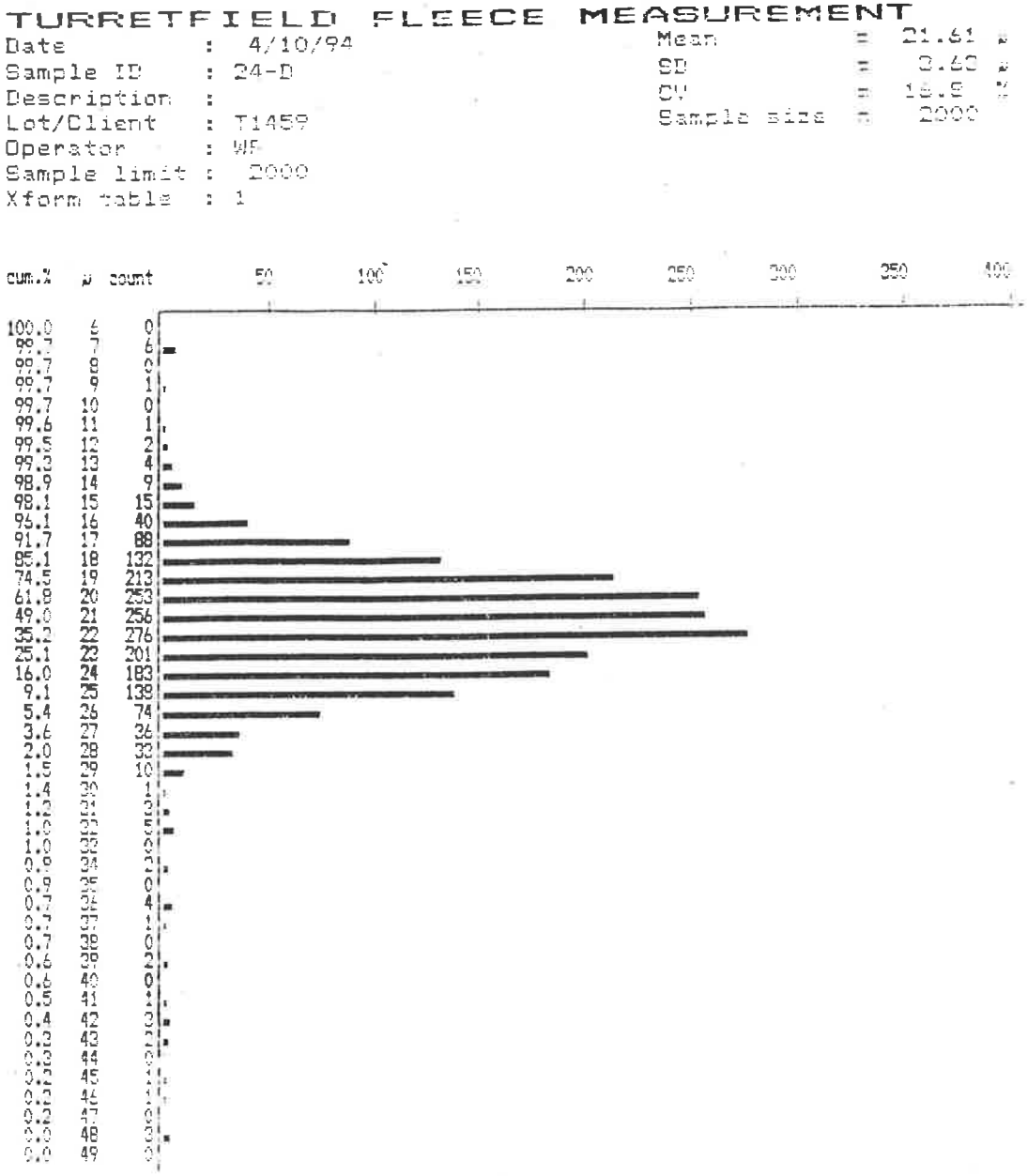


Figure 7.2: An example of fibre diameter (sheep no.14, group B) measurement using the FFDA analyser

7.6. Results

Voluntary dry matter intake (DMI): Results of DMI of the whole rations are given in Figures 7.3 and 7.4 and Appendix 7.1. The results of DMI of individual feedstuffs are given in Table 7.7. These data are also presented graphically for each treatment throughout the experimental period in Figure 7.6. The results of statistical analysis of the data is shown in Appendices 7.1 and 7.3.

Total DMI and intake of individual ingredients during about the first 6 weeks of the experiment for all rations (A, B,C, D and E) increased weekly, while during the subsequent weeks it was nearly constant. This might have been because during the first weeks of the experiment the sheep were still adapting to the feedlot conditions and also were gradually getting used to ammonia-treated diets (Figures 7.3 to 7.6). While there were different DMI of diets A, B and C during the experiment (Figure 7.3) these differences were not significant. Similar results were obtained for diets D and E as well (Figure 7.6). Figure 7.5 shows that the DMI of treated seagrass in diet A during the first 3 weeks was lower than the DMI of straw in diet B; was the same in week 4, but was again lower during the following weeks.The same figure shows that the DMI of straw in diet C initially was lower than that in diet B, but sharply increased after week 3 and this increase continued up to week 7 when it was higher. There was no significant difference however in average DMI of seagrass in diet A and straw in diet B and C over the experimental periods (Table 7.7). Average weekly DMI of lucerne in diets A and B, and chicken litter in diet C are also shown in Table 7.7. These data show that there was no significant difference between them. Figure 7.6 in general shows that the DMI of diets containing lucerne (A and B) and chicken litter (C) were significantly higher than for diets D and E which did not contain lucerne nor chicken litter.

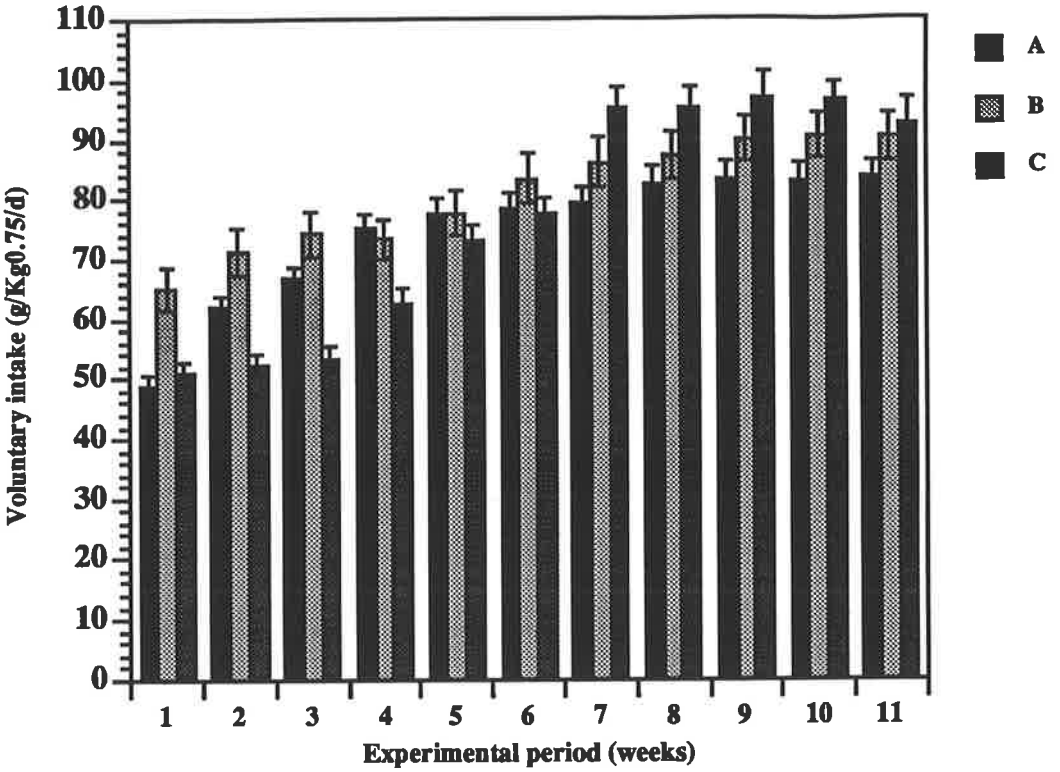


Figure 7.3: Average daily dry matter voluntary intake of diets A, B and C

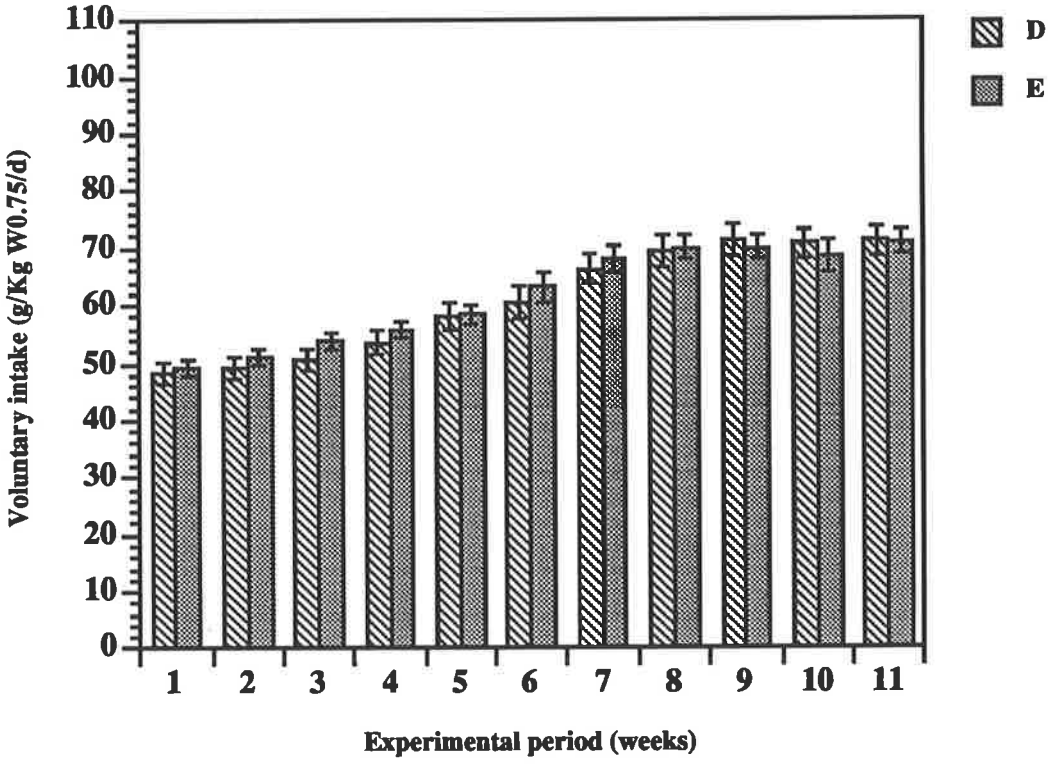


Figure 7.4: Average daily dry matter voluntary intake of diets D and E

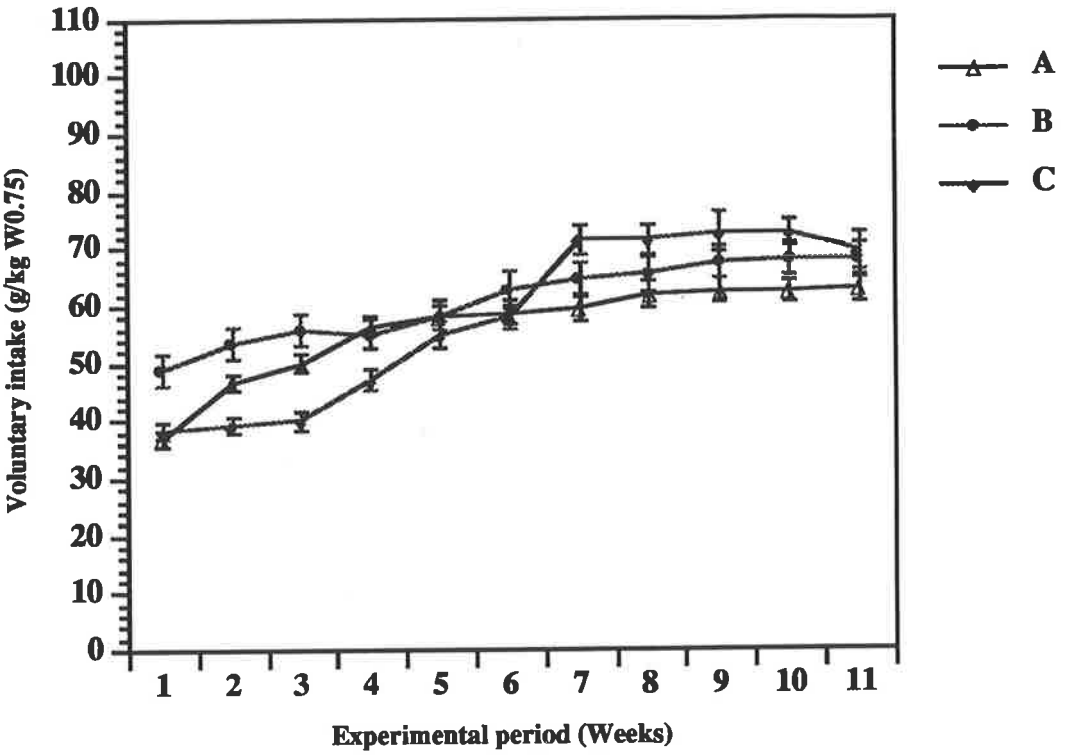


Figure 7.5: Trend of average voluntary intake of straw (Diets B and C) and seagrass (Diet A)

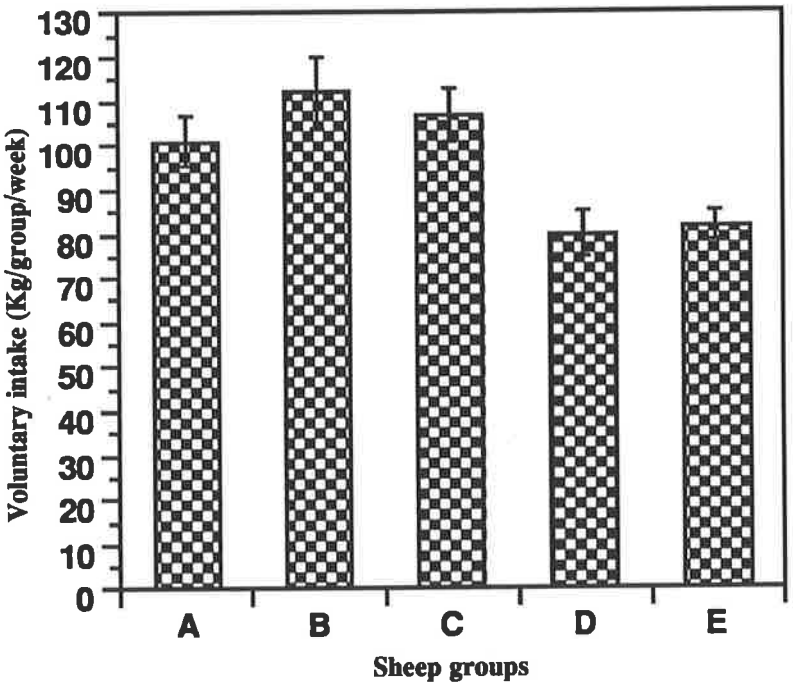


Figure 7.6: Average weekly voluntary intake of whole diets (A, B, C, D and E) by the individual groups of sheep

Table 7.7: Average voluntary intake (Kg/sheep/week) of different diet ingredients over the whole experiment (Data show means ± SE on dry matter basis)

Sheep groups	Diets ingredients			
	Lucerne	Treated Straw	Treated Seagrass	Chicken litter
A	2.5 ± 0.15	-	7.6 ± 0.44	-
B	2.79 ± 0.20	8.4 ± 0.61	-	-
C	-	8.0 ± 0.45	-	2.7 ± 0.15
D	-	4.0 ± 0.26	4.0 ± 0.26	-
E	-	8.2 ± 0.33	-	-

Body weight changes: Weekly mean body weight and body weight changes of the five groups of experimental sheep are summarised in Figures 7.7 and 7.8 and Appendices 7.3 and 7.4. In all treatments (except groups D and E in week 1) sheep body weight increased steadily over the experimental period.

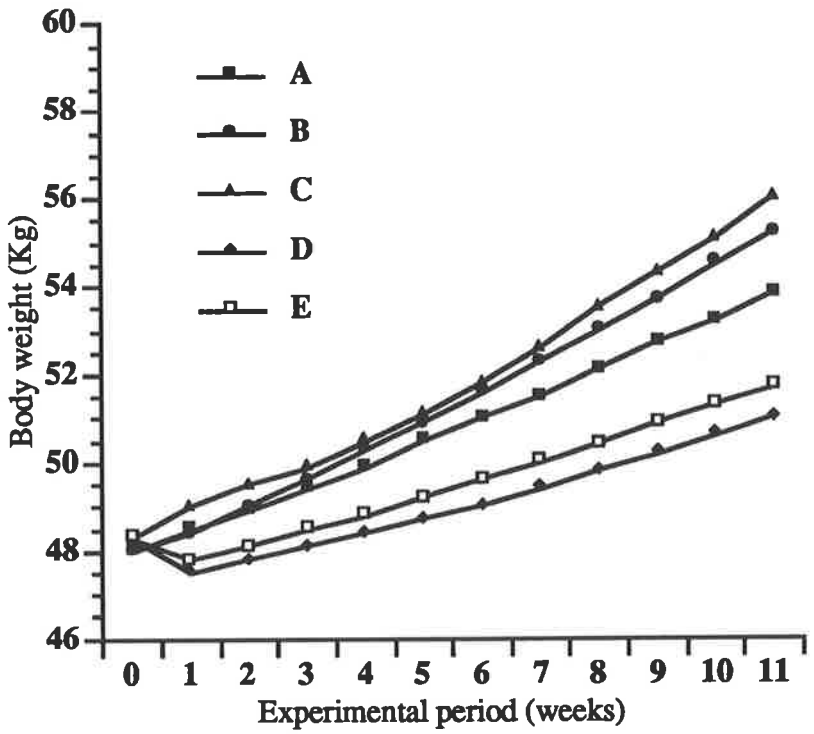


Figure 7.7: Trends in mean body weight of the five groups of sheep over the experimental period.

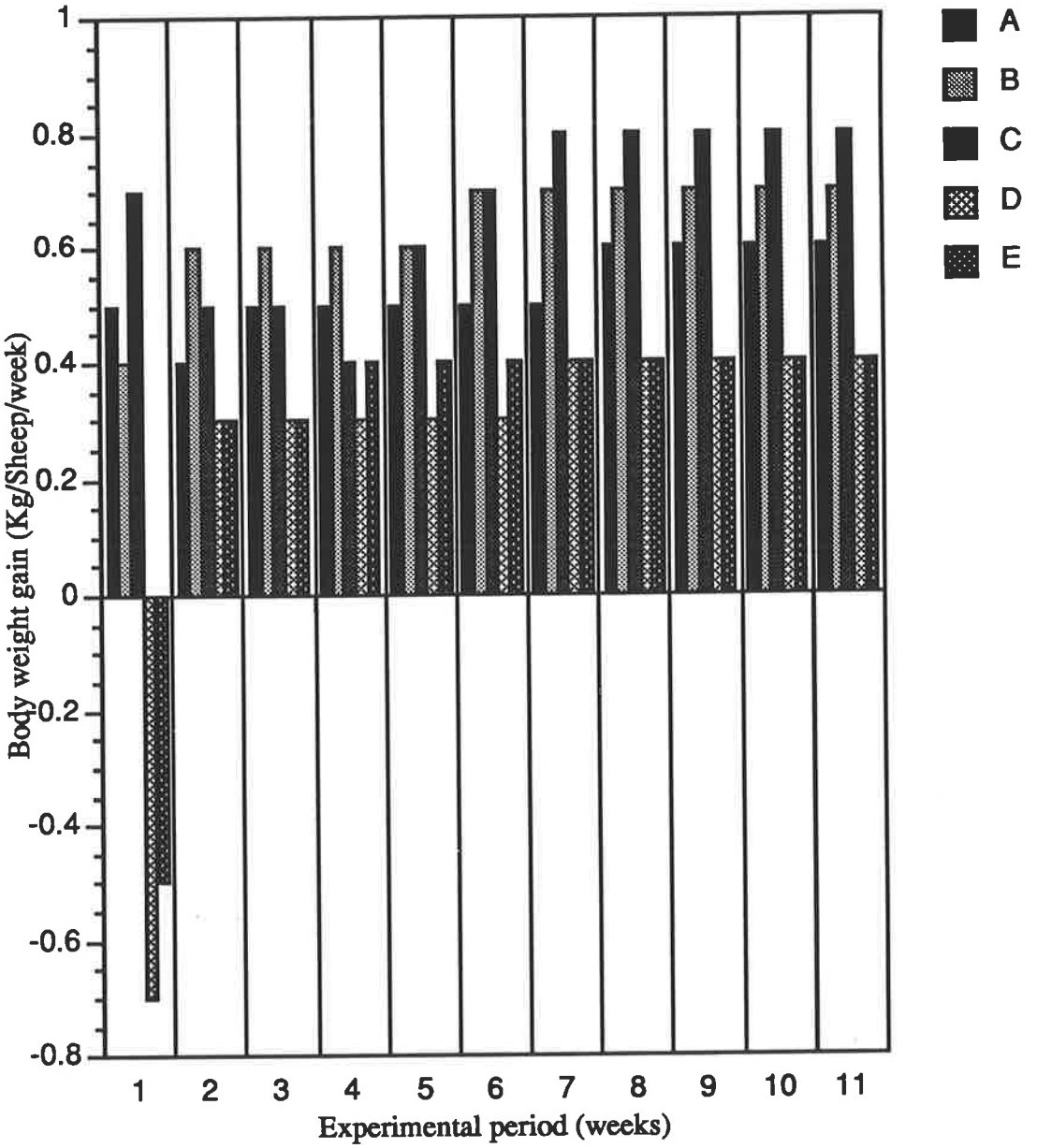


Figure 7.8: Body weight changes of five groups sheep over the experimental periods

This negative body gain of sheep in groups D and E in the beginning of the experiment might have been due to inadequate intake of protein and energy during the adaptation period. The levels of body gain in sheep on diets D and E were significantly less than of those on diets A, B and C througout the experiment (Figure 7.7 and 7.8); because of lower crude protein and energy intakes. Although there were some differences in body weight gain between the sheep in groups D and E in some weeks the difference in total body gain during the entire experimental period was not significant (Table 7.9). Between sheep of groups A, B and C total body gain of the sheep in group A was significantly less than that of the other

two groups. This might have been due to less voluntary intake of diet A (112 vs 122 and 117 Kg/sheep/77 days).

The total body gain of sheep which had been grazing on green pasture the experiment is graphically compared with that of the experimental sheep in Appendix 7.7. According to this data the body weight gain of sheep grazed on pasture was significantly less than that of the sheep groups B and C, more than for the sheep in groups D and E ($P < 0.05$) and equal that of the to sheep group A.

Fat score: The mean weekly fat scores of the experimental sheep are shown in Figure 7.12. It is clear from the data presented there and in Appendix 7.6 that there were no significant difference between mean scores in week 0, 1, 2, 3, and 4. During the following weeks, however, significant differences ($P < 0.05$ in weeks 5, 6, 7, 8 and 10; $P < 0.01$ in weeks 9 and 11) in fat scores developed. The general trends of mean fat scores were similar to those of body weight (Figures 7.10 and 7.11).

Feed efficiency: Feed conversion ratios (Kg feed intake : Kg body gain) for the five diets during the experimental period are shown in Table 7.8. According to these results diet C (with chicken litter) showed the best feed efficiency and diet D showed the poorest. These differences in feed conversion ratios between the diets (Table 7.8) are similar to the differences in metabolizable energy supplied (Table 7.4).

Feed cost: Daily feed costs for growth and maintenance levels are shown in Table 7.9. This data shows that in order to produce 100 g body gain per day the cost of diet C (containing chicken litter) is lowest in, comparison with both diets A and B (containing lucerne) and diets D and E (without lucerne). The differences of feed cost in term of maintenance for the experimental diets are similar to those for growth. A comparison of feed cost by computer program and by practice shows some intersting differences. It is supposed that in practice it is almost impossible to compound rations (even with a computer program) that meet exactly the nutrient requirements of pre-defined classes of animals. Different individual performances between animals, variation in nutrient content of feedstuffs, feeding

management and environmental factors such as heat or cold stress all the important factors that can affect the animal responses to the computer-based balanced rations.

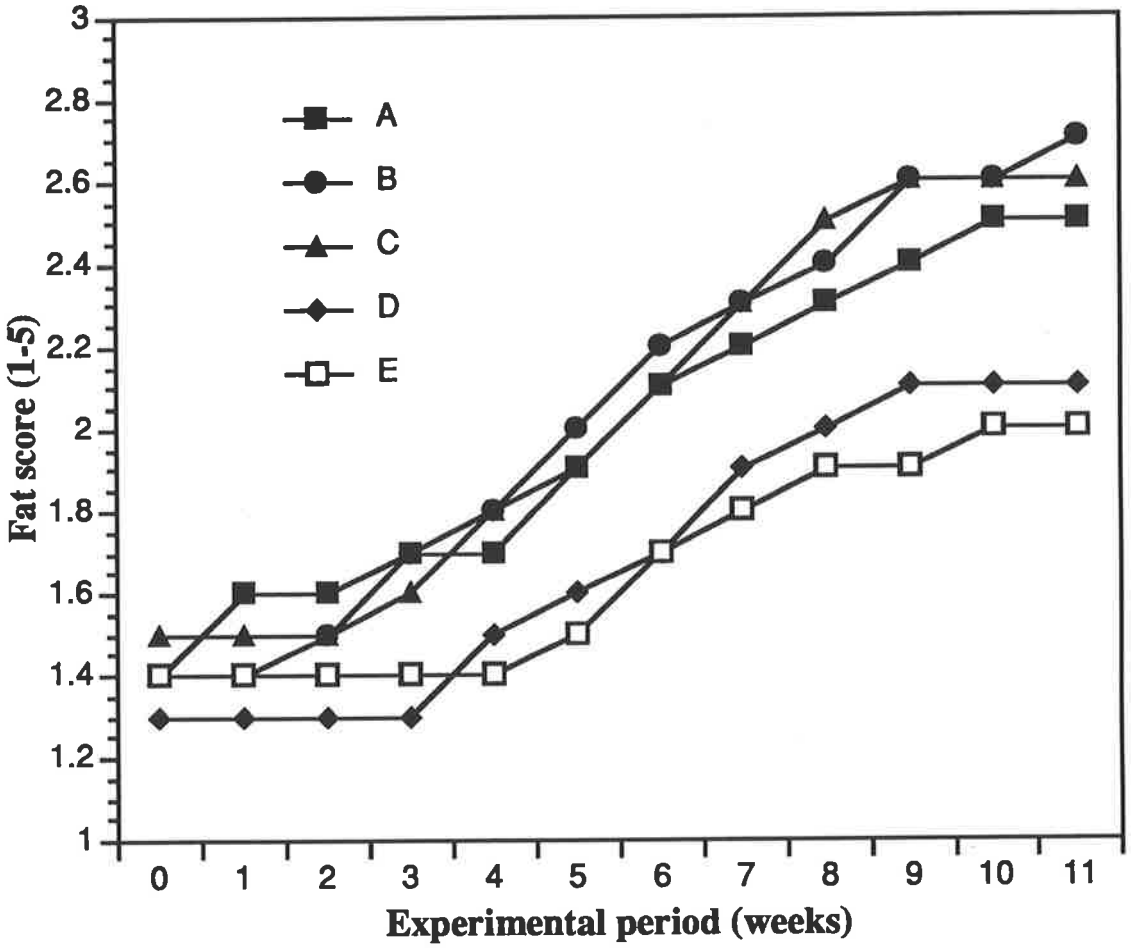


Figure 7.9: Mean fat score of the five groups of sheep over the experimental periods

Table 7.8: Feed conversion ratios in five groups of experimental sheep*

Diet	Wt. gain (Kg/sheep/ 77d)	DMI** (Kg/sheep/ 77d)	Feed conversion ratio (Kg feed intake/Kg body gain)
A	5.9^{b}	111.2^{b}	19.6^{a}
B	7.2^{c}	122.7^{b}	17.8^{a}
C	7.5^{c}	117.7^{b}	15.7^{a}
D	2.8^{a}	88.0^{a}	33.7^{c}
E	3.1^{a}	89.7^{a}	27.8^{b}
LSD (P<0.05)	0.85	17.2	5.2

* Mean within each column that share no common superscript differ significantly (P<0.05)
** DMI= Dry matter intake

Table 7.9: Daily feed costs (cents/sheep/d) for South Australian adult Merino wethers (50 Kg) (from TAKE AWAY computer program)

Animal status	Rations	Price	
		By computer	In practice
Growth (100 g/d):			
	A	47	57
	B	47	62
	C	42	51
	D	69	105
	E	64	97
Maintenance:			
	A	25	-
	B	26	-
	C	23	-
	D	32	-
	E	32	-

Wool production: The data obtained from wool measurements are shown in Table 7.10 and Appendix 7.8. In general there were few statistical differences, except that sheep on diet A showed consistently lowest values for greasy and clean fleece weight, staple growth rate, length:diameter ratio and staple strength.

A comparison of these characteristics of the experimental sheep and those of sheep grazed for the same time is also shown in Appendix 7.7. Important results seen from this table relate to clean wool growth rate, standard deviation of fibre diameter and L:FD ratio. There was not a significant difference in clean fleece growth rate between sheep grazed on pasture and sheep in diet groups B, C, D and E, but this value was higher than for sheep in group A. In spite of a lower FD of sheep grazed on pasture the variation (SD) of this measurement was still considerably less than for all experimental sheep. There was also a significant difference between sheep grazed on pasture and the experimental sheep in terms of L:FD ratio.

Health condition: There were no apparent health problems with any of the sheep during the course of the experiment.

Table 7.10: Wool production characteristics of experimental sheep

component	Sheep groups					LSD*	
	A	B	C	D	E	P<0.01	P<0.05
Greasy fleece weight (g/sheep/9weeks)	705	806	846	767	863	141.7	105.6
Clean scoured yeild (%)	70.2	72.0	71.4	74.7	70.8	6.5	4.9
Clean fleece weight (g/sheep/9 weeks)	495	579	614	572	609	102.7	76.6
Clean wool growth rate (g/sheep/d)	8.0	9.3	9.9	9.2	9.8	1.7	1.2
Staple length (mm)	13.0	14.2	13.9	14.9	15.8	2.3	1.7
Fibre diameter (mean µm)	20.6	21.7	21.5	20.9	22.4	1.9	1.5
Fibre diameter (st.dev. µm)	1.4	1.7	1.1	1.6	1.9	-	-
Length:diameter (µm:µm)	10.2	10.6	10.4	11.5	11.4	0.01	0.10
Staple strength (N/Ktex)	41.8	51.1	46.5	55.9	47.8	9.9	7.4

*: Least Significant Difference

7.7: Discussion

As already discussed in the introduction to this chapter sheep fed the seagrass *P. australis* as a whole diet or as a high proportion of their diet lost weight. The major reason for this was apparently a low voluntary intake, although contributing factors were low digestibility and a limited supply of protein. Both the level of intake and the digestibility of seagrass were

increased following chemical treatment (NH_3) and by providing supplementary nutrients (molasses, green forage and chicken litter). The final target of the project was to determine wether or not farmers could use the seagrass as a alternative feedstuff for their sheep in case of short term feed scarcity or drought, for both maintenance and production. To approach this target four major criteria were examined, namely: voluntary intake, body weight gain, feed efficiency, feed cost and wool growth rate. To examine the use of seagrass as a possible feedstuff in terms of commercial realities straw (a more conventional lignocellulosic feedstuff) was used as a control and seagrass containing chicken litter (diet C) was used in comparison with lucerne hay for its nutritive value (in diet A and B).

In general the intake of seagrass and seagrass/litter containing rations (diets A and C) were comparable with that of straw and lucerne respectively (Diet B) (Table 7.8). The feed efficiency of seagrass and seagrass/litter in production rations was the same as for straw and lucerne (diet A, B and C) but feed efficiency of seagrass in case of maintenance (diet D) was lower than that of straw (diet E) (Table 7.8).

Voluntary intake: Forage supplements are already commonly fed on farms in other countries, particularly in Asia, because they are cheap relative to purchased concentrates. Improvements in voluntary intake and nutritive value of poor quality roughage by green forage and chicken litter has been discussed by several research workers (eg. Moran et al. 1983; Suriyajantratong and Wilaipon 1985; Devendra 1983; Vearaslip 1981 and Jakhmola R.C. et al. 1988). The results here also indicate that seagrass can compare favourably with straw when both are treated and supplemented with NH_3 and molasses. Overall it can be stated that an improved intake of seagrass by NH_3-molasses treatment and forage/chicken litter supplementation led to increased utilization of required nutrients by sheep.

Body gain and feed conversion efficiency: As was presented in Figures 7.7 and 7.8 and Table 7.8 all groups of sheep gained weight over the experimental period. Total body weight gains of sheep fed the different diets were qualitatively similar to their voluntary intake, except that sheep fed with chicken litter containing diet (C) showed higher body gain than sheep fed lucerne containing diet (B) despite their lower voluntary intake. As expected

sheep in groups A, B and C achieved higher body gains than sheeps in groups D and E (no lucerne nor litter).

Feed conversion efficiency of seagrass and seagrass/litter in diets formulated for production (A and C) was similar to that of the straw and lucerne containing diets (B) (Table 7.8). In term of maintenance, however, seagrass (D) was less efficient than straw (E) but could nevertheless meet the requirements of sheep for more than just maintenance. Both diets of course could be adjusted for intake to meet exactly body weight maintenance. High efficiency in Diets B, and C in converting feed to body gain was due to high content of metabolisable energy. Regarding Table 7.8 and Appendix 7.1, the intake level of diets A and C could meet 100 g body gain requirement of sheep, which was similar to diet B.

As was presented in Appendix 7.6 the body gain of the 5 groups of sheep during the experimental period was compared with a group of sheep grazed for the same time on green pasture. In terms of body gain the nutritive value of green pasture was less than that of the diets containing straw-lucerne (B) and straw-seagrass/litter (C), equal to that of the diet containing seagrass-lucerne and more than for diets D and E, which were formulated for maintenance alone. An important point than can be concluded from this result is that even in the presence of existing good quality pasture diet B and diet C (that is with seagrass/litter) in a lot-feeding system can be preferred for fattening sheep.

Wool growth and characteristics: In terms of quantity and quality of the wool produced by sheep is affected by the genotype, environmental and dietary factors (Brown 1976b; Allden 1979; Hynd 1982). In general, in all groups the rate of wool growth was in the range reported by Hall (1987). According to his finding clean wool growth rates in grazing sheep range from 3.6 g per day in winter to 15.3 g per day in spring, which is comparable with the result of this experiment, i.e from 8 g per day in group A to 9.9 g per day in group C (seagrass and seagrass/litter containing diet). There was no significant difference in the clean fleece weight of sheep fed a diet containing seagrass or straw, or between a diet containing lucerne hay and chicken litter (B and C). Finding relative to staple strength, fibre diameter and length:diameter ratio in sheep on the experimental diets were similar to the differences found in clean wool growth rate.

Comparison of the results of wool measurements of experimental sheep in lot-feeding with the sheep on pasture showed the latter achieved clean fleece weight growth equal to that in the feed lot, with the exception that wool growth on pasture was greater than on diet A (seagrass/lucerne), except group A had a lower body weight than the pasture group of sheep. An intersting result seen from the data is that the standard deviation of mean fibre diameter of the wool of the sheep on pasture was significantly less than from all feed-lot groups. This higher value of standard deviation in feed-lot groups could be due to a more variable voluntary intake over the experimental periods. The cost of diets presented in Table 7.9 include delivery cost, that in this experiment seagrass and straw were delivered to the experimental location from 500 and 100 Km respectively away, so considering this fact it can be concluded that the cost of seagrass and chicken litter based diets are much less than that for straw and lucerne based diets.

Feed cost: The costs of the experimental diets were calculated both by using the TAKE AWAY computer program and by observation in practice (Table 7.5 and 7.8). In terms of the relative cost of body weight gains diet C (containing chicken litter) proved to be the most economical. Clearly diets D and E involved higher costs in term of body weight gain, but these were formulated for maintenance only.

In summary, seagrass, when mixed with green forage (e.g. lucerne) can compete with straw in terms of sheep body weight gain. In addition, when seagrass is used first as a bedding material for broiler chicken the resultant litter can be used proportionally in a mixed diet to provide enough nutrients and energy for sheep production compete comercially with lucerne.

In terms simply of maintenance of body weight although all 5 diets would be successful, but in time of drought or feed scarcity it is suggested that either of the cheaper diets D (seagrass containing) or E (straw only) could be used with equal efficiency in a lot-feeding system.

CHAPTER 8

General discussion

CHAPTER 8

General discussion

The results of the various experiments of this project and their appropriate interrelationships have already been reviewed and discussed in the separate chapters of this thesis. This final chapter summarises and interprets the main points of the various sections and makes suggestions for further research.

According to many scientists there is a need to double animal protein production in the next 20 to 25 years in order to improve the protein intake status of the world's rapidly growing human population (Minson 1990). In the agricultural sector the sheep industry is one of the major and largest enterprises. To provide the protein required for adequate human nutrition maximum protein production of sheep must be achieved, but can be only if the animals are supplied with sufficient quantities of the raw materials required for the synthesis of protein products. In order to nourish adequately the world population the nutrient yield per hectare needs to be some three times higher than at present (Boda 1990). This situation could theoretically be achieved by several solutions including: breeding more efficient animals, breeding plants which are able to fix more than the current maximum of 3% of solar energy and finding new protein and energy sources.

Among these solutions the use of non-traditional resources should be examined, for both animal and human nutrition. Due to the superiority of ruminants in their digestive system a strategy for the utilisation of non-conventional raw materials can be in this way extended much further throughout the world.

One of the most important non-conventional resources that should be considered seriously throughout the world, and especially in Australia for animal nutrition, is aquatic plant life. The saline waters which cover about 71% of our planet's surface support many different kinds of plants. Amongst these plants seaweeds and seagrasses are the important constituents of the marine vegetation (Christianson *et al.* 1988). There are thousands of

known species of seaweeds and seagrasses (Womersley 1980; den Hartog 1970), which are possibly the world's most productive plants (Schopf *et al.* 1978; Westlake 1963). They can contribute both to food webs in coastal waters and to terrestrial animals. Therefore the use of marine plant for animal nutrition, especially ruminant nutrition, should be placed on the agenda of world animal nutrition programs to solve world-wide feed deficiency. Nutritional studies of marine plants should be carried out based on their local availability.

Primary study on the nutritive value of the more readily available marine plants from the area of Kingston, South Australia, showed that the chemical composition and digestibility *in vitro* of various species of seaweeds and seagrass were very different. In general the chemical composition of aquatic plants are always linked to their respective morphological characteristics and growth stages, which at any one time of collection can differ substantially between species (Pirc 1989). Clearly the two most important factors which can greatly affect the commercial utilisation of aquatic plants for animal production are cost of handling and nutritive value. In this study these two factors lead the project to screen the species *Posidonia australis*, which dislodges from the sea bed during storms and is cast onto the beach, where it is massed and exposed to the sun for a long time. In order to use this and other aquatic plants in animal nutrition intensively, however, the establishment and development of appropriate harvesting methods can be regarded as an urgent priority.

Results arising from the chemical analysis of four different physical forms (green, fresh, dry-unwashed, dry-washed) of *Posidonia australis* showed that there are no great differences among these different forms. So it can be proposed that in simple economic terms the utilisation of the more available dry-unwashed form of *Posidonia* is preferred to the other forms, at least from Kingston, South Australia.

The studies reported here in chapter 3 also indicated that *Posidonia* is rich in NDF (45.2%) including 23.4% non-starch polysaccharides and 18.6% uronic acid, contains 14.5% lignin, is rich in ash (20%), including 14.6% soluble and 5.4% insoluble ash, poor in crude protein (5.6%) and ether extract (1.1%), and of low dry-matter digestibility *in vitro* (34.7%). In addition it contains about 1.7% tannin, which is proportionally high. The quantity of major nutrients in the composition of *Posidonia* and its digestibility make it a typical lignocellulosic

feed resource for ruminants. According to normal international classification, animal feedstuffs with a crude fibre content more than 18% and a low crude protein content are grouped as roughages (AFIC 1987).

The results of the experiments *in vivo* presented in the first section of chapter 4 showed that both voluntary intake and digestibility of *Posidonia* were low. This result confirmed that this material can be regarded generally as a poor lignocellulosic feedstuff. Accordingly, from both this finding and the observation of its low protein content it can be proposed that the application of some useful and practical treatments to improve the nutritive value of *Posidonia* should be examined in order to establish its potential for sheep nutrition.

In the subsequent experiments *in vivo* of Chapter 4 the effects of natural decomposition and of supplementation with green hay (lucerne), chicken manure and molasses were studied.

Natural composting of *Posidonia* is both a physical and a chemical process. The process causes losses of organic matter, in particular neutral detergent fibre (from 34% to 8%) and proportional increase in ash content (from 20.5% to 40.5%), but no change in the proportion of protein. An increased ash content due to decomposition is in agreement with the results of Odum (1984), who pointed out that processing of seagrass causes remineralization. Another important possibility for the increased ash content could be just mixing of the material with coastal sands over time. A loss of crude fibre during composting was mentioned by Doyle *et al.* (1986). The explanation of constant protein could be that the intensity of biological decay on this seagrass was low, and/or the mixing up of coastal sands was high, so that any increases due to biological processes did not result in any increase in total crude protein content. The data here also showed that the intake of *Posidonia* decreased following decomposition. That was in some contrast with the work of Garnsworthy and Cole (1990) who pointed out that in ruminants when a roughage offered is changed to a smaller particle size its voluntary intake generally increases, due to the faster rate of passage of material from the rumen. This contrast here might be explained by the change in size due to decomposition as being different from that affected by simply chopping or grinding, or decomposition increased oxidation of phenolic compounds and made it unpalatable, or more likely the increase in ash content affected palatability and, as a result,

voluntary intake decreased. Therefore because of these negative effects of natural decomposition on the nutritive value of *Posidonia* there is no preference for using this rather than fresh material.

The results of Chapter 4 also showed that *Posidonia*, after its use as a bedding material for broiler chickens, greatly increased in crude protein content (from 5.6% to 18%), voluntary intake and digestibility. In addition to this, the chickens on the seagrass bedding had as normal growth as these on regular sawdust bedding. Several references (Krishna Reddy and Raj Reddy 1989; Jakhmola *et al.* 1988 and Muler 1980) indicate that poultry excreta can improve the nutritive value of bedding material. It is strongly recommended that seagrass massed on Kingston, South Australia beaches should be considered as a relatively inexpensive and readily-available bedding material for broiler chickens. After the rearing period the material, with its high content of crude protein, high digestibility and intake can be used as a partial ruminant feedstuff.

Molasses treatment at a high enough concentration improved both voluntary intake of *Posidonia* by sheep and its digestibility. It has been known for a long time that molasses is a useful supplement (Mc Donald *et al.* 1988) and inclusion of it into animal diets of low quality feed increases voluntary intake (Schiere *et al.* 1988). Concerning the ash content of both decomposed *Posidonia* litter and *Posidonia*, which is high, molasses supplementation of these two materials can be able not only to control the dust problem but also to increase palatability. Generally the results of Chapter 4 of this thesis draw the conclusion that *Posidonia* alone, because of its physical and chemical nature, is not able to meet the maintenance and production requirements of sheep. So its supplementation with protein and energy sources (such as green hay, chicken manure or molasses) is recommended.

In the experiments *in vitro* of Chapter 5 various alkalis and fungal treatments were examined in order to find a more practical way to use *Posidonia* more effectively for sheep nutrition. The data presented in that Chapter indicate that sodium hydroxide, calcium hydroxide, urea/ammonia and fungi are all effective treatments in increasing dry matter digestibility of *Posidonia*. The increment in dry matter digestibility and cell-wall degradability following treatment of *Posidonia* with sodium hydroxide was higher than for other lignocellulosic

materials. This is in agreement with the conclusion of Owen (1978) that greater increases in digestibility can be expected following treatment of roughages with low initial digestibility. The 4% concentration of sodium or calcium hydroxide in 24 hours reaction time and 6% concentration of urea solution in 20 days gave better results than other levels tested. As there was previously no information available on the effect of alkali treatment on the digestibility of seagrass the level applied in this project was similar to that used in experiments *in vivo* and *in vitro* reported elsewhere for other roughages (Klopfenstein 1978). The results indicate that the effect of alkali treatments on different lignocellulosic material are basically the same. Although the treatment of lignocellulosic material with some species of white-rot fungi causes considerably increase in digestibility of structural carbohydrates, due to degradation of lignin,(Jung *et al.* 1992) the species of fungi used in this study, *C. versicolor* and *P. gigantea* had not great effect on *Posidonia.*

Data from experiments *in sacco* (Chapter 5) showed that in general the ruminal degradability of *Posidonia* in 72 hours was close in value to the results *in vitro* and *in vivo* presented in Chapters 3 and 4. As a rough guide Ørskov *et al.* (1980) suggested that poor quality roughages require 48-72 hours incubation time to achieve maximum degradability.

These results also showed that the effect of alkalis on the degradability of structural carbohydrates (NDF, ADF) of *Posidonia* was similar to that on dry matter degradability. Generally NDF degradability was substantially increased up to 6.3 percentage units by fungi (*P. gigantea*) 18.8 by urea/ammonia, 28.7 by $Ca(OH)_2$ and 35.4 by NaOH. The action of the treatment could be explained by solubilization of hemicellulose increasing the extent and rate of both cellulose and hemicellulose digestion (Wanapat *et al.* 1985).

Generally for efficient utilisation by ruminants of lignocellulosic materials like *Posidonia* it is essential that the rate of microbial digestion be maximised. Both nitrogen and energy are limiting factors. While the energy availability of fibrous feeds can be increased by alkali treatment, nitrogen deficiency remains as an important limiting factor for materials such as *Posidonia*, Although NaOH was more effective in increasing digestibility and consequently increasing available energy of *Posidonia*, the N limitation removed any advantage. Thus it is strongly recommended that the urea/ammonia treatment can overcome more than any other

treatment both energy and N deficiency of *Posidonia*. Fungal treatment, because of its low effect on digestibility, difficulties in large scale operation and high cost, is not suggested for *Posidonia*.

In Chapter 6 the effects *in vivo* of NaOH, urea/ammonia, molasses and ammonia/molasses treatments on the nutritive value of *Posidonia* were examined for two reasons: firstly to compare with results *in vitro* described in Chapter 5 and secondly to evaluate rumen ecosystem parameters in relation to this novel plant, *Posidonia*. In summary, all alkali treatment methods confirmed *in vivo* the results of methods *in vitro*, i.e. in terms of increased digestibility.

The results of N-balance studies in this thesis indicate that the feeding of chemically-treated seagrass significantly improves the proportion of crude protein (N x 6.25) retained by sheep. The improvement in nitrogen balance for treated seagrass may be a result of the presence of more readily fermentable carbohydrate derived from available cellulose and/or improved microbial nitrogen capture of the soluble and cell-wall nitrogen in the treated seagrass. This would be in agreement with Moss *et al.* (1992). Sheep fed molasses-treated seagrass retained more N than did controls and it seems reasonable to infer that this might have resulted from lower losses of ammonia from the rumen (Thomas and Thomas 1985), a possible result of giving more readily-fermentable carbohydrate.

One important rumen parameter which is expected to be affected by a change of diet is pH. In this study the pH of rumen digesta was higher when sheep were fed the alkali treated *Posidonia* diet than when fed the un-treated material. This was in contrast with the result of Kerley *et al.* (1986). The possibility exists that because of the low level of moisture used more of the alkaline solution was retained on the *Posidonia* in the present study.

In this study considerable diurnal variation in ruminal NH_3 concentration was observed in sheep fed both untreated and treated seagrass. As expected NH_3 and NH_3/molasses treated seagrass increased the average rumenal concentration of NH_3, although NaOH and molasses alone did not. These results are generally in agreement with those of Drori and Loosi (1961), Leng and Nolan (1984) and de Wall and Biel (1989).

Data showed that the average ruminal total VFA concentration in sheep fed untreated seagrass was 86.6 mM; which is comparable to that reported by Jayasuriya (1981) for sheep fed untreated rice straw, namely 101.6 mM. Among the diets all treated seagrasses resulted in lower levels of mean total VFA, in spite of increased digestibility of cell wall contents such as cellulose. In considering this result it must be remembered that the level of any substances in the rumen of any one time is determined not only by its rate of production but also by its rates of metabolism, absorption and passage from the rumen, as well as by any dilution factors. In this study the significantly-low concentration of total VFA in the rumen of sheep receiving treated seagrass could be due both to the effect of increased rumen volume, resulting from higher level of intake and higher consumption of water due to alkali treatments, and to increased absorption and metabolism. The average relative proportions of the main individual VFAs (acetic:propionic:butyric) in rumen of sheep fed untreated seagrass was 60:16:30 and on treated (NaOH, molasses and NH3/molasses) seagrass diet this proportions in first 3 hours after feeding was 70:19:11. That there was an increase in acetate proportion could be a result of the presence of more available or fermentable carbohydrate, such as cellulose and hemicellulose, due to break down of lignin by chemical treatment (Moss *et al.* 1992).

All microorganisms including total, viable, cellulolytic and proteolytic bacteria and protozoa of rumen of sheep fed with treated seagrass increased after 12 hours feeding in all groups of sheep. Treatment of the seagrass also caused significant increases in the average numbers of these microorganisms. These result are in agreement with these of Bryand and Robinson (1961).

Rumen microorganisms are either highly specialised, intermediate or very broad in relation to the type of nutrients that they will use. In some respects it can be said that when the ruminant animal has consumed its food the rest of the digestive process is left essentially to the rumen microbes. Therefore this study of rumen micro-organisms can give some basic estimation of the relative nutritive value of seagrass. In general the examination of some important rumen ecosystem parameters showed that the effect of both untreated and treated seagrass on rumen condition were similar to those of other traditional lignocellulosic

feedstuffs. Thus it seems both possible and logical that this novel roughage can be added to the list of more conventional feedstuffs to meet some aspects of ruminant nutritional requirements.

In an area with a Mediterranean-type climate, such as southern Australia, with its regular summer dry months and periodic droughts, in order to cope with recurring droughts and to reduce accompanying sheep damage to annual pasture seed reserves and soil farmers must adopt appropriate drought feeding management systems. A recommended management is lot-feeding, or hand-feeding of sheep in a confined area, during late summer and early autumn. The study reported in Chapter 7 of this thesis was an attempt in this direction. In this chapter a practical commercial guide was recommended for locally-available *Posidonia*, with special concern to its nutritional characteristics as obtained from the results of the previous experiments.

In a survey conducted over the agricultural districts of South Australia (Valizadeh 1994) over 75% of the responding Livestock Officers and Agronomists recommended lot-feeding as a management strategy for reducing the effects of short term and long-term drought on sheep and pasture, avoiding soil erosion, securing newly-emerged seedlings following the break of season and maintaining an adequate pod and seed reserve of annual legumes. Some 90 percent recommended lot-feeding during the late summer-early autumn period. This information shows that lot-feeding is regarded as important in the cereal-livestock zone, where both soils and pastures are most prone to damage by excessive grazing in summer and autumn.

Generally the hand feeding of sheep requires careful feed selection, ration good formulation and appropriate feeding management. Computer formulations of least-cost rations (such as with the TAKE-AWAY ration formulation program developed by the South Australian Department of Primary Industry) are excellent tools in this regard, in order to achieve maximum benefit at minimum cost.

Ammonia/molasses-treated *Posidonia* can be fed to sheep in considerable amounts (50% of the required dry matter), with wheat straw mainly at maintenance or drought-feeding levels.

These sheep may lose considerably body weight first but weight can be recovered very soon after adaptation. The low crude protein content of fibrous feedstuffs like seagrass can reduce its feeding value by restricting normal microbial activity in the rumen (ICARDA 1991). In such circumstances feeding ruminants with urea/ammonia-treated *Posidonia* can improve nitrogen retention and dry matter digestibility of the ration. These were the main reasons for choosing urea/ammonia treatment for the final feed-lot experiment. Additionally supplementation of treated *Posidonia*-based diets with the inclusion of around 25% lucerne hay can meet body weight gain requirements of about 80 g/sheep/day. A very useful and commercially recommendable result from the studies of chapter 7 is that when *Posidonia* is used as a bedding material for broiler chicken for 6 weeks then the resultant litter, with amounts of 25% in the ration, could meet the body gain requirement of 100g/sheep/day. This result in terms of feed efficiency and cost, statistically, was equal to the value of lucerne hay at the same level in the ration.

In general, in groups of sheep fed with *Posidonia*-based diets the rate of wool growth was in the range reported by Hall (1987) and comparable with other more traditional diets. Also wool growth and its characteristics in experimental sheep in this lot-feeding trial were comparable with those from sheep grazed in green pasture. In summary from the results presented in Chapter 7 it can be recommended that lot-feeding of sheep with treated *Posidonia* and *Posidonia* litter-based diets during the dry period of southern Australia can be successfully established with simple and inexpensive facilities at the farm level, for both maintenance and production (meat and wool) purposes.

As a final conclusion it can be stated that this project is the only known study using the seagrass, *Posidonia australis* as, an unconventional marine plant in ruminant nutrition. Clearly more studies on both nutritive and possible anti-nutritive value of this potential feedstuff are required. Apart from this species of marine plant, which was selected primarily on its readily availability at South Australia beaches, there are thousands of other species of aquatic plant throughout the world with different potential nutritive value (poor to rich) that could be considered as new sources of animal feed. For any extending study on the utilisation of any aquatic plants the two important overall issues of concern would be the :

negative and positive environmental effects of collection and the economic realities of the processing industry from harvesting to utilisation.

9

Appendices

9 APPENDICES

Appendix 3.1: Ingredients and chemical composition of the ration* fed to the experimental sheep

(A) Feed ingredients

Ingredients	%
Oat hulls	40
Oat (grain)	39
Triticale (grain)	15
Bentonite	2
Salt	1
Limestone	2
Urea	0.5
Vitamin and minerals	1

(B) Composition of the vitamin and minerals

Vitamin and mineral	IU/Kg	mg/Kg	Active ingredient
Vitamin A	8250	-	-
Vitamin D3	1650	-	-
Iron	-	93	Ferrous soulphate
Manganese	-	48	Manganous oxide
Zinc	-	52	Zinc oxide
Cobalt	-	1	Cobalt sulphate
Molybdenum	-	0.4	Sodium molybdate
Iodine	-	0.6	potassium iodide

(C) Chemical composition

Nutrient	KJ/Kg	%
Digestible energy	9.4	-
Crude fibre	-	17.00
Crude protein	-	7.80
Calcium	-	0.66
Phosphorus	-	0.24

* Australian Feed Services Pty. Ltd., South Australia

Appendix 3.2: Approximate chemical composition of 12 species of seaweeds and 1 species of seagrass in comparison with lucerne hay (%DM).

Plant* samples	Ash	Organic matter	Crude protein	Crude fibre	Ether extract	Nitrogen free extract
Seaweeds:						
AP	33.0	67.0	7.3	8.8	1.1	49.8
CD	24.7	75.3	4.8	8.9	1.7	59.9
CM	19.1	80.9	5.1	10.1	1.6	64.1
CR	23.8	76.2	6.5	3.7	1.1	64.9
CS	20.5	79.5	5.3	4.9	1.7	67.5
ER	28.6	71.4	5.6	5.8	1.2	58.7
SA	19.5	80.5	4.4	7.7	1.1	67.3
SB	28.3	71.7	4.8	7.8	1.1	58.0
SD	31.5	68.5	5.4	6.6	1.7	54.8
SL	40.0	60.0	5.5	6.4	1.3	46.7
SVa	28.3	71.7	4.6	5.9	1.5	59.7
SVe	34.3	65.7	6.0	6.2	1.1	52.4
Seagrass:						
PA	19.8	80.2	5.5	34.4	1.1	39.2
Legume:						
Luc.	8.3	91.7	17.9	29.8	2.0	42.0
LSD 1%	3.5	3.5	1.2	2.5	0.3	4.7
LSD 5%	2.6	2.6	0.9	1.8	0.2	3.5

* See abbreviation on section 3.1.2

Appendix 5.1: Mean dry-matter digestibility *in vitro* of *Posidonia australis* treated with different concentrations of NaOH for different incubation times

NaOH concentration	Reaction time (days)				
(% dry matter)	1	2	3	4	Mean
0	34.7	34.7	34.7	34.7	34.7^{a}
2	40.0	41.2	42.0	42.4	41.4^{b}
4	50.0	52.4	54.0	54.3	52.7^{c}
6	57.0	59.2	60.1	60.4	59.2^{d}
8	60.0	61.4	63.2	63.5	62.0^{e}
Mean	48.3^{a}	49.8^{ab}	50.8^{b}	51.1^{b}	

Significance:	:	LSD*(0.01)
	Interaction (NaOH concentration x time)	NS
	NaOH concentration	1.7
	Incubation time	1.5

Means on the same line or column having different superscripts are significantly different ($P<0.01$); * LSD= Least significant difference; NS= Non significant

Appendix 5.2: Mean organic-matter digestibility *in vitro* of *Posidonia australis* treated with different concentrations of NaOH for different incubation times

NaOH concentration	Reaction time (days)				
(% dry matter)	1	2	3	4	Mean
0	23.5	23.5	23.5	23.5	23.5[a]
2	34.5	35.5	36.1	36.4	35.6[b]
4	44.7	46.8	48.2	48.5	47.0[c]
6	52.6	54.6	55.4	55.7	54.6[d]
8	55.4	57.0	58.7	59.0	57.5[e]
Mean	42.1[a]	43.5[ab]	44.4[b]	44.6[b]	

Significance:	LSD*(0.05)
Interaction (NaOH concentration x time)	NS
NaOH concentration	1.5
Reaction time	1.3

Means on the same line or column having different superscripts are significantly different ($P<0.01$); * LSD= Least significant difference; NS= Non significant

Appendix 5.3: Mean dry-matter digestibility *in vitro* of *Posidonia australis* treated with different concentrations Ca $(OH)_2$ for different incubation times

Ca $(OH)_2$ concentration	Reaction time (days)				
(% dry matter)	1	2	3	4	Mean
0	34.7	34.7	34.7	34.7	34.7^{a}
2	43.0	43.6	44.0	44.8	43.9^{b}
4	47.0	48.2	49.0	50.2	48.6^{c}
6	49.0	49.6	50.0	50.8	49.9^{cd}
8	50.2	50.4	51.0	51.2	50.7^{d}
Mean	37.3^{a}	37.7^{ab}	38.1^{ab}	38.6^{b}	

Significance:	LSD*(0.01)
Interaction (Ca $(OH)_2$ concentration x time)	NS
Ca $(OH)_2$ concentration	1.6
reaction time	1.4

Means on the same line or column having different superscript are significantly different ($P<0.01$); * LSD= Least significant difference; NS= Non significant

Appendix 5.4: Mean organic-matter digestibility *in vitro* of *Posidonia australis* treated with different concentrations of ($Ca(OH^2)$) for different incubation times

($Ca(OH^2)$) concentration (% dry matter)	Reaction time (days)				
(% dry matter)	24	48	72	96	Mean
0	23.5	23.5	23.5	23.5	23.5^{a}
2	39.3	39.8	40.1	40.8	40.0^{b}
4	39.8	40.8	41.5	42.5	41.2^{b}
6	47.4	48.0	48.4	49.1	48.2^{c}
8	47.8	48.0	48.6	48.8	48.3^{c}
Mean	33.0^{a}	33.4^{ab}	33.7^{ab}	34.1^{b}	

Significance:	LSD*(0.01)
Interaction ($Ca(OH)_2$ concentration x time)	NS
$Ca(OH)_2$ concentration	0.9
Incubation time	0.7

Means on the same line or column having different superscripts are significantly different ($P<0.01$); * LSD= Least significant difference; NS= Non significant

Appendix 5.5: Mean dry-matter digestibility *in vitro* of *Posidonia australis* treated with different concentrations of aqueos urea for different incubation time

Urea concentration	Reaction time (days)				
(% dry matter)	1	2	3	4	Mean
0	34.7	34.7	34.7	34.7	34.7[a]
2	36.4	37.6	38.1	40.0	38.0[b]
4	38.6	39.9	40.4	42.4	40.3[c]
6	39.6	40.0	45.2	54.0	44.7[d]
8	41.1	41.5	46.9	56.0	46.4[e]
Mean	38.1[a]	38.7[a]	41.1[b]	45.4[c]	

Significance:	LSD*(0.01)
Interaction (NH_3 concentration x time)	NS
NH_3 concentration	1.1
Incubation time	0.9

Means on the same line or column having different superscripts are significantly different ($P<0.01$); *LSD= Least significant difference; NS= Non significant

Appendix 5.6: Mean organic-matter digestibility *in vitro* of *Posidonia australis* treated with different concentrations of aqueos urea for different incubation time

NaOH concentration	Intraction time (days)				
(% dry matter)	1	2	3	4	Mean
0	23.5	23.5	23.5	23.5	23.5^{a}
2	28.5	29.4	29.8	31.3	29.8^{b}
4	30.4	31.4	31.8	33.4	31.8^{c}
6	31.2	31.5	35.6	42.5	35.2^{d}
8	32.4	32.7	35.2	42.0	35.6^{d}
Mean	29.2^{a}	29.7^{a}	31.2^{b}	34.5^{c}	

Significance:	LSD*(0.01)
Intraction (NH3 concentration x time)	NS
NH_3 concentration	0.9
Reaction time	0.7

Means on the same line or column having different superscripts are significantly different ($P<0.01$); * LSD= Least significant difference; NS= Non significant

Appendix 7.1: Average daily dry matter intake (g/kg $W^{0.75}$) of whole diets by sheep*

Experimental	Experimental diets					Siginificance
period(weeks)	A	B	C	D	E	(LSD; P<0.05)
1	48.9^{a}	65.2^{b}	51^{a}	48.4^{a}	49.3^{a}	6.2
2	62.1^{b}	71.3^{c}	52.4^{a}	49.5^{a}	51.4^{a}	6.8
3	66.7^{b}	74.3^{c}	53.2^{a}	50.7^{a}	54.0^{a}	7.0
4	75.2^{c}	73.4^{c}	62.7^{b}	53.7^{a}	55.9^{ab}	7.1
5	77.5^{b}	77.7^{b}	73.0^{b}	58.2^{a}	58.6^{a}	7.8
6	78.2^{b}	83.4^{b}	77.5^{b}	60.5^{a}	63.1^{a}	8.6
7	79.3^{b}	85.9^{b}	95.2^{c}	66.3^{a}	68.0^{a}	9.1
8	82.4^{b}	87.4^{bc}	95.3^{c}	69.3^{a}	70.1^{a}	8.8
9	83.3^{b}	89.9^{bc}	96.8^{c}	71.1^{a}	70.1^{a}	9.7
10	83.0^{b}	90.5^{bc}	96.5^{c}	70.6^{a}	68.6^{a}	8.8
11	83.6^{b}	90.4^{b}	92.3^{b}	71.1^{a}	71.0^{a}	9.4
Average	74.6^{b}	80.8^{b}	76.9^{b}	60.8^{a}	61.8^{a}	7.6

* Mean within each column that share no common superscript differ significantly (P<0.05)

Appendix 7.2: Dry matter intake (g/d/sheep) of different feedstuffs and rations by five experimental group of sheep

	Experimental diets & ingredients	Experimental period (weeks)											
		1	2	3	4	5	6	7	8	9	10	11	Mean
	Seagrass (treared)	682	871	940	1075	1112	1123	1158	1211	1222	780	1249	1038
A	lucerne	227	290	314	358	371	374	386	404	408	263	417	346
	Total	909	1161	1254	1433	1483	1497	1549	1614	1630	1053	1666	1384
	Straw (treated)	898	998	1045	1066	1087	1207	1255	1289	1343	1364	1376	1175
B	Lucerne	300	333	348	355	363	402	419	430	448	455	459	392
	Total	1198	1331	1393	1421	1450	1609	1674	1719	1791	1819	1835	1567
	Straw (treated)	709	734	757	888	1051	1127	1402	1417	1393	1468	1498	1131
C	Chicken litter	236	245	253	296	350	376	467	473	464	489	499	377
	Total	945	979	1010	1184	1401	1503	1869	1890	1857	1957	1997	1508
	Seagrass (treated)	439	451	464	494	538	564	620	653	671	671	679	568
D	Straw (treated)	439	451	464	494	538	564	620	653	671	671	679	568
	Total	878	902	928	988	1076	1128	1240	1306	1342	1342	1358	1136
E	Straw (treated)	902	942	993	1034	1087	1155	1275	1326	1339	1354	1373	1162
	total	902	942	993	1034	1087	1155	1275	1326	1339	1354	1373	1162

Appendix 7.3: Mean body weight of sheep (Kg) in feed lot over the experimental periods

Period (week)	Experimental groups					Significance level
	A	B	C	D	E	
0	48	48	48.3	48.3	48.3	NS
1	48.5	48.4	49.0	47.5	47.8	NS
2	48.9	49	49.5	47.8	48.1	NS
3	49.4	49.6	49.9	48.1	48.5	NS
4	49.9	50.3	50.5	48.4	48.8	NS
5	50.5	50.9	51.1	48.7	49.2	NS
6	51	51.6	51.8	49	49.6	NS
7	51.5	52.3	52	49.4	50	NS
8	52.1	53.0	53.5	49.8	50.4	NS
9	52.7	53.7	54.3	50.2	50.9	*
10	53.2	54.5	55.1	50.6	51.3	*
11	53.8	55.2	56.0	51.0	51.7	*

NS = Non significant ; * = P< 0.05

Appendix 7.4: Body weight changes of five group sheep over the experimental periods (Kg/sheep/week)

Period (week)	groups A	B	C	D	E	LSD (P < 0.05
1	0.5	0.4	0.7	-0.7	-0.5	0.34
2	0.4	0.6	0.5	0.3	0.3	0.21
3	0.5	0.6	0.5	0.3	0.3	0.19
4	0.5	0.6	0.4	0.3	0.4	0.29
5	0.5	0.6	0.6	0.3	0.4	0.14
6	0.5	0.7	0.7	0.3	0.4	0.32
7	0.5	0.7	0.8	0.4	0.4	0.29
8	0.5	0.7	0.8	0.4	0.4	0.31
9	0.6	0.7	0.8	0.4	0.4	0.32
10	0.6	0.7	0.8	0.4	0.4	0.22
11	0.6	0.7	0.8	0.4	0.4	0.29
Average	5.9	7.2	7.5	2.8	3.4	0.86

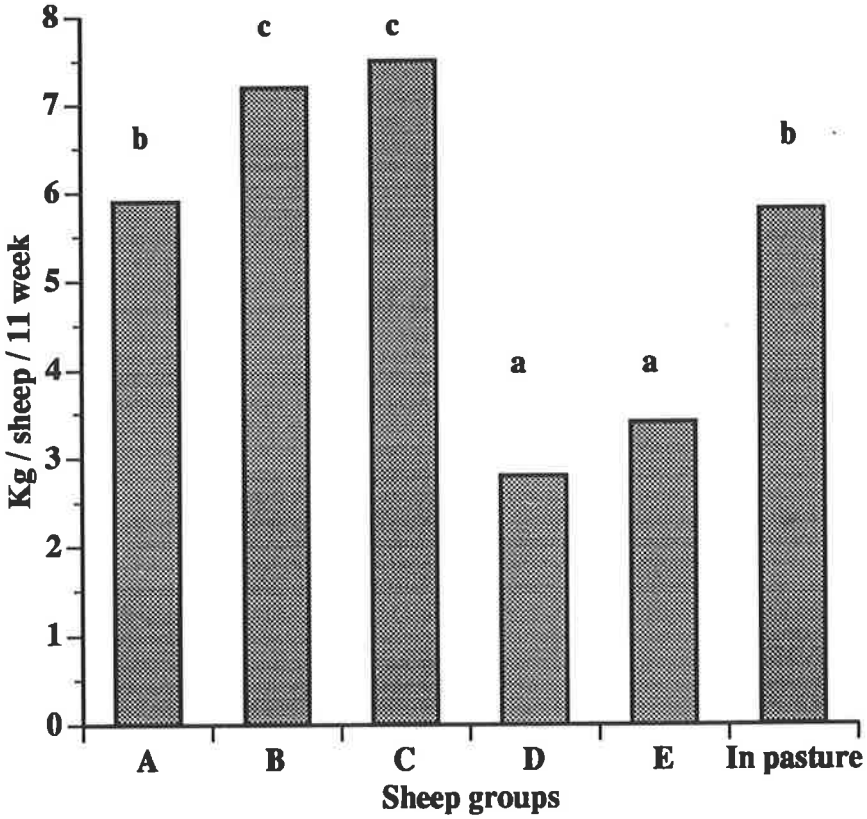
LSD = Least Significant difference

Appendix 7.5: Average weekly fat score of experimental sheep in feed lot

Period (week)	groups A	B	C	D	E	LSD (P < 0.05
0	1.4	1.4	1.3	1.5	1.4	NS
1	1.6	1.4	1.3	1.5	1.4	NS
2	1.7	1.7	1.3	1.6	1.4	NS
3	1.7	1.7	1.3	1.6	1.4	NS
4	1.7	1.8	1.5	1.8	1.4	NS
5	1.9	2.0	1.6	1.9	1.5	*
6	2.1	2.2	1.7	2.1	1.7	*
7	2.2	2.3	1.9	2.3	1.8	*
8	2.3	2.4	2.0	2.5	1.9	*
9	2.4	2.6	2.1	2.6	1.9	**
10	2.5	2.6	2.1	2.6	2.0	*
11	2.5	2.7	2.1	2.6	2.0	**

NS = None significant ; * = P < 0.05 ; ** = P < 0.01

LSD (P < 0.05) =



Appendix 7.6: Total body gain comparison of experimental sheep and sheep released in green pasture

Appendix 7.7: wool production comparison of experimental sheep and sheep in pasture*

component	Sheep groups					
	A	B	C	D	E	In pasture
Greasy Fleece weight (g/sheep/9weeks)	705^{a}	806^{ab}	864^{b}	767^{ab}	863^{b}	890^{b}
Clean scoured yeild (%)	70.2^{a}	72.0^{a}	71.4^{a}	74.7^{a}	70.8^{a}	71.2^{a}
Clean fleece weight (g/sheep/9 weeks)	495^{a}	579^{b}	614^{b}	572^{b}	609^{b}	634^{b}
Clean fleece weight (g/sheep/d)	8.0^{a}	9.3^{b}	9.9^{b}	9.2^{b}	9.8^{b}	10.2^{b}
Staple length (mm)	13.0^{a}	14.2^{abc}	13.9^{ab}	14.9^{bcd}	15.8^{cd}	16.8^{d}
Fibre diameter (µm)	20.6^{a}	21.7^{ab}	21.5^{ab}	20.9^{a}	22.4^{b}	20.4^{a}
Fibre diameter (St.dev. µm)	1.4^{a}	1.7^{ab}	1.1^{a}	1.6^{c}	1.9^{bc}	0.6^{d}
Length:diameter (µm:µm)	10.2^{a}	10.6^{ab}	10.4^{a}	11.5^{b}	11.4^{bc}	13.4^{d}
Staple strength (N/Ktex)	41.8^{a}	51.1^{bc}	46.5^{ab}	55.9^{c}	47.8^{ab}	41.8^{a}

* Mean within each row that share no common superscript differ significantly ($P<0.05$)

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Bibliography

10 BIBLIOGRAPHY

ABARE (1992). ABARE's Commodity Statistical Bulletin. Australian Bureau of Agricultural and Resource Economics,Canberra.

ABE, A. and HORRI, S. (1978). Application of the detergent analysis to the feed ingredients and to the various formula feeds. *Jpns. J. Zootech. Sci.* 49: 733-738.

ABE, M., IRIKI, T., TOBE, N and SHIBUI, H. (1981). Sequestration of holotrich protozoa in the reticulo-rumen of cattle. *Appl. Environ. Microbiol.* 41: 758-65.

ABE, M., SHIBUI, H., IRIKI, T. and KUMENO, F. (1973). Relation between diet and protozoal populations in the rumen. *Br. J. Nutr.* 29:197-202.

ADAS (Agricultural Development and Advisory Service (1984). Energy allowances and feeding system for ruminants. Ministry of Agriculture Fisheries and Food, Department of Agriculture and Fisheries for Scotland, Department of Agriculture for Northern Ireland, London. p. 85.

ADEBOWALE, E.A., ØRSKOV, E.R. and HOTTEN, P.M. (1989). Rumen degradation of straw. 8. Effect of alkaline hydrogen peroxide on degradation of straw using either sodium hydroxide or gaseous ammonia as source of alkali. *Anim. Prod.* 48: 553-559.

AFIC (Australian Feeds Information Centre) (1987). Australian feed composition tables. Ostrowski-Meissner, H. (Ed.). CSIRO, Sydney, Australia. 447p.

AGRAWAL, I.S. (1975). The effect of curing time on the digestibility of poddy and wheat straw. In "Improved utilisation of agricultural waste material and industrial by-products as livestock feed (research progress report, 1969-1974)". Pant, G.B. (Ed), University press, Pantnagar. pp. 28-31.

AITCHISON, E.M., MURRAY, P.J. and ROWE, J.B. (1986). Improving the nutritive value of round bales of oat sraw by treatment with urea or by supplementation with lupins. *Proc. Aust. Soc. Anim. Prod.* 16:123-126.

AKIN, D.E. (1975). Microscopic evaluation of forage digestion by rumen microorganisms (A review). *J. Anim. Sci.* 48:701-710.

ALEXANDER, B.W., GORDON, A.H., LOMAX, J.A. and CHESSON, A. (1987). Composition and rumen degradability of straw from three varieties of oilseed rape before and after alkali, hydrothermal and oxidative treatment. *J. Sci. Food Agric.* 41: 1-15.

ALLDEN, W.G. (1959). The summer nutrition of weaner sheep. The relative roles of available energy and protein when fed as supplements to sheep grazing mature pasture herbage. *Aust. J. Agric. Res.* 10: 219-236.

ALLDEN, W.G. (1979a). Feed intake, diet composition and wool growth. In "Physiological and environmental limitations to wool growth". Black, J.L. and Reis, D.J. (Eds.) CSIRO, Sydney, Australia. pp. 61-77.

ALLDEN, W.G. (1979b). Nutritional studies on grain legume crops for grazing livestock. Biennial Report, 1978-79, Waite Agricultural Research Institute, The University of Adelaide. pp. 53-56.

ALLEN, R.E. (Ed.). (1984). The pocket Oxford dictionary. Clarendon Press, Oxford. London. p. 651.

ALWASH, A.H. and THOMAS, P. C. (1974). Effect of the size of hay particles on digestion in the sheep. *J. Sci. Food Agric.* 25: 139-147.

AMAN, P. and GRAHAM, H. (1990). Chemical evaluation of polysaccharides in animal feeds. In "Feedstuff evaluation". Wiseman, J. and Cole, D.J.A. (Eds.). Butterworths. London. pp. 161-178.

AMOS, H. E. and AKIN, D. E. (1978). Rumen protozoal degradation of structurally intact forage tissues. *Appl. Environ. Microbiol.* 36: 513-522.

AOAC (Association of Official Analytical Chemist) (1980). Official methods of analysis. William H. (Ed). Washington, USA. PP: 125-132.

AOAC (Association of Official Analytical Chemists). (1990). Official methods of analysis. Helrich, K. (Ed.).Arlington, Virginia, USA. pp: 69-83.

ARBER, A. (1920). Water plants (a study of aquatic angiosperms). Cambridge University Press, Cambridge. 436p.

ARC (Agricultural Research Council). (1980). The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux, Slough, UK. 351p.

ARCHER, K., READ, J. and MURRAY, G. (1993). Pasture decline - real or imagined?. *Proc. 8th. Annual. Conf. Grassl. Soc. NSW.* pp. 8-13.

ARGYLE, J.L. and FORSTER, R.J. (1989). Effect of ciliate protozoa on rumen bacterial populations. In "The roles of protozoa and fungi in ruminant digestion". Nolan, J.V., Leng, R.A. and Demeyer, D.I. (Eds.). Penambul Books, Armidale, Australia. pp. 317-319.

ARMSBY, H.P. (1917). The nutrition of farm animals. The Macmillan Company. NY. 743p.

ARMSTRONG, D. G. and SMITHARD, R. R. (1979). The fate of carbohydrates in the small and large intestines of the ruminant. *Proc. Nutr. Soc.* 38: 283-293.

ARNASON, J. (1979). Results from Norwegian experiments with treated straw as feed for ruminants. In "Straw decay and its effect on disposal and utilisation". Grossbard, E. (Ed.). John Wiley and Sons.. London. pp. 199-205.

ARNOLD, G.W. (1970). Regulation of feed intake in grazing ruminants. In "Physiology of Digestion and Metabolism in the Ruminant". Phillipson A.T. (Ed.). Oriel Press, England. pp. 264-276.

ARNOLD, G.W. (1981). Grazing behaviour. In "World Animal Science, B1: Grazing animals". Morley, F.H.W. (Ed.). Elsevier Sci. Pub. Company. pp. 79-104.

ASADPOUR, P. and KLOPFENSTEIN, T.J. (1979). Calcium and ammonium hydroxide treatment of wheat straw. *J. Anim. Sci.* 49: 105 A.

ASHTON, B. (1984). Lot-feeding sheep through a drought. Fact Sheet., No. FS 3/84. Dept. Agric. South Australia. 4p.

ASHTON, B. (1986). Lot-feeding of lambs. Fact Sheet, FS 126/76. Dept. Agric. South Australia. 4p.

ASHTON, B. (1989). Feedlots have lots to offer. In "Farmer and Stockowner". 29: 26.

ASHTON, B. (1990). The use of straw in sheep feedlots. Fact sheet, FS 3/90. Dept. Agric., South Australia. 4p.

ASHTON, B.L. and KING, A.E. (1990). The value of cereal straw in drought rations for lotfed ewes. *Proc. Aust. Soc. Anim. Prod.* 18: 449.

AUGIER, H., CALVERT, H., WOLLASTON, E. and SANTIMONE, M. (1982). A comparison of the C, H, N, protein and amino acid composition of *Posidonia australis* with that of *Posidonia oceanica* and several other marine phanerograms. *Aquat. Bot.* 12: 69-80.

AWC (1989). The Australian wool industry. Australian Wool Corporation. Melbourne.

BACON, J.S.D. (1988). Structure and chemistry. In "World Animal Science, B4. Feed science". Ørskov, E.R. (Ed.). Elsevier Sci. Pub. Company. pp. 23-50.

BAE (1983). Australian agricultural and grazing industries survey 1979-80. Bureau of Agricultural Economics. Canberra, Australia. 146p.

BAE (1984). Wool situation and outlook. Bureau of Agricultural Economics. Canberra, Australia. 35p.

BAGNALL, L.O. and HENTGES, J.F. (1979). Processing and conservation of water hyacinth and hydrilla for livestock feeding. In "Aquatic plants, lake management, and ecosystem consequences of lake harvesting. Breck, J.E., Prentki, R. T. and Loucks O. L. (Eds.). University of Wisconsin, Madison. pp. 367-374.

BAGNALL, L.O., CASSELMAN, T. W., KESTERSON, J. W., Easley, J.F. and Hellwing, R. E. (1977). Aquatic forage processing in Florida. *Trans. ASAE.* 20: 221-225

BAKER, A.J., MOHAUPT, A.A. and SPINO, D.F. (1973). Evaluating wood pulp as feedstuff for ruminants and substrate for *Aspergillus famingatus*. *J. Anim. Sci.* 37: 179-182.

BAKER, S. K. (1985). The rumen as an ecosystem. In "Ruminant physiology- concepts and consequences". Baker, S.K., Gawthorne, J.M., Mackintosh, J.B. and Purser, D.B. (Eds). University of Western Australia, Perth. pp. 149-160.

BALASUMBRAMANYA, R.H. and BHATAWDEKAR, S.P. (1980). Semi-solid microbial fermentation of rice and wheat straw for protein enrichment and increased digestibility. *Indian. J. Agric. Sci.* 50: 965-970.

BALCH, C.C. and CAMPLING, R.C. (1962). Regulation of voluntary food intake in ruminants. *Nutr. Abst. Rev.* 32: 669-686.

BALCH, C.C. and JOHNSON, V.W. (1950). Factors effecting the utilisation of food by dairy cows. 2. Factors influencing the rate of break down of cellulose in the rumen of the cow. *Br. J. Nutr.* 4: 389-394.

BARBER, A.A. (1990). TAKE-AWAY: A ruminant nutrition software package. *Proc. Aust. Soc. Anim. Prod.* 18: pp. 136-139.

BARBOSA, C., ROVERSO, E.A., DE CAMPOS, B.D.E.S. and PEREIRA, W.M. (1980). Comparison of rations with groundnut shells or soy straw combined with poultry litter for rearing beef cattle in yards. *Nutr. Abstr. Rev.* 50 (B) : 2827.

BARNES, R.S.K., and MANN, K.H. (1980). Fundamentals of aquatic ecosystems. Blackwell, Oxford. 229p.

BARRY, T.N. and DUNCAN, S.J. (1984). The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. *Br. J. Nutr.* 51: 485-491.

BARTON, F. E. and AKIN, D. E. (1977). Digestibility of delignified forage cell walls. *Agric. Food. Chem.* 25:1299-1303.

BARTON, F.E. (1988). Chemistry of lignocellulose: Methods of analysis and consequences of structure. *Anim. Feed Sci. Technol.* 21: 279-289.

BARTON, F.E. and Akin, D.E. (1977). Digestibility of delignified forage cell walls. *Agric. Food. Chem.* 25: 1299-1303.

BAUCHOP, T. (1979). Rumen anaerobic fungi of cattle and sheep. *Appl. Environ. Microbiol.* 38:148-58.

BECKER, E.R. (1929). Methods of rendering the rumen and reticulum of ruminants free from their normal infusorian fauna. *Proc. Nation. Acad. Sci.* U.S.A. 15: 435-438.

BECKER, E.R., SCHULZ, J.A. and EMMERSON, M.A. (1930). Experiments on the physiological relationships between the stomach infusoria of ruminants and their hosts, with a bibliography, *Iowa St. J. Sci.* 4: 215-51.

BECKMANN, E. (1922). Conversion of grain straw and lupins into feeds of high nutrient value. *Chem. Abst.* 16: 765.

BELL, A., SIMPSON, I. and GILBERT, D. (1986). Lamb lot-feeding budget sheet. Ag. Fact. A. 3.5.2. (Agdex 431/55). Dept. Agric. NSW.

BELL, A., SIMPSON, I. and GILBERT, D. (1991a). Watch hidden lot-feeding costs. In "Kondinin Group Talk", Australia's National Farm Improvement Group. pp. 11-12.

BELL, A., SIMPSON, I. and GILBERT, D. (1991b). Finishing off with a good start. In "Kondinin Group Talk". Australia's National Farm Improvement Group. pp. 15-16.

BEN-GHEDALIA, D., and MIRON, J. (1981). Effect of sodium hydroxide, ozone and sulphur dioxide on the composition and *in vitro* digestibility of wheat straw. *J. Sci. Food Agric.* 32: 224-228.

BEN-GHEDALIA, D., and MIRON, J. (1983). The response of wheat straw varieties to mild sulphur dioxide treatment. *Anim. Feed Sci. Technol.*10: 269-276.

BEN-GHEDALIA, D., and MIRON, J. (1984). The digestibility of wheat straw treated with sulphur dioxide. *J. Agric. Sci. Camb.* 102: 517-520.

BEN-GHEDALIA, D., MIRON, J., EST, Y. and YOSEF, F. (1988). SO_2 treatment for converting straw into a concentrate-like feed: A growth study with lambs. *Anim. Feed Sci. Technol.*19: 219-229.

BEN-GHEDALIA, D., SHEFET, G. and MIRON, J. (1980). Effects of ozone and ammonium hydroxide treatments on the composition and *in vitro* digestibility of cotton straw. *J. Sci. Food Agric.* 13: 1337- 1342.

BEN-GHEDALIA, D., SHEFET G., MIRON, J. and DROR, Y. (1982). Effect of ozone and sodium hydroxide treatments on some chemical characteristics of cotton straw. *J. Sci. Food Agric.* 33: 1213-1218.

BEN-GHEDALIA, D. and RUBINSTEIN, A. (1986). The response of screened manure fibre to ozone and sodium hydroxide treatments. *Anim. Feed Sci. Technol.* 15: 47-55.

BIRCH, W.R. (1975). Some chemical and calorific properties of tropical marine angiosperms compared with those of other plants. *J. Appl. Ecol.* 12: 201-12.

BIRD, S.H., HILL, M.K. and LENG, R.A. (1979). The effect of defaunation of the rumen on the growth of lambs on low-protein high-energy diets. *Brit. J. Nutri.* 42: 81-87.

BJORNDAL, K.A. (1980). Nutrition and grazing behavior of the green turtle *Chelonia mydas. Marine Biol.* 56: 147-54.

BLACK, J.L. and KENNEY, P.A. (1984). Factors affecting diet selection by sheep. II. Height and density of pasture. *Australian J. Agric. Res.* 5: 565.

BLACKBURN, T.H. and HOBSON, P.N. (1960). Proteolysis by whole and fractionated rumen contents. *J. Gen. Microbiol.* 22: 272-281.

BLAKENEY, A.B., HARRIS P.J., HENRY, R.J. and STONE B.A. (1983). *Carbohydr. Res.* 113: 291.

BLAXTER, K.L. (1960). The utilisation of the energy of grassland products. *Proc. XIII. Int. Grassld. Cong.* pp. 479-484.

BLUMENKRANTZ, N. and ASBE-HAANSEN, G. (1973). New method for quantitative determination of uronic acids. *Anal. Biochem.* 54: 489-9.

BLUNDEN, G., BINNS, W.W. and PERKS, F. (1975). Commercial collection and utilization of maerl. *Econ. Bot.* 29: 140-145.

BODA, K. (Ed.). (1990). Non-conventional feedstuffs in the nutrition of farm animals. Elsevier, Amsterdam. pp. 13-87.

BORGIOLI, E. and TOCCHINI, M. (1969). Sterilized poultry litter for feeding young cattle. *Aliment Anim..* 13: 263. *Nutr. Abstr. Rev.* 40: 3871 (1971).

BOSMAN, S.W. (1973). Chicken litter in fattening rations for cattle and sheep. *South African J. Anim. Sci.* 3: 57.

BOYD, C. E. (1968). Fresh-Water plants: A potential source of protein. *Econ. Bot.* 22: 359-368.

BOYD, C. E. (1974). Utilization of aquatic plants. In "Aquatic vegetation, its use and control". Mitchell, D.S. (Ed.). UNESCO, Paris. pp. 107-115.

BOYNE, A.W., EADIE, J.M. and RAITT, K. (1957). The development and testing of a method of counting rumen ciliate protozoa. *J. Gen. Microbiol.* 17: 414-423.

BRAMAN, W.L. and ABE, R.K. (1977). Laboratory and *in vivo* evaluation of the nutritive value of NaOH-treated wheat straw. *J. Anim. Sci.* 46: 496-505.

BREWER, D., DUNCAN, J.M., SAFE, S. and TAYLOR, A. (1972). Ovine ill-thrift in Nova Scotia. 4. The survival at low oxygen partial pressure of fungi isolated from the contents of the ovine rumen. *Can. J. Microbiol.* 18: 1119-28.

BROSCH, A., SNEH, B. and SHKOLNOK, A. (1983). Effect of severe dehydration on the activity of the rumen microbial population of black bedouin goats. *J. Agric Sci .* 100: 413-421.

BROWN, T.H. (1976a). Effect of deferred autumn grazing and stocking rate of sheep on pasture production in a Mediterranean-type climate. *Aust. J. exp. Agric. Anim. Husb.* 16: 181-188.

BROWN, T.H. (1976b). The effect of stocking rate and deferred autumn grazing of pasture on live weight and wool production of Merino wethers in a Mediterranean-type climate. *Aust. J. exp. Agric. Anim. Husb.* 16: 189-196.

BRUHN, H.D. (1955). Pelleting grain and hay mixtures. *Agric. Engineer.* 36: 330-331.

BRUHN, H.D. (1957). Engineering problems in pelletised feed. *Agric. Enginee.* 38: 322-325.

BRYANT, M.P. and BURKEY, L. A. (1953a). Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. *J. Dairy Sci.* 36: 205-217.

BRYANT M.P. and ROBINSON, I.M. (1961). An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. *J. Dairy Sci.* 44: 1446-56.

BRYANT, M. P. (1959). Bacterial species of the rumen. *Bacteriol. Rev.* 23: 125-153

BRYANT, M. P. (1963). Identification of groups of anaerobic bacteria active in the rumen. *J. Anim. Sci.* 22: 801-813.

BRYANT, M.P. and BURKEY, L.A. (1953b). Numbers and some predominant groups of bacteria in the rumen of cows fed different rations. *J. Dairy Sci.* 36: 218-24.

BRYANT, M.P. and ROBINSON, I.M. (1968). Effects of diet, time after feeding, and position sampled on numbers of viable bacteria in the bovine rumen. *J. Dairy Sci.* 51: 1950-5.

BRYANT, T.H. (1983). National lamb marketing and production conference (Industry Overview). In "Implications of developments in meat science, production and marketing for lamb production systems". Thatcher, L.P. and Harris, D.C. (Eds.). Paper No. 2. Victorian. Dept. Agric. and NSW Dept. Agric., Australia. pp. 1-5.

BUNT, J.S. (1975). Primary productivity of marine ecosystems. In "Primary productivity of the biosphere". Lieth, H. and Whittaker, R.H. (Eds.). Springer-Verlay, New York. pp: 169-183.

BUNTING, L.D., RICHARDSON, C.R. and TOCK, R.W. (1984). Digestibility of ozone-treated sorghum stover by ruminants. *J. Agric. Sci. Camb.* 102: 747-750.

BURNS, J.C. (1981). Integration of grazing with other feed resources. In "Nutritional limits to animal production from pastures". Hacker, J.B. (Ed.). CAB (Commonwealth Agricultural Bureaux), Farnham Royal, UK. pp. 455-471.

BURROWS, I., SEAL, K.J. and EGGINS, H.O.W. (1979). The biodegradation of barley straw by *Coprinus cinereus* for the production of ruminant feed. In "Straw decay and its effect on disposal and utilisation". Grossbard, E. (Ed.). John Wiley & Sons. London. pp. 147-154.

BUTLER, J.L. and McCOLLY, H.F. (1959). Factors affecting the pelleting of hay. *Agric. Engineer.* 40: 442-446.

CAB (Commonwealth Agricultural Bureaux) (1980). The nutrient requirements of ruminant livestock. The Gresham Press, Surrey, UK. 351p.

CALDWELL, D.R. and BRYANT, M.P. (1966). Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Appl. Microbiol.* 14: 794-801.

CALLOW, C. (1992). The New South Wales feedlot industry: An overview. Yanco. Institute, NSW. 82p.

CAMPLING, R. C. , FREER, M. and BALCH, C. C. (1963). Factors affecting the voluntary intake of food by cows 6. A preliminary experiment with ground-pelleted hay. *Br. J. Nutr.* 17: 263-272.

CAMPLING, R.C. and FREER, M. (1966). Factors affecting the voluntary intake of food by cows 8. experiments with ground pelleted roughages. *Br. J. Nutr.* 20: 229-244.

CAPPER, B.S. (1988). The role of food legume straw and stubble in feeding livestock. In "The role of legumes in the farming systems of Mediterranean areas". Osman, A.E., Ibrahim, M.H. and Jones, M.A. (Eds). Klower Academic Pub. pp. 151-162.

CARTER, E.D. (1981a). Seed and seedling dynamics of annual medic pastures in South Australia. *Proc. XIV Int. Grassld. Cong.* Lexington, Ky, USA. pp. 447-450.

CARTER, E.D. (1981b). The role of medics in livestock and crop production. Symposium Roseworthy Agric. College, South Australia. Sept. 1981.

CARTER, E.D. (1982). The need for change in making the best use of medics in the cereal-livestock farming systems of South Australia. *Proc. 2nd Aust. Agron. Conf.*, Wagga Wagga, NSW. p. 180.

CARTER, E.D. (1993). Principles of sustainable pasture production. *Proc. 8th. Annual. Conf. Grassl. Soc. NSW.* pp. 18-24.

CARTER, E.D., CHAICHI, M.R., GRIERSON, I.T., PORTER, R.G. and VALIZADEH, R. (1993). The potential for lot feeding of sheep on cereal farms to protect medic seed reserves and surface soil. *Proc. 7th. Aust. Agron. Conf.* Adelaide. p. 440.

CARTER, E.D., WOLFE, E.C. and FRANCIS, C.M. (1982). Problems of maintaining pastures in the cereal-livestock areas of southern Australia. *Proc. 2nd Aust. Agron. Conf.*, Wagga Wagga, NSW. pp. 68-82.

CASTILLO, L.S. (1983). Current utilisation of fibrous residues in Asian countries. In "The utilisation of fibrous agricultural residues". Pearce, G.R. (Ed.). Australian Development Assistance Bureau, Canberra, Australia. pp. 33-49.

CASTRILLO, C., FONDEVILA, M., ALIBES, X. and JOY, M. (1991). Chemical treatments for upgrading lignocellulosic resources and strategies for their utilisation in ruminant feeding. In "Production and utilisation of lignocellulosics". Galletti, G.C. (Ed.). Elsevier Appl. Sci. New York, pp. 339-373.

CATE, H.A., LEWIS, J.M., WEBB, R.J., MANSFIELD, M.E. and GARRIGUS, U.S. (1954). The effect of pelleting rations of varied quality on feed utilisation by lambs. *J. Anim. Sci.* 13: 979A.

CHANDRA, S. and JACKSON, M.G. (1971). A study of various chemical treatments to remove lignin from coarse roughages and increase their digestibility. *J. Agric. Sci. Camb.* 77: 11-17.

CHAPMAN, P.G. and NORTON, B.W. (1982). The effect of sample preparation on the digestion of chopped, masticated and ground siratro and pangola grass in nylon bags. *Proc. Aust. Soc. Anim.* 14: 580-583.

CHAPMAN, R.E. and WHEELER, J.L. (1963). Dye-banding: A technique for fleece growth studies. *Aust. J. Sci.* 26: 53-54.

CHAPMAN, V.J., and CHAPMAN, D.J. (1980). Seaweeds and their uses. 3rd ed. Chapman and Hall, London. 344pp.

CHATURVEDI, M.L., SINGH, U.B. and RANJHAN, S.K. (1973). Effect of feeding water-soaked and dry wheat straw on feed intake, digestibility of nutrients and VFA production in growing zebu and buffalo calves. *J. Agric. Sci. Camb.* 80: 393-397.

CHENG, K. J. and COSTERTON, J.W. (1980). Adherent rumen bacteria - their role in the digestion of plant material, urea and epithelial cells. In "Digestive physiology and metabolism in ruminants (Proceedings of the Fifth International Symposium on Ruminant Physiology)". Ruckebusch, Y. and Thivend, p. (Eds.). MTP Press, Lancaster. pp. 227-250.

CHENG, K.J. , AKIN, D.E. and COSTERTON, J.W. (1977). Rumen bacterial interaction with particulate dietary components and response to dietary variation. *Fed. Proc.* 36: 193-197.

CHENSON, M., GRENET, E., DEMARQUILLY, C. and JARRIGE, R. (1970). The use of the nylon bag technique for the study of forage digestion in the rumen and for predicting feed value. *Proc. XI Int. Grassld. Cong.* Qld, Australia. pp. 697-701.

CHESHIRE, A.C., and HALLAM, N.D. (1985). The environmental role of alginates in *Durvillaea potatorum* (*Fucales, phaeophyta*). *Phycologia.* 24: 147-153.

CHEVA-ISARAKUL, B. (1988). Performance of sheep fed urea-treated or urea-molasses supplemented straw with or without fresh leucaena supplement as compared with fresh grass. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 225-231.

CHEVA-ISARAKUL, B. and KANJANAPRUTJIPONG, J. (1987). A comparison of urea-treated rice straw with urea-molasses sprayed rice straw as a base diet for growing cattle. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp 191-198.

CHOMYSZYN, M., ZIOLECKA, A., KUZDOWKS, M. and BIELINSKI, K. (1961). The use of ammoniated fodder in the feeding of ruminants 1. The use of ammoniated sugar beet pulp in the feeding of wethers. *Nutr. Abst. Rev.* 31: 306.

CHRISTIANSEN, W.C. , KAWASHIMA, R. and BURROUGHS, W. (1965). Influence of protozoa upon rumen acid production and liveweight gains in lambs. *J. Anim. Sci.* 24: 730-734.

CHRISTIANSEN, W.C., WOOD, W. and BURROUGHS, W. (1964). Ration characteristics influencing rumen protozoal populations. *J. Anim. Sci.* 23: 984-988.

CHRISTIANSON, I.G., CLAYTON M.N., ALLENDER, B.M. (1988). Seaweeds of Australia. Reed Books, Sydney. 112pp.

CHURCH, D. C. (1979). The importance of ruminants. In ". Digestive physiology and nutrition of ruminants". Church, D. C. (Ed.). Volume 1- Digestive Physiology (Second Edition). O & B Books, Corvallis, Oregon. pp. 1-6.

CHURCH, D.C. (1988). The ruminant animal, digestive physiology and nutrition. Oxford Press, Oregan, USA. 564p.

CLARK, T. (1985a). Beef cattle feedlots (facilities). Fact sheet 15/85. Dept. Agric. South Australia. 2p.

CLARK, T. (1985b). Beef cattle feedlots, (health). Fact sheet 17/85. Dept. Agric. South Australia. 2p.

CLARKE, R. T. J. and DIMENNA, M.E. (1961). Yeasts from the bovine rumen. *J.Gen. Microbiol.* 113-17.

CLARKE, R.T.J. (1964). Ciliates of the rumen of domestic cattle (*Bos taurus L.*). *NZ J. Agric. Res.* 7: 248-57.

CLARKE, R.T.J. (1965). Diurnal variation in the numbers of rumen ciliate protozoa in cattle. *NZ J. Agric. Res.* 18: 1-9.

CLARKE, R.T.J., ULYATT, M.J. and JOHN, A. (1982). Variation in number and mass of ciliate protozoa in the rumen of sheep fed chaffed alfalfa (*Medicago sativa*). *Appl. Environ. Microbiol.* 43: 1201-1204.

CLAYTON, M.N. and KING, R.J. (1975). Marine botany: an Australian perspective. Longman Cheshire, Melbourne 307p.

CLOETE, S.W.P., de VILLIERS, T.T. and KRITZINGER, N. M. (1983). The effects of ammoniation by urea on the nutritive value of wheat straw for sheep. *S. Afr. J. Anim. Sci.* 13(3): 143-146.

COLEMAN, G.S. (1980). Rumen ciliate protozoa. In "Advances in Parasitology". Lumsden, W.H.R., Muller, R. and Backer J.R. (Eds.). Academic Press, New York, pp. 121-73.

COLEMAN, G.S. (1983). Hydrolysis of fraction 1 leaf protein and casein by rumen entodiniomorphid protozoa. *J. Appl. Bacteriol.* 55: 111-118.

COLEMAN, G.S. (1985). Possible causes of the high death rate of ciliate protozoa in the rumen. *J. Agric. Sci.* 105: 39-43.

CONACHER, J.J., LANZING, W. J. and LARKUM, A. W. (1979). Ecology of Botany Bay. ii. Aspects of the feeding ecology of the fanbellied leather jacket *Monacanthus chinensis* in *Posidonia australis* seagrass beds in Quibray Bay, Botany Bay, N. S. W. *Aust. J. Mar. Freshwater Res.* 30: 387-400.

COOMBE, J.B. (1980). Utilisation of low-quality residues. In "World Animal Science, B1, Grazing animals". Morley, F.H.W. (Ed.). Elsevier, New York. 319-334.

COOMBE, J.B. and TRIBE, D.E. (1962). The effects of urea supplements on the utilisation of straw plus molasses diets by sheep. *Aust. J. Agric. Res.* 1: 70-91.

COOMBE, J.B., DINIUS, D.A. and WHEELER, W.E. (1979). Effect of alkali treatment on intake and digestion of barley straw by beef steers. *J. Anim. Sci.* 49: 169-176.

CORBETT, J.L. (1969). The nutritional value of grassland herbage. In "Nutrition of animals of agricultural importance. Part 2. Assessment of and factors affecting requirements of farm livestock". Cuthbertson, D. (Ed.). Pergamon Press, Melbourne. pp. 593-644.

COSTERTON, J. W., GEESEY, G. G. and CHENG, K. (1978). How bacteria stick. *Sci. Am.* 238: 86-95.

COTTLE, D.J. (1991). The sheep industry. In "Australian Sheep and Wool Handbook". Cottle, D.J. (Ed.). Inkata Press. Melbourne. pp. 1-18.

COTTYN, B.G. and DeBOEVER, J.L. (1988). Upgrading of straw by ammoniation. *Anim. Feed Sci. Technol.* 21: 287-294.

CRAMPTON, E.W. and HARRIS, L.E. (1969). Applied animal nutrition, the use of feedstuffs in the formulation of livestock rations. W.H. Freeman and Company. San Francisco, USA. pp. 312-395.

CRAWLEY, M.J. (1983). Herbivory. The dynamics of animal-plant interactions. Studies in ecology, Vol. 10, Blackwell Scientific Publications, Oxford. 437pp.

CROSS, H.H., SMITH, L.W. and DeBARTH, J.V. (1974). Rates of *in vitro* forage fibre digestion as influenced by chemical treatment. *J. Anim. Sci.* 39: 808-812.

CROSTHWAITE, C., ISHIHARA, M. and RICHARDS, G.N. (1984). Acid-ageing of lignocellulosics to improve ruminant digestibility: Application to bagasse, wheat and rice straws and oat hulls. *J. Sci. Food Agric.* 35: 1041-1050.

CZERKAWSKI, J.W. (1986). An introduction to rumen studies. Pergamon Press, Oxford. 236p.

CZERKAWSKI, J.W. and BRECKENRIDGE, G. (1979). Experiments with the long-term rumen simulation technique (Rusitec); response to supplementation of basal rations. *Br. J. Nutr.* 42: 217-28.

CZERKAWSKI, J.W. and BRECKENRIDGE, G. (1979). Experiments with the long-term rumen simulation technique (Rusitec); use of soluble food and an inert solid matrix. *Br. J. Nutr.* 42: 229-245.

DALAL-CLAYTON, D.B (1985). Black's agricultural dictionary. A&C Black. London. p 312.

DALTON, H.L., HUFFMAN, C.F. and RALSTON, N.P. (1953). The effect of feeding concentrates with different degrees of fineness and water contents on the eating and milking time in dairy cattle *J. Dairy Sci.* 39: 1279-1284.

DANIELS, L.B., SMITH, M.J., STALLCUO, O.T. and RAKES, J.M. (1983). Nutritive value of ensiled broiler litter for cattle. *Anim. Feed Sci. Technol.* 8: 19-34.

DAVIS, C.H. (1983). Experiences in Bangladesh with improving the nutritive value of straw. In "The utilisation of fibrous agricultural residues". Pearce, G.R. (Ed.). Australian Development Assistance Bureau, Canberra, Australia. pp. 123-128.

DAWES, C.J. and LAWRENS, J.M. (1980). Seasonal changes in the proximate constituents of the seagrasses *Thalassia testudinum, Halodule wrightil*, and *Syringodium filiforme. Aquat. Bot.* 8: 371-80.

de BOER, G., MURPHY, J.J. and KENNELLY, J.J. (1987). A modified method for determination of *in situ* rumen degradation of feedstuffs. *Can. J. Anim. Sci.* 67: 93-102.

de BOEVER, J.L., COTTYN, B.G., ANDRIES, J.I, BUYSSE, F.X. and VANACKER, J.M. (1988). The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. *Anim. Feed Sci. Technol.* 19: 247-260.

de KONING, C.T. and CARTER, E.D. (1989). Losses of subterranean clover seed from dry pasture residues during grazing by sheep in summer-autumn. *Proc. 5th. Aust. Agron. Conf.*, Perth. p. 408.

de WAAL, H.O. and BIEL, L.C. (1989). Supplementation of lactating Dorper and Merino ewes with *Themeda cymbopogon veld*, 3. Seasonal and diurnal variation in rumen pH and ammonia concentration. *J. Anim. Sci.*, South Africa, Tydskr. Veek. 19(4): 156-164.

DEAR, B.S. and LOVELAND, B. (1985). A survey of seed reserves of subterranean clover pastures on the southern tableland of New South Wales. *Proc. 3rd Aust. Agron. Conf.*, Tasmania. p. 214.

DEARTH R.N., DEHORITY, B.A. and POTTER, E.L. (1974). Rumen microbial numbers in lambs as affected by level of feed intake and dietary diethylstilbesterol. *J. Anim. Sci.* 38: 991-6.

DEHORITY, B. A. and JOHNSON, R. R. (1961). Effect of particle size upon the *in vitro* cellulose digestibility of forages by rumen bacteria. *J. Dairy Sci.* 44: 2242-2249.

DEHORITY, B.A. (1970). Occurrence of the ciliate protozoa *Butschlia parva schuberg* in the rumen of the ovine. *Appl. Microbiol.* 19: 179-81.

DEHORITY, B.A. (1978). Specificity of rumen ciliate protozoa in cattle and sheep. *J. Protozool.* 25: 509-13.

DEHORITY, B.A. (1979). Ciliate protozoa in the rumen of Brazilian water buffalo, *Bubalus bubalis Linnaeus. J. Protozool.* 26: 536-44.

DEHORITY, B.A. (1984). Evaluation of subsampling and fixation procedures used for counting rumen protozoa. *Appl. Environ. Microbiol.* 48: 182-5.

DEHORITY, B.A. (1986). Microbes in the foregut of arctic ruminants. In "Control of digestion and metabolism in ruminants". Milligan L.P. , Grovum W.L. and Dobson A. (Eds.). Prentice Hall, Englewood Cliffs, New Jersey. pp. 307-25.

DEHORITY, B.A. and GRUBB, J.A. (1980). Effect of short term chilling of rumen contents on viable bacterial numbers. *Appl. Environ. Microbiol.* 39: 376-81.

DEHORITY, B.A. and MATTOS, W.R.S. (1978). Diurnal changes and effect of ration on concentrations of the rumen ciliate *Charon ventriculi. Appl. Environ. Microbiol.* 36: 953-8.

DEHORITY, B.A. and PURSER, D.B. (1970). Factor affecting the establishment and numbers of holotrich protozoa in the ovine rumen. *J. Anima.Sci.* 30: 445-449.

DEINUM, B. and VAN SOEST, P.J. (1969). Prediction of forage digestibility from some laboratory procedures. *Neth. J. Agric. Sci.* 17: 119-127.

DELFOSSE-DEBUSSCHER, J. THINES-SEMPOUX, D. , VANBELLE, M. and LATTEUR B. (1979). Contribution of protozoa to the rumen cellulolytic activity. *Ann. Rech. Vet.* 10: 255-257.

DEMEYER D. (1981). Rumen microbes and digestion of plant cell walls. *Agric. and environ.* 6: 295-337.

DEMEYER, D. and VAN NEVEL, C. (1986). Influence of substrate and microbial intraction on efficiency of rumen microbial growth. *Reprod. Nutr. Dev.* 26: 161-79.

DEMEYER, D. and VAN NEVEL, C.J. (1979). Protein fermentation and growth by rumen microbes. *Ann. Rech. Vet.* 10: 275-279.

DEMEYER, D. I. (1973). Lipidstoffwechsel im Pansen. In "Biochemie und Biologie der mikrobiellen Verdauung. Giesecke D., Henderickx, H. (Eds.). BLV Verlagsgesell-Schaft, Munchen. pp. 209-234.

DEMEYER, D.I. and VAN NEVEL, C.J. (1979a). Effect of defaunation on the metabolism of rumen microorganisms, *Br. J. Nutr.* 42: 515-524.

den HARTOG, C. (1970). The seagrasses of the world. North Holland Publ. Co., Amsterdam. 275p.

DENNIS, S.M., ARAMBEL, M.J., BARTLEY, E.E. and DAYTON, A.D. (1983). Effect of energy concentration and source of nitrogen on numbers and types of rumen protozoa. *J. Dairy Sci.* 66: 1248

DEVENDRA, C. (1983). Physical treatment of rice straw for goats and sheep and the response to substitution with variable levels of Cassava (*Manihot Esculata Crantz*), Leucaena (Leucaena Leucocephala) and Gliricidia (*Gliricidia Maculata*) forages. *MARDI Research Bulletin* 11. 3: 272-290.

DIXON, R.M. (1987). Maximising the rate of fibre digestion in the rumen. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp 49-67.

DIXON, R.M. and EGAN, A.R. (1988). Strategies for optimising use of fibrous crop residues as animal feeds. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 11-26.

DJAJANEGARA, A. , MOLINA, B. T. , and DOYLE.P. T. (1984/85). The utilization of calcium hydroxide treated wheat straw by sheep. *Anim. Feed. Sci. Technol.* 12: 141-150.

DJAJANEGARA, A. and DOYLE, P. T. (1989a). Digestion rates and outflow rates from the rumen of sheep fed untreated or calcium hydroxide treated wheat straw. *Anim. Feed. Sci. Technol.* 25: 179-191.

DJAJANEGARA, A. and DOYLE, P. T. (1989b). Urea supplementation compared to pretreatment, 2. Effect on ruminal and post- ruminal digestion in sheep fed a rice straw. *Anim. Feed. Sci. Technol.* 27: 31-47.

DJAJANEGARA, A., DOYLE, P.T. and MOLINA, B.T. (1985). Fibre digestion and mineral balances in sheep fed calcium hydroxide-treated wheat straw. In "The utilisation of fibrous agricultural residues as animal feeds". Doyle, P.T. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 94-102.

DOBIE, J.B. (1959). Engineering appraisal of hay pelleting. *Agric. Engin.* 40: 76-77.

DONEFER, E., (1968). Effect of sodium hydroxide treatment on digestibility and voluntary intake of straw. *Proceeding of 2nd World Conference on Animal Production,* University of Maryland. P. 446.

DONEFER, E., LLOYD, L.E. and CRAMPTON, E.W. (1963). Effect of varying alfalfa: barley rations on energy intake and volatile fatty acid production by sheep. *J. Anim. Sci.* 22: 425-428.

DOYLE, P.T. (1983). Digestion of treated crop residues and the need for nutrient additions in balanced rations using such residues. In "The utilisation of fibrous agricultural residues". Pearce, G.R. (Ed.). Australian Development Assistance Bureau, Canberra, Australia. pp. 69-84.

DOYLE, P.T. and EGAN, J.K. (1987). Palatability of mature subterranean clover. In "Temperate pastures, their production, use and management". Wheeler, J.L., Pearson, C.J. and Robards, G.E. (Eds.). Australian Wool Corporation, CSIRO. Aust. pp. 328-330.

DOYLE, P.T. and PANDAY, S.B. (1990). The feeding value of cereal straw for sheep. III, Supplementation with minerals or minerals and urea. *Anim. Feed Sci. Technol.* 29: 29-43.

DOYLE, P.T., DEVENDRA, C. and PEARCE, G.R. (1986). Rice straw as a feed for ruminants. International Development Program of Australian Universities and Colleges. Canberra, Australia. 117p.

DRORI D. and LOOSLI J.K. (1961). Urea and carbohydrates versus plant protein for sheep. *J. Anim. Sci.* 20: 233.

DULPHY, J.P. and DEMARQUILLY, C. (1983). Voluntary feed consumption as an attribute of feeds. In "Feed information and animal production" Robards, G.E. and Packham, R.G. (Eds.). CAB (Commonwealth Agricultural Bureau). UK. pp. 135-156.

DULPHY, J.P., REMOND, B., THERIEZ, M. (1980). Ingestive behaviour and related activities in ruminants. In "Digestive physiology and metabolism in ruminants". Ruckebusch, Y. and Thivend, P. (Eds.). Lancaster, UK. pp. 103-122.

DUNLOP, A.C. and McDONALD, C.L. (1986). Protein enrichment of cereal grain for livestock. *West. Aust. J. Agric.* 27: 64-67.

DURAKO, M.J. and DAWS, C.J. (1980). A comparative study of two populations of *Hypnea musciformis* from the East and west coast of Florida, USA. I. Growth and chemistry. *Mar. Biol.* 59: 151-156.

DURAND, M. and KAWASHIMA, R. (1979). Influence of minerals in rumen microbial digestion. In "Digestive physiology and metabolism in ruminants". Ruckebush, Y. and Thivend, P. (Eds.). *Proc. 5th Int. Symp. Ruminant Physiol. Clermont-Ferrand.* MTP press, Lancaster, Great Britain. pp. 375-408.

EADIE, J.M. and GILL, J.C. (1971). The effect of the absence of rumen ciliate protozoa on growing lambs fed on a roughage-concentrate diet. *Br. J. Nutr.* 26: 155-67.

EADIE, J.M., HYLDEGAARD-JENSEN, J., MANN, S.O., REID, R.S. and WHITELAW, F.G.(1970). Observations on the microbiology and biochemistry of the rumen in cattle given different quantities of a pelleted barley ration. *Bri. J. Nutr.* 24: 157-177.

EASLEY, J.F. and SHIRLEY, R.L. (1974). Nutrient elements for livestock in aquatic plants. *Hyacinth Control J.* 12: 82-85.

EGAN, A. R. (1990). Strategies in modification of plant attributes and rumen environment to increase utilization of lignocellulose. In "Microbial and plant opportunities to improve lignocellulose utilization by ruminants". Akin, D. E. , Ljungdahl, L. G.,Wilson, J. R. , Harris, P. J. (Eds.). Elsevier, New York. pp. 33-39.

EGAN, A. R. FREDERICKS, F. and DIXON, R. M. (1987). Improving the efficiency of use of supplements by manipulation through management procedures. In "ruminant feeding systems utilizing fibrous agricultural residues". Dixon, R. M. (Eds.). IDP, Canbera. pp. 69-81.

EGAN, A.R. (1986). Principals of supplementation of poor-quality roughages with nitrogen. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 49-57.

EL-SABBAN, F.F., BRATZLER, J.W., LONG, T.A., FREAR, D.E.H. and GENTRY, R.F. (1970). Value of processed poultry waste as a feed for ruminants. *J. Anim. Sci.* 31: 107-111.

ELLIOTT, R.C. (1967a). Voluntary intake of low protein diets by ruminants. I. Intake of feed by cattle. *J. Agric. Sci. Camb.* 69: 375-382.

ELLIOTT, R.C. (1967b). Voluntary intake of low protein diets by ruminants. II. Intake of feed by sheep. *J. Agric. Sci. Camb.* 69: 383-390.

ELLIS, W.C., WYLIE, M.J. and MATIS, J.H. (1988). Dietary digestive interactions determining the feeding value of forages and roughages. In "World Animal Science, B4. Feed science". Ørskov, E.R. (Ed.). Elsevier, New York. pp. 177-229.

ENGLYST, H., WIGGINS, H.S. and CUMMINGS, J.H. (1982). Determination of the nonstarch polysaccharides in plant food by gas liquid chromotography of constituent sugars as a alditol acetates. *Analyst*, 107: 307-18.

ENTWISTLE, K.W. and BAIRD, D.A. (1976). Studies on the supplementary feeding of sheep consuming mulga (*Acacia aneura*). 2. Comparative levels of molasses and urea supplements fed under pen conditions. *Aust J. Exp. Agric. Anim. Husb.* 16: 174-180.

ERICSON, L.E. (1952). Uptake of radioactive cobalt and vitamin B12 by some marine algae. *Chem. and Ind.* pp. 829-30.

ERNST, A.J., LIMPUS, J.F. and O'ROURKE, P.K. (1975). Effect of supplements of molasses and urea on intake and digestibility of native pasture hay by steers. *Aust J. Exp. Agric. Anim. Husb.* 15: 451-455.

ERWIN, E.A. and ELLISON, N.G. (1959). Rapid method of determining digestibility of concentrates and roughages in cattle. *J. Anim. Sci.* 18: 1518A.

ERWIN, E.S., MARCO, G.J. and EMERY, E.M. (1961). Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J. Dairy Sci.*, 1968-1770.

EVANS, P.J. (1979). Chemical and physical aspects of the interaction of sodium hydroxide with the cell wall components of straw. In "Straw decay and its effect on disposal and utilisation". Grassbard, E. (Ed.). John Wiley & Sons. London. pp. 187-229.

FAICHNEY, G.J. and GRIFFITHS, D.A. (1978). Behaviour of solute and particle markers in the stomach of sheep given a concentrate diet. *Br. J. Nutr.* 40: 71-81.

FAO (1990). FAO. Production Yearbook. FAO statistics series. No. 99. Rome, Italy. pp. 71-87.

FAU (Feedlot Advisory Unit). (1990). The feedlot manual. Dept. Agric. Fish. NSW., Australia.

FEIST, W.C., BAKER, A.J. and TARKOW, H. (1970). Alkali requirements for improving digestibility of hard woods by rumen micro organisms. *J. Anim. Sci.* 30: 832-835.

FELS, H.E. (1980). Principles of lot-feeding and the design and operation of feedlots. In Proc. of a seminar on grain feeding of sheep for survival and growth. Hunter, R.A. (Ed.). *Aust. Soc. Anim. Prod.* pp. 76-89.

FENCHEL, T. (1972). Aspects of decomposer food chains in marine benthose. *Verh. Dtsch. Zool.* 65: 14-22.

FENCHEL, T. (1977). Aspect of the decomposition of seagrass. In "Seagrass ecosystems". McRoy, C.P. and Helfferich, C. (Eds.). Marcel Dekker, New York. pp. 123-145.

FENCHEL, T. and Blackburn, T.H. (1979). Bacteria in detritus food chains. In: "Bacteria and mineral cycling". Fenchel, T. and Blackburn, T.H. (Eds). Academic press, London. PP. 52-78.

FENCHEL, T.M and JORGENSEN, B.B., (1977). Detritus food chains of aquatic ecosystems: the role of bacteria. *Advanc. Microbial Ecol.* 1: 1-58.

FERNANDEZ CARMONA, J. and GREENHALGH, J.F.D. (1972). The digestibility and acceptability to sheep of chopped or milled barley straw soaked or sprayed with alkali. *J. Agric. Sci. Camb.* 78: 477-485.

FLACHOWSKY, G., TIROKE, K. and SCHEIN, G. (1991). Botanical fractions of straw of 51 cereal varieties and *in sacco* degradability of various fractions. *Anim. Feed Sci. Technol.* 34: 279-289.

FLATT, W.P. (1988). Feed evaluation systems: Historical background. In "World Animal Science, B4. Feed science". Ørskov, E.R. (Ed.). Elsevier Sci. Pub. Company. NY. pp. 1-22.

FONTENOT, J. P. and WEBB, K. E. (1974). Poultry wastes as feedstuffs for ruminants. *Fed. Proc.* 33: 1936-1937.

FONTY, G., JOUANY, J.-P., THIVEND, P, GOUET, Ph. and SENAUD, J. (1983). A descriptive study of rumen digestion in meroxenic lambs according to the nature and complexity of the microflora. *Reprod. Nutri. develop.* 23: 857- 873.

FORD, C.W. (1978). Effect of particle de-lignification on the *in vitro* digestibility of cell wall polysaccharides in *Digitaria decumbens* (Pangola grass). *Aust. J. Agric. Res.* 29: 1157-1161.

FORD, C.W. (1983). Effect of particle size and de-lignification on the rate of digestion of hemicellulose and cellulose by cellulase in mature pangola grass stems. *Aust. J. Agric. Res.* 34: 241-248.

FORD, C.W., ELLIOTT, R. and MAYNARD, P.J. (1987). The effect of chlorite de-lignification on digestibility of some grass forages and on intake and rumen microbial activity in sheep fed barley straw. *J. Agric Sci. Camb.* 108: 129-136.

FRANCIS, C.M. (1973). The influence of isoflavone glycosides on the taste of subterranean clover leaves. *J. Sci. Food Agric.* 24: 1235-1240.

FRANCIS, G. L. , Gawthorne, J. B. (1978). Factors affecting the activity of cellulases isolated from the rumen digesta of sheep. *Appl. Environ. Microbiol.* 36: 643-649.

FREER, M. (1981). The control of food intake by grazing animals. In "World Animal Science, B1, Grazing animals". Morley, F.H.W. (Ed.). Elsevier Sci. Pub. Company. pp. 105-124.

GADDEN, W. (1920). The digestibility of straw after treatment with soda. *J. Agric. Sci. Cambr.* 10: 437-465.

GARNSWORTHY, P.C. and COLE, D.J.A. (1990). The importance of intake in feed evaluation. "Feedstuff evaluation". Wiseman, J. and Cole, D.J.A. (Eds.). Butterworths. UK. pp. 147-160.

GARRETT, W.N., WALKER, Jr., H.G., KOHLER, G.O., HART, M.R. and GRAHAM, R.P. (1981). Steam treatment of crop residues for increased ruminant digestibility. II. Lamb feeding studies. *J. Anim. Sci.* 51: 409-413.

GHARIB, F.H., GOODRICH, R.D., MEISKE, J.C. and ELSERAFY, A.M. (1975). Effects of grinding and sodium hydroxide treatment on poplar bark. *J. Anim. Sci.* 40: 723-733.

GIESECKE, D. and VAN GYLSWYCK, N.O. (1975). A study of feeding types and certain rumen functions in six species of South African wild ruminants. *J. Agric. Sci.* 85: 75-83.

GILCHRIST, F. M. C. , HENNING, P. A. , VAN DER LINDEN, Y. , MATTEYE, M. E., NAUHAUS, W. K. and SCHWARTZ, H.M. (1979). Factors affecting digestion of grain-supplemented straw. *Ann. Rech. Vet.* 10: 320-322.

GILCHRIST, F.M.C. and KISTNER, A. (1962). Bacteria of the ovine rumen. I. The composition of the population on a diet of poor teff hay. *J. Agric. Sci.* 59: 77-83.

GILLESPIE, D.J. (1983). Pasture deterioration causes and cures. *West. Aust. J. Agric.* 1: 3-8.

GOERING, H.K., SMITH, L.W., VAN SOEST, P.J. and GORDON, C.H. (1973). Digestibility of roughages materials ensiled with sodium chlorite. *J. Dairy Sci.* 56: 233-240.

GOERING, H.K., VAN SOEST P.J. (1970). Forage fibre analyses. (Agriculture Hand book No. 379). Agricultural research service. Dept. of Anim. Sci., U.S.A., 20p.

GOMEZ CABRERA, A. and VAN der MEER, J.M. (1988). Rate of degradation of organic matter and neutral detergent fibre in barley straw: Effect of genetic variation and treatment with ammonia on degradation *in sacco* and *in vitro*. *Neth. J. Agric. Sci.* 36: 108-110.

GOULD, J. M. (1984). Alkaline peroxide delignification of agricultural residues to enhance enzymic saccharification. *Biotechnol. Bioeng.* 26: 46-52.

GOULD, J.M. (1985). Studies on the mechanism of alkaline peroxide de-lignification of agricultural residues. *Anim. Feed Technol.* 17: 179-199)

GREENHALGH, J.F.D. and REID, G.W. (1973). The effects of pelleting various diets on intake and digestibility in sheep and cattle. *Anim. Prod.* 16: 223-233.

GREENHALGH, J.F.D. and REID, G.W. (1974). Long and short term effects on intake of pelleting roughage for sheep. *Anim. Prod.* 19: 77-86.

GREENHALGH, J.F.D. and WAINMAN, F.W. (1972). The nutritive value of processed roughages for fattening cattle and sheep. *Proc. Br. Soc. Anim. Prod.* pp. 61-72.

GROVUM, W.L. (1988). Appetite, palatability and control of feed intake. In "The ruminant animal, digestive physiology and nutrition". Church, D.C. (Ed.). A Reston Book, Prentice Hall, Englewood, Cliffs. NJ. pp. 202-216.

GRUBB, J.A. and DEHORITY, B.A. (1975). Effects of an abrupt change in ration from all roughage to high concentrate upon rumen microbial numbers in sheep. *Appl. Microbiol.* 30: 404-12.

GRUBB, J.A. and DEHORITY, B.A. (1976). Variation in colony counts of total viable anaerobic rumen bacteria as influenced by media and cultural methods. *Appl. Environ. Microbiol.* 31: 262-7.

GRUBY, D. and DELAFOND, H.M.O. (1843). Recherches sur des animalcules se devellopant en grand nombre dans Lestomac et dans les intestins pendant la digestion des animaux herbivores et carnivores. Comptes Rendus hebdomadaire des Seances de L Academie des Sciences, cited In"The rumen protozoa". Springer-verlag, New York, 441p. Paris. 17: 1304-1308.

GRUMMER, R.R., STAPLE, C.R. and DAVIS, C.L. (1983). Effect of defaunation on ruminal volatile fatty acids and pH of steers fed a diet high in dried whole whey. *J. Dairy Sci.*. 66: 1738-1741.

GUGGOLZ, J., KOHLER, G.O. and KLOPFENSTEIN, T.J. (1971). Composition and improvement of grass straw for ruminant nutrition. *J. Anim. Sci.* 33: 151-156.

GUPTA, A.K. and VERMA, M.L. (1980). In "Abstracts of papers, National Symposium on Recycling of Residues of Agriculture and Industry". PAU, Ludhiana (abstract), pp. 26-27.

HACK, W, ASHTON, B. and GILES, B. (1988a). Production feeding of sheep; Management and facilities. Fact Sheet, FS 10/88. Dept. Agric. South Australia. 4p.

HACK, W., ASHTON, B., GILES, B. (1988b). Production feeding of sheep; Health. Fact Sheet., FS 11/88. Dept. Agric. South Australia. 2p.

HACK, W., ASHTON, B., GILES, B. (1988c). Production feeding of sheep; Rations. Fact Sheet, .FS. 12/88. Dept. Agric. South Australia. 4p.

HALL, D.G. (1987). Seasonality of growth and quality of wool from crossbred ews on improved pastures in southern New South Wales. In "Temperate pastures: their production, uses and management". Wheeler, J. L., Pearson C.J., Robard G. E. (Eds.). East Melborne, Victoria, Australia.Commonwealth Scientific and Industrial Research Organization, pp: 486-88.

HALL, D.G. and MULHOLLAND, J.G. (1982). Some problems of introducing feedlot rations to lambs. *Proc. Aust. Soc. Anim. Prod.* 14: p. 650.

HAN, Y.W. (1978). Microbial utilisation of straw (a review). *Advanc. Appl. Microbiol.* 23: 119-153.

HAN, Y.W. and ANDERSON, A.W. (1975). Semisolid fermentation of ryegrass straw. *Appl. Microb.* 30: 930-934.

HAN, Y.W. and CALLIHAN, C.D. (1974). Cellulose fermentation; effect of substrate pre-treatment on microbial growth. *Appl. Microbio.* 27: 159-165.

HARISON, P.G. and MANN, K.H. (1975). Chemical changes during the seasonal cycle of growth and decay in eelgrass on the Atlantic coast of Canada. *J. Fish. Res. Bd. Can.*. 32: 615-21.

HARKIN, J.M. (1973). Lignin. In "Chemistry and biochemistry of herbage". Vol. 1. Butler, G.W. and Bailey, R.W. (Eds.) Academic Press. NY. pp. 323-373.

HARMON, B.W., FONTENOT, J.P. and WEBB, K.E. (1975). Ensiled broiler litter and corn forage. 1. Fermentation characteristics. *J. Anim. Sci.* 40: 144-155.

HARRIS, L.E. (1970). Nutrition research techniques for domestic and wild animals. Vol. 1. Utah State University, Logan, Utah, USA. 5501p.

HARRISON, D. G. and McALLAN, A. B. (1979). Factors affecting microbial growth yield in the reticulo rumen. In "Digestive physiology and metabolism in ruminants. Ruckebush, Y., Thived, P. (Eds). *Proc. 5th Int. Symp. Ruminant Phsiol.*, Clermont-Ferrand. MTP press, Lancaster, Great Britain, pp. 205-226.

HARRISON, D.G., BEEVER, D.E., THOMSON, D.J. and OSBOURN, D. F. (1975). Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen. *J.Agric. Sci.* 85: 93-101.

HART, M.R., WALKER, Jr., H.G., GRAHAM. R.P., HANNI, P.J., BROWN, A.H. and KOHLER, G.O. (1981). Steam treatment of crop residues for increased ruminant digestibility. 1. Effects of process parameters. *J. Anim. Sci.* 51: 402-408.

HARTLEY, R.D. (1981). Chemical constitution, properties and processing of lignocellulosic wastes in relation to nutritional quality for animals. *Agric. Environ.* 6: 91-113.

HARTLEY, R.D., JONES, E.C., KING, N.J. and SMITH , G.A. (1974). Modified wood waste and straw as potential components of animal feeds. *J. Sci. Food Agric.* 25: 433-437.

HAUG, A. and JENSON, A. (1954). Seasonal variations in the chemical composition of *Alaria esculenta, Laminaria saccharina, Laminaria hyperborea* and *Laminaria digitata* from Northern Norway. Norsk institutt for tang-og tareforskning, rep. No. 4. pp. 1-14.

HEANEY, D.P. and PIGDEN W.J. (1963). Interrelationships and conversion factors between expression of the digestible energy value of forages. *J. Anim. Sci.* 22: 956-960.

HECKER, J.F (1974). Experimental surgery in small ruminants. Butterworths, London, 322p.

HEGARTY, M.P. (1981). Deleterious factors in forages affecting animal production. In "Nutritional limits to animal production from pastures". Hacker, J.B. (Ed.). CAB (Commonwealth Agricultural Bureaux), Farnham, Royal, UK. pp. 133-150.

HESPELL, R. B. and BRYANT, M. P. (1979). Efficiency of rumen microbial growth: influence of some theoretical and experimental factors on ATP. *J. Anim. Sci.* 49: 1640-1659.

HINKSON, R.S., STERN, M.D. and GRAY, H.G. (1976). Effect of defaunation on acid concentrations in the rumen of the mature bovine. *Sci. Biol. J.* 2: 116- 123.

HIRONAKA, R., KIMURA, N. and KOZUB, G. (1979). Influence of feed particle size on rate and efficiency of gain, characteristics of rumen fluid and rumen epithelium, and rumen protozoa. *Can. J. Anim. Sci.* 59: 395-402.

HOBSON, P. N. and WALLACE, R. J. (1982). Microbial ecology and activities in the rumen: Part i. *CRC Crit. Rev. Microbiol.* 9: 165-225.

HOBSON, P.N., MANN, S.O. and SUMMERS, R. (1976). Rumen micro-organisms in red deer, hill sheep and reindeer in the Scottish highlands. *Proc. Roy. Soc. Edinburgh, B.* 75: 171-80.

HODGSON, J. C., THOMAS, P. C. and WILSON, A. G. (1976). The influence of the level of feeding and flaked maize. *J. Agric. Sci., Camb.* 87: 297-302.

HOGAN J.P. and LECHE, T.F. (1983). Types of fibrous residues and their characteristics. In "The utilisation of fibrous agricultural residues". Pearce, G.R. (Ed.). Australian Development Assistance Bureau, Canberra, Australia. pp. 3-13.

HOGAN, J. P. and WESTON, R. H. (1969). The digestion of pasture plants by sheep. III. The digestion of forage oats varying in maturity and in content of protein and soluble carbohydrate. *Aust. J. Agric. Res.* 20: 347-363.

HOGAN, J.P., WESTON, R.H. and LINDSAY, J.R. (1969). The digestion of pasture plants by sheep. IV. The digestion of *Phalaris tuberosa* at different stages of maturity. *Aust. J. Agric. Res.* 20: 925-949.

HOLDEMAN, L.V., CATO, E.P., and MOORE, W. E. C. (1977). Anaerobe laboratory manual, 4th ed. , Virginia Polytch. Inst. and State Univ. , Blacksburg, Virginia. pp. 1-156.

HOLZER, Z., LEVY, D., TAGARI, H. and VOLCANI, R. (1975). Soaking of complete fattening rations high in poor-roughage. 1. The effect of moisture content and spontaneous fermentation on nutritional value. *Anim. Prod.* 21: 323-335.

HOLZER, Z., TAGARI,H. LEVY, D. and VOLCANI, R. (1976). Soaking of complete fattening ration. 2. The effect of moisture content on the performance of male cattle. *Anim. Prod.* 22: 41-53.

HOOVER, W.H. (1978). Digestion and absorption in the hindgut of ruminants. *J. Anim. Sci.* 46: 1789-1799.

HOVELL, F.D., NGAMBI, J.W.W., BARBER, W.P. and KYLE, D.J. (1986). The voluntary intake of hay by sheep in relation to its degradability in the rumen as measured in nylon bags. *Anim. Prod.* 42: 111-118.

HOWARD, K. and PLASTO, T. (1991). Feedlotting (a guide for beef producers). Department of Primary Industries, Brisbane, Queensland, Australia. 80p.

HOWARTH, R.E. (1988). Antiquality factors and non-nutritive chemical components. In "Alfalfa and alfalfa improvement". *Amer. Soc. Agron.* 29: 493-514.

HUFFMAN, J.G., KITTS, W.D. and KRISHNAMURTI, C.R. (1971). Effects of alkali treatment and gamma irradiation on the chemical composition and *in vitro* rumen digestibility of certain species of wood. *Can. J. Anim. Sci.* 51: 457-464.

HUGHES, A.D., EGAN, J.P. and GLAETZER, B.M. (1983). Computer-based least-cost ration formulation for ruminants. In "Feed information and animal production". Robards, G.E. and Packham, R.G. (Eds). Commonwealth Agricultural Bureaux, UK. pp. 369-374.

HUME, I. D. (1984). Evolution of herbivores - The ruminant in perspective. In "Ruminant physiology - concepts and consequences". Baker, S. K., Gawthorne, J. M. , Macckintosh, J. B. and Purser, D. B. (Eds.) University of Western Australia, Perth. pp. 15-25.

HUME, I. D. and WARNER, A.C.I. (1980). Evolution of microbial digestion in mammals. In "Digestive physiology and metabolism in ruminants (Proceeding of the fifth international symposium on ruminant physiology)". Ruckebusch, Y. and Thivend, P. (Eds.) MTP press limited, London. pp. 665-684.

HUNGATE, R. E. (1966). The rumen and its microbes. Academic press, New York. pp. 533.

HUNGATE, R.E. (1942). The culture of *Eudiplodinium neglectum* with experiments on the digestion of cellulose. *Biol. Bull.* (Woods Hole). 83: 303-319.

HUNGATE, R.E. (1943). Further experiments on cellulose digestion by the protozoa in the rumen of cattle. *Biol. Bull.* (Woods Hole). 84: 157-163.

HUNGATE, R.E. (1975). The rumen microbial ecosystem. *Annu. Rev. Ecol. Syst.*. 6: 39-66.

HUNGATE, R.E. (1988). The ruminant and the rumen. In "The rumen microbiology ecosystem". Hobson, P.N. (Ed.). Elsevier Applied Science., London. pp. 1-19.

HUNTER, R.A. (1988). Some aspects of the role of concentrates in increasing feed intake and productivity of cattle fed fibrous diets. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 37-48.

HUPP, E.W. and LEWIS, R.C. (1958). Effect of adding water to the concentrate mix on maximum milking rate, average milking rate and eating rate. *J. Dairy Sci.* 724 A.

HVELPLUND, T., STIGSEN, P., MØIIER, P.D. and JENSEN, K., (1978). Propionic acid production rate in the bovine rumen after feeding untreated and sodium treated straw. *Z. Tierphysiol. Tierenahr. Futtermittelkd.* 40: 183-190.

HYND, P.I. (1982). Wool growth efficiency: a study of the effects of live weight status and diet on wool growth. Ph. D. Thesis, The University of Adelaide, Australia. 211p.

IBRAHIM, M.N.M, KETELAAR, R.S., TAMMINGA, S. and ZEMMELINK, G. (1988). Degradation characteristics of untreated and urea-treated rice straw in the rumen. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 123-126.

IBRAHIM, M.N.M. (1983). Physical, chemical, physico-chemical and biological treatments of crop residues. In "The utilisation of fibrous agricultural residues". Pearce, G.R. (Ed.). Australian Development Assistance Bureau, Canberra, Australia. pp. 53-68.

IBRAHIM. M.N.M. (1985). Effects of *Gliricidia maculata* leaves on the duration of urea-ammonia treatment of rice straw. In "The utilisation of fibrous agricultural residues as animal feeds". Doyle, P.T. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 77-80.

ICARDA (1984). Research highlights. International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria. pp. 60-64.

ICARDA (1991). Annual report. International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria. pp. 52-55.

ICARDA. (1990-1991). Pasture, forage and livestock program, Annual report for 1990/1991. ICARDA, Alippo, Syria. 192p.

JACKSON, M.G. (1977). Review article: The alkali treatment of straws. *Anim. Feed Sci. Technol.* 2: 105-130.

JACOBS G.J.L. and LEIBHOLZ, J. (1977). Effect of including ensiled broiler-house litter in rations of sheep on the digestibility of nutrients and retention of nitrogen. *Aust. J. Exp. Agric. Anim. Husb.* 17: 43-47.

JAKHMOLA, R.C., KUNDA, S.S., PUNGI, M.L., SINGH, K., KAMRA, D.N. and SINGH, R. (1988). Animal excreta as ruminant feed - scope and limitation under Indian conditions. *Anim. Feed Sci. Tech.* 19: 1-23.

JAMES L.F., ALLISON, M.J. and LITTLEDLIKE, E.T.(1975). Production and modification of toxic substances in the rumen. In "Digestion and metabolism in the ruminant". McDonald, I.W. and Warner, A.C.I. (Eds.).University of New England publishing unit, Armidale. pp. 576-590.

JAMIESON, B. (1984). Fat scores for sheep and lamb (Department of Agriculture, South Australia)., Government printer, South Australia. pp. 19-20.

JARRIGE, R. (1980). Place of herbivores in the agricultural ecosystems. In "Digestive physiology and metabolism in ruminants". Ruckebusch, Y. and Thivend, p. (Eds.). MTP Press limited, Lancaster. pp. 763-823.

JAYAL, M.M., JAIN, V.K. and PATHAK, N.N. (1981). Studies on the utilization of ensiled poultry excreta as a ration for replacement stock of crossbred dairy cattle. *Agric. Wastes*, 3: 157-163.

JAYASURIYA, M.C.N. (1981). Effect of urea-ensiling of rice straw on digestibility, voluntary intake and VFA production in growing buffalo calves. Paper presented to the: *"Second Research Coordination Meeting of the coordinated Research Programme on the Use of Nuclear Techniques to improve Domestic buffalo Production in Asia"*, International Atomic Energy Agency - Food and Agriculture Organization of the United Nation. 13pp.

JAYASURIYA, M.C.N. (1983). Problems for treating crop residues at the village level. In "The utilisation of fibrous agricultural residues". Australian Development Assistance Bureau, Canberra, Australia. pp. 100-106.

JEFFERIES, B.C. (1961). Body condition scoring and its use in management. *Tasm. J. Agric.* 32: 19-21.

JEFFERIES, B.C. and NASH, H.M. (1989). Sheep husbandry in South Australia. 98p.

JOANING, S.W., JOHNSON, D.E. and BARRY, B.P. (1981). Nutrient digestibility depressions in corn silage-corn grain mixture fed to steers. *J. Anim. Sci.* 53: 1095-1103.

JOHANSON, R.R., RICKETTS, G.E., KLOSTERMAN, E.W. and MOXON, A.L. (1964). Studies on the utilisation and digestion of long, ground and pelleted alfalfa and mixed hay. *J. Anim. Sci.* 23: 94-99.

JOHNSON, R. R. (1976). Influence of carbohydrate solubility on non-protein nitrogen utilization in the ruminant. *J. Anim. Sci.* 43: 184-191.

JOHNSTON, I.M. (1982). Ecology and distribution of seagrasses. *Monographi.Biologi.* 42: 497-512.

JONES, M.J. and KLOPFENSTEIN, T.J. (1967). Chemical treatment of poor-quality roughages. *J. Anim. Sci.* 26: 1492 A.

JONES, R.M. and CARTER, E.D. (1989). Demography of pasture legumes. In "Persistence of forage legumes". Marten, G.C., Matches, A.G., Barnes, R.F., Brougham, R.W., Clements, R.J. and Sheath, G.W. (Eds.). Amer. Soc. Agron. pp. 139-156.

JUNG, H.G. (1989). Forage lignins and their effects on fibre digestibility. *Agron. J.* 81: 33-38.

JUNG, H.G., VALDEZ, F.R., ABAD, A.R., BLANCHETLE R.A. and HATFIELD, R.D. (1992). Effect of white rot *basidiomycetes* on chemical composition and *in vitro* digestibility of oat straw and alfalfa stems. *J. Anim. Sci.* 70: 1928-1935.

KANDYLIS, K. and NIKOKYRIS, P. (1991). A reassessment of the nylon bag technique. *World Rev. Anim. Prod.* XXVI: 23-32.

KAUFMANN, W., HAGEMEISTER, H. AND DIRKSEN, G. (1980). Adaptation to changes in dietary composition, level and frequency of feeding. In digestive physiology and metabolism, Lancaster, PP. 587-602.

KAVANAGH, W.J., and BOW, M.R. (1985). Estimating the wool content of staples sample for staple strength measurement. IWTO Technical Committee, Report of the paris meeting, January 1985.

KEMPTON, T.J. (1980). The use of nylon bags to characterise the potential degradability of feeds for ruminants. *Trop. Anim. Prod.* 5: 107-116.

KENNEDY, P.M., CHRISTOPHERSON, R.J. and MILLIGAN, L.P. (1976). The effect of cold exposure of sheep on digestion, rumen turn over time and efficiency of microbial synthesis. *Br. J. Nutr.* 36: 231-241.

KERLEY, M.S., FAHEY, G.C., BERGER, L.L., MERCHEN, N.R. and GOULD, J.M. (1986). Effect of alkaline hydrogen peroxide treatment of wheat straw on site and extent of digestion in sheep. *J. Anim. Sci.* 63: 868-878.

KERNAN, J.A., COXWORTH, E.C. and SPURR, D.T. (1981). New crop residues and forages for western Canada: assessment of feeding value *in vitro* and response to ammonia treatment. *Anim. Feed Sci. Technol.* 6: 257-271.

KETELAARS, J.J.M.H. and TOLKAMP, B.J.(1992). Toward a new theory of feed intake regulation in ruminants. 1. Causes of differences in voluntary feed intake: Critique of current views. *Livestock Prod. Sci.* 30: 269-296.

KIANGI, E.M.I. and KATEGILE, J.A. (1981). Different sources of ammonia for improving the nutritive value of low-quality roughages. *Anim. Feed Sci. Technol.* 6: 377-386.

KIFLEWAHID, B. (1983). An overview of research methods employed in the evaluation of by-products for use in animal feed. In "By-product utilisation for animal production". Kiflewahid, B., Potts, G.R. and Drysdale, R.M. (Eds.). pp. 93-115.

KIKUCHI, T. and PERES, J.M. (1977). Consumer ecology of seagrass bed. In "Seagrass ecosystems: a scientific perspective". McRoy, C.P. and Helfrich, C. (Eds.). Marcell Dekker Ink. New Yourk and Basel. pp. 147-193.

KING, J.O.L. (1982). Effect of processing on nutrient content of foods and feeds; Water treatment. In "Handbook of nutritive value of processed food". Vol. II. Animal feedstuffs. Milosan, R. Jr. (Ed.). CRC, Press, Inc., Boca Raton, Florida, USA. pp. 129-134.

KING, R.J. (1980). Marine angiosperms: seagrass. In "Marine botany: an australian perspective". Clayton, M.N. and King, R.J. (Eds.). Longman Cheshire. pp. 201-210.

KIRBY, R.H. (1953). Seaweeds in commerce. H.M.S.O., London. 130p.

KIRK, T.K. (1984). Degradation of lignin. In "Microbial degradation of organic compounds. Gibson, D.T. (Ed.). Marcel dekker, inc. New York. pp. 399-437.

KLEIN, D.R. (1962). Rumen contents analysis as an index to range quality. *Transactions of the North American Wildlife conference* . 27: 150-164.

KLEIN, D.R. (1965). Ecology of deer range in Alaska. *Ecolog. Monographs* . 35: 259-284.

KLOPFENSTEIN, T.J. (1978). Chemical treatment of crop residues. *J. Anim. Sci.* 46: 841-848.

KLOPFENSTEIN, T.J. and BOLSEN, K.K. (1971). High temperature pressure treated crop residues. *J. Anim. Sci.* 33: 290 A.

KLOPFENSTEIN, T.J. and OWEN, F.G. (1981). Value and potential of crop residues and by products in dairy rations. *J. Dairy Sci.* 64: 1250.

KLOPFENSTEIN, T.J., PURSER, D.B. and TYZNIK, W.J. (1966). Effect of defaunation on feed digestibility, rumen metabolism and blood metabolites. *J. Anim. Sci.* 25: 765-773.

KLOPFESTEIN, T.J. (1978). Chemical treatments of crop residues. *J. Anim. Sci.* 46: 841-848.

KLOPFESTEIN, T.J., KRAUSE, V.E., JONES, M.J. and WOODS, W. (1972). Chemical treatment of low-quality roughages. *J. Anim. Sci.* 35: 418-422.

KLUMPP, D.W. and NICHOLS P.D. (1983). Utilization of the seagrass *Posidonia australis* as food by the Rock crab *Nectocinus integrifons* (Latrille) (Crustacea: Decapoda: Portunidae). *Mar. biol. letters.* 4: 331-339.

KLUMPP, D.W. and VAN der VALK, A. (1984). Nutritional quality of seagrass (*Posidonia australis and Heterozostera tasmanica*): Comparison between species and stages of decomposition. *Mar. Biol. letter.* 5: 67-83.

KOES, R.M. and PFANDEN, W.H. (1974). Increased utilisation of bluestem hay with the addition of water or certain protein sources. *J. Anim. Sci.* 38: 662-668.

KRISHNA REDDY, G.V. and RAJ REDDY, M. (1989). Nutritive value of rice straw (*Oryza sativa*) ensiled with animal excreta and rumen digesta. *Anim. Feed and Technol.* 24: 69-81.

KRISTENSEN, V.F. (1982) Effect of processing on nutrient content of feeds: Alkali treatment. In "Handbook of nutritive value of processed food, Vol., II. Animal feedstuffs". Rechcigl, Jr. M. (Ed.). CRC Press. Inc. Boca Raton, Florida. pp. 65-101.

KUMANOV, S., PALIEV, H. and JANKOV, B. (1969). Use of deep litter from broiler production as a feed for fattening calves with a complete feed pelleted or as meal. Cited in "*Nutr. abstr. Rev*". (1970). 40: 3872

KUO, J. and Cambridge, M.L. (1978). Morphology, anatomy and histochemistry of the Australian seagrasses of the genus *Posidonia* Konig (Posidoniaceae). II. Rhizome and root of *Posidonia australis*. *Aqat. Bot.* 5: 191-206.

KUO, J., McCOMB, A.J. and CAMBRIDGE, M.L. (1981). Ultrastructure of the seagrass rhizosphere. *New Phytol.* 89: 139-43.

KURIHARA, Y., TAKECHI, T. and SHIBATA, F. (1978). Relationship between bacteria and cilliate protozoa in the rumen of sheep fed on a purified diet. *J. Agric. Sci.*, Camb. 90: 373-381.

KYLIN, K. (1915). Biochemistry of sea algae. *Ztschr. Physiol. Chem.*, Bd. 94: 337-425.

LANGER, R.H.M. (1972). Growth of grasses and clovers. In "Pastures and pastoral plants". Langer, R.H.M. (Ed.). A.H. & A.W. Reed, Wellington. pp. 41-63.

LANGER, R.H.M. and HILL, G.D. (1991). Agricultural plants. Cambridge University Press, Cambridge. 344p.

LANGLANDS, J.P. and WHEELER, J.L. (1968). The dye-banding and tattooed patch procedures for estimating wool production and obtaining samples for the measurement of fibre diameter. *Aust. J. Exp. Agric. Anim. Husb.* 8: 265-269.

LANGMAN, M. (1988). Worm control in sheep, "the cereal zone". Fact Sheet., FS 26/88. Dept. Agric. South Australia. 4p.

LANGMAN, M., ASHTON, B., MORBEY, T. (1990). Health of sheep lotfed for maintenance. Fact Sheet., FS 5/90. Dept. Agric. South Australia. 5p.

LARKUM, A.W.D. and den HARTOG, C. (1989). Evolution and biogeography of seagrasses. In "Biology of seagrasses". Larkum, A.W.D., Mc Comb, A.J.M. and Shephered, S.A. (Eds.). Elsevier, The Netherlands. pp. 113-156.

LARKUM, A.W.D. and WEST, R.J. (1990). Long term changes of seagrass meadows in botany bay, Australia. *Aquat. Bot.* 37: 55-70.

LATHAM, M.J. (1979). Pre-treatment of barley straw with white-rot fungi to improve digestion in the rumen. In "Straw decay and its effect on disposal and utilisation". Grossbard, E. (Ed.). John Wiley and Sons, London. pp. 131-137.

LATHAM, M.J., BROOKER, B.E., PETTIPHER, G.L. and HARRIS, P.J. (1978). Adhesion of *Baccteroides succinogenes* in pure culture and in the presence of *Ruminoccus flavefaciens* to cell wall in leaves of perennial ryegrass (*Lolium perenne*). *Appl. Environ. Microbiol.* 35: 1166-1173.

LATHAM, M.J., HOBBS, D. G. (1979). Adhesion of rumen bacteria to alkali treated plant stems. *Ann. Rech. Vet.* 10: 244-245.

LATHAM, M.J., SHARPE, M.E. and SUTTON, J.D. (1971). The microbial flora of the rumen of cows fed hay and high cereal rations and its relationship to the rumen fermentation. *J. Appl. Bacteriol.* 34: 425-34.

LEATHERWOOD, J.M., MOCHZIC, R.D. and THOMAS, N.E. (1960). Some effects of a supplementary cellulose preparation on feed utilisation by ruminants. *J. Dairy Sci.* 43: 1460-1464.

LEEDLE, J.A.Z. and HESPELL, R.B. (1980). Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohyrate-utilizing subgroups in rumen bacterial populations. *Appl. Environ. Microbiol.* 39: 709-19.

LEEDLE, J.A.Z., BRASUHN, K. and HESPELL, R.B. (1986). Postprandial trends in estimated ruminal digesta polysaccharides and their relation to changes in bacterial groups and ruminal fluid characteristics. *J. Anim. Sci.* 62: 789-803.

LEEDLE, J.A.Z., BRYANT, M. P. and HESPELL, R.B. (1982). Diurnal variations in bacterial numbers and fluid parameters in ruminal contents of animals fed low - or high forage diets. *Appl. Environ. Microbiol.* 44: 402-12.

LEHMANN, F. (1895). Uber die Moglichkeit, Stroh Hoher Verdaulich zu Machen. (Cited by BODA 1990), Nonconventional feedstuffs in the nutrition of farm animals, Elsevier, Amsterdom, pp. 13-87.

LEIBHOLZ, J. (1983). Practical aspects of utilizing livestock manures as feeds for animals. In"The utilization of fibrous agricultural residues". Pearce, G.R. (Ed.). Australian Government Publishing Services, Canberra, 178p.

LEISOLA, M., ULEMER, D. and FIECHER A. (1983). Problem of oxygen transfer during degradation of lignin by *Phanerochaete chrysosporium. Eur. J. Appl. Microbiol. Biotechnol.* 17: 113.

LENG, R. A. (1974). Salient features of the digestion of pastures by ruminants and other herbivores. In "Chemistry and biochemistry of herbage". Bailey, R.W., and Bulter, G.W. (Eds.). . Academic press, New York, pp. 295.

LENG, R.A. (1985). Microbial interaction in the rumen. In "Ruminant physiology-Concepts and consequences". Baker, S.K. , Gawthorne, J. M. , Mackintosh, J. B. and Purser, D. B. (Eds.). University of Western Australia, Perth, pp. 161-173.

LENG, R.A. and NOLAN, J.V. (1984). Nitrogen metabolism in the rumen (Symposium: Protein nutritionof the lacting dairy cow). J. *Dairy Sci.* 67: 1072.

LENG, R.A., (1976). Factors influencing net protein production by the rumen microbiota. . In " Reviews in rural science". Sutherland, T.M.T., and Leng, R.A. (Eds.). Univ. New England, Armidale, N.S.W., pp. 85-91.

LESOING, G. and KLOPFENSTEIN, T.J (1981). Chemical treatment of wheat straw. *J. Anim. Sci.* 51: 263-269.

LESOING, G., RUSH, I., KLOPFENSTEIN, T.J., WARD, J. (1981). Wheat straw in growing cattle diets. *J. Anim. Sci.* 51: 257-262.

LEWIS, S.M., HOLZGRAEFE, D.P., BEGNE, L.L., FAHEY Jr. G.C., GOULD, J.M. and FANTA, G.F. (1987). Alkali-hydrogen peroxide treatments of crop residues to increase ruminal dry matter disappearance *in sacco. Anim. Feed Sci. Technol.* 17: 179-199.

LIENER, I.E. (1990). Naturally occurring toxic factors in animal feedstuffs. In "Feedstuff evaluation". Wiseman, J. and Cole, D.J.A. (Eds.). Butterworths. UK. pp. 377-393.

LINDBERG, J.E. (1985). Estimation of rumen degradability of feed protein with the *in sacco* technique and various *in vitro* methods: A review. *Acta Agric. Scand. Suppl.* 25: 64-97.

LINDBERG, J.E. and KNUTSSON, P.G. (1981). Effect of bag pore size on the loss of particular matter and on the degradation of cell wall fibre. *Agric and Environ.* 6: 171-182.

LIZAMA, L.C., MARION, J.E. and McDOWELL, L.R. (1988). Utilization of aquatic plants *Elodea canadensis* and *Hydrilla verticillata* in broiler chick diets. *Anim. Feed Sci. Technol.* 20: 155-161.

LOOSLI, J.K. and McDONALD, I.W. (1968). Non-Protein nitrogen in the nutrition of ruminants. FAO agricultural studies, No. 75, FAO, Rome, p. 94.

LOWE, S.E., THEODORU, M.K. and TRINCI, A.P.J. (1987). Cellulases and xylanase of an anaerobic rumen fungus grown on wheat straw, wheat straw holocellulose, cellulose and xylan. *Appl. Environ. Microbial.* 53: 1216-23.

LOWMAN, B.G. and KNIGHT, D.W. (1970). A note on the apparent digestibility of energy and protein in dried poultry excreta. *Anim. Prod.* 12: 525-528.

LOWTON, E.J., BELLANY, W.D., HUNGATE, R.T., BRYANT, M.P. and HALL, E. (1951). Some effects of high velocity electrons on wood. *Science*. 113: 380-382.

LUBBERDING, H.J., GIJZEN, H.J., GERHARDUS, M.J. and VOGELS, G.D. (1987). Fibre degradation and activities in an artifitial rumen system in the presence and the absence of rumen ciliates. In "Physiology of Ruminant Nutrition". Boda, K. (ed.). Slovak Acadamy of Science, Kosice. pp. 301-315.

LUND, A. (1974). Yeast and moulds in the bovine rumen. *J.Gen. Microbiol.* 81: 453-62.

LUNING, K. (1990). Seaweeds, their environment, biogeography, and ecophysiology. John Willy and Sons, Inc., New York. 483pp.

LUTHER, R. TRENKLE, A. and BURROUGHS, W. (1966). Influence of rumen protozoa on volatile fatty acid production and ration digestibility in lambs. J. *Anim. Sci.* 25: 1116-1122.

LYLE, R.R., JOHNSON,R.R., WILHITE, J.V. and BACKUS, W.R. (1981). Ruminal characteristics in steers as affected by adaptation from forage to all-concentrate diets. *J. Anim. Sci.* 53: 1383-1390.

LYNCH, L.J. , and MITCHIE, N.A. (1976). An instrument for the rapid automatic measurement of fibre fineness distribution. *Text. Res. J.* 46: 635-60.

MACKIE, R.I. and GILCHRIST, F.M.C. (1979). Changes in lactate-producing and lactate-utilizing bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high-concentrate diet. *Appl. Environ. Microbiol.* 38: 422-30.

MACKIE, R.I., GILCHRIST, F.M.C., ROBERTS, A.M., HANNAH, P.E. and SCHWARTZ, H.M. (1978). Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. *J. Agric.* 90: 241-54.

MACQUARIE LIBRARY (1990). The Macquarie Everyday Dictionary. Delbridge, A.; Bernard, J.; Blair, D.; Butler, S.; Peters, P.; Tardif, R. and Butler, J. (Eds). Macquarie University, NSW. Australia. p. 589.

MAKI, L.R. and FOSTER, E.M. (1957). Effect of roughage in the bovine ration on types of bacteria in the rumen. *J. Dairy Sci.* 40: 905-13.

MAKKAR, G.S., CHAUHAN, T.R., GILL, R.S., MALIK, N.S. and ICCHPONANI, J.S. (1980). Feeding value of wheat straw based poultry litter for buffalo heifers. *Indian J. Dairy Sci.* 33: 83-86

MALES, J.R., MUNSINGER, R.A. and JOHNSON, R.P. (1979). *In vitro* and *in vivo* ammonia release from " slow- release" urea supplements. *J. Anim. Sci.* 48: 887-892.

MANN, K.H. (1972). Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. II. Productivity of the seaweeds. *Mar. Biol.* 14: 199-209.

MANN, K.H. (1976). Decomposition of marine macrophytes. In "The role of terrestrial and aquatic organisms in decomposition processes". Anderson, J.M. and Macfadyen, A. (Eds). Blackwell Scientific, London, pp. 247-267.

MANN, K.H. (1982). Ecology of coastal waters. A system approach. Blackwell, Oxford. 322p.

MANN, K.H. (1988). Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystem. *Limnol. Oceanogr.* 33: 910-930.

MAPOON, L.K. (1980). Degradability of some high protein forages in the rumen. *Trop. Anim. Prod.* 5: 53-56.

MARGOLIN, S. (1930). Methods for the cultivation of cattle ciliates. *Biologi. Bull.* 59: 301-305.

MAYNARD, L.A. and LOOSLI, J.K. (1956). Animal nutrition. McGraw-Hill, Book Company. Inc. NY. 484p.

McALLISTER, T.A., CHENG,K.J., RODE, L.M. and BUCHANA-SMITH, J.G. (1990). Use of formaldehyde to regulate digestion of barley starch. *Can. J. Anim. Sci.* 70: 581-90.

McCASKEY, T.A. and ANTHONY, W.B. (1979). Human and animal health aspects of feeding livestock excreta. *J. Anim. Sci.* 48: 163-177.

McCOMB, A.J., CAMBRIDGE, M.L., KIRKMAN, H. and KUO, J. (1981). Biology of seagrasses. In "Biology of Australian plants". Pate, J.S. and McComb, A.J. (Eds.). The University of Western Australia Press, Nedlands. pp. 258-93.

McDONALD, C.L., NORRIS, R.T., SPEIJERS, E.J. and RIDINGS, H. (1990). Feeding behaviour of Merino wethers under conditions similar to lot-feeding before live export. *Aust. J. Exp. Agric.* 30: 343-348.

McDONALD, I.W. (1954). The extent of conversion of food protein to microbial protein in the rumen of sheep. *Biochem. J.* 56: 120-125.

McDONALD, I.W. and HALL, R.J. (1957). The conversion of casein into microbial protein in the rumen. *Biochem. J.* 67: 400-406.

McDONALD, P., EDWARDS R.A. and GREENHALGH J.F.D. (Ed.) (1988). Animal nutrition. Longman, New Yourk. 479pp.

McDOUGALL, E.I. (1948). Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochem. J.* 43: 99.

McMANUS, W.R. (1978). Alkali effects on agricultural wastes and their cell wall fraction. *Aust. J. Exp. Agric. Anim. Husb.* 18: 231-242.

McMANUS, W.R., MANTA, L., McFARLANE, J. D. and GRAY, A. C. (1972). The effect of diet supplements and gamma-irradiation on dissimilation of low-quality roughages by ruminants. III. Effects of feeding gamma-irradiated based diets of wheaten straw and rice straw to sheep. *J. Agric. Sci. Camb.* 79: 55-66.

McROY, C.P. and HELFFRICH, C. (1980). Applied aspects of seagrasses. In "Hand book of seagrass biology- An ecosystem approach". Phillips, C. and McRoy, C.P. (Eds.). Garland Publications, New York. pp. 297-342.

McSWEENEY, C.S., MACKIE, R.I. and WHITE, B.A. (1994). Transport and intracellular metabolism of major feed compounds by ruminal bacteria: The potential for metabolic manipulation. *J. Agric. Res.*, 45: 731-56.

MEESKE, R., MEISSNER, H.H. and PIENAAR, J.P. (1993). The upgrading of wheat straw by alkaline hydrogen peroxide treatment: The effect of NaOH and H_2O_2 on the site and extent of digestion in sheep. *Anim. Feed Sci. and Technol.* 40: 121-133.

MEHREZ, A.Z. and ØRSKOV, E.R. (1977). A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *J. Agric. Sci. Camb.* 88: 645-650.

MEHREZ, A.Z., ØRSKOV, E.R. and McDONALD, I. (1977). Rates of rumen fermentation in relation to ammonia concentration. *Br. J. Nutr.* 38: 437-443.

MERCHEN, N.R. (1988). Digestion, absorption and excretion in ruminants. In "The ruminant animal, digestive physiology and nutrition". Church, D.C. (Ed.).Prentice Hall, Eaglewood Cliffs. New Jersey. pp. 172-201.

METENS, D.R. (1977). Dietary fibre components: relationship to rate and extent of ruminal digestion. *Fed. Proc.* 36: 187-192.

MEYER, J.H., GASKILL, R.C., STOEWSAND, G.S. and WEIR., W.C. (1959). Influence of pelleting on the utilisation of alfalfa. *J. Anim. Sci.* 18: 336-346.

MICHAILOWSKI, T. and MUSZYNSKI, P., (1978). Diurnal variations in number of ciliate protozoa in the rumen of sheep fed once and twice daily. *J. Agric. Sci.* 90: 1-5.

MICHALET-DOREAU, B. and CERNEAU, P. (1991). Influence of foodstuff particle size on *in situ* degradation of nitrogen in the rumen. *Anim. Feed Sci. Technol.* 35: 69-81.

MICHALOWSKI, T. (1975). Effect of different diets on the diurnal concentrations of ciliate protozoa in the rumen of water buffalo. *J. Agric. Sci.* 85: 145-50.

MICHALOWSKI, T. (1977). Diurnal changes in concentration of rumen ciliates and in occurrence of dividing forms in water buffalo (*Bubalus bubalus*) fed once daily. *Appl. Environ. Microbiol.* 33: 802-4.

MICHALOWSKI, T. (1987). The volatile fatty acid production by ciliate protozoa in the rumen of sheep. *Acta protozoologi.* 26: 335-345.

MICHANECK, G. (1975). Seaweed resources of the ocean. FAO Fisheries Tech. Paper. 138, Rome, 82p.

MILLER, B.L., FAHEY, Jr., G.C., RINDSIG, R.B., BERGER, L.L. and BOTTJE, W.G. (1979). *In vitro* and *in vivo* evaluations of soy-bean residues ensiled with various additives. *J. Anim. Sci.* 49: 1545-1551.

MILLETT, M.A., BAKER, A.J., FEIST, W.C., MELLENBERGER, R.W. and SATTER, L.D. (1970). Modifying wood to increase its *in vitro* digestibility. *J. Anim. Sci.* 31: 781-785.

MINATO, H. and SUTO, T., (1978). Techniqe for fractionation of bacteria in rumen microbial ecosystem. II. Attachment of bacteria isolated from bovin rumen to cellulose powder *in vitro* and elution of bacteria attached therefrom. *J. Gen. Appl. Microbiol.* 24: 1-16.

MINSON D.J. and MILFORD R. (1967). The voluntary intake and digestibility of diets containing different proportions of legume and mature pangola grass. *Aust. J. exp. Agric. Anim. Husb.* 7: 546-551.

MINSON, D.J. (1963). The effect of pelleting and wafering on the feeding value of roughage. A review. *J. Br. Grassld. Soc.* 18: 39-49.

MINSON, D.J. (1980). Nutritional differences between tropical and temperate pastures. In "World Animal Science, B1, Grazing animals". Morley, F.H.W. (Ed.). Elsevier Sci. Pub. Company. pp. 143-157.

MINSON, D.J. (1981). Effects of chemical and physical composition of herbage eaten upon intake. In "Nutritional limits to animal production from pastures". Hacker, J.B. (Ed.). CAB (Commonwealth Agricultural Bureaux), Farnham, Royal, UK. pp. 167-182.

MINSON, D.J. (1987). Estimation of the nutritive value of forage. In "Temperate pastures, their production, use and management". Wheeler, J.L., *et al.* (Eds.). Australian Wool Corporation, CSIRO. Australia. pp. 415-422.

MINSON, D.J. (1990). Forage in ruminant nutrition. Academic Press, Inc. San Diego. USA. 483p.

MOE, P.W. (1981). Energy metabolism of dairy cattle. *J. Dairy Sci.* 64: 1120-1139.

MOIR, K.W., LAWS, L. and BLIGHT, G. (1975). The relative importance of the total cell wall and quantity of digested cell wall in the regulation of the voluntary intake of grass hays by sheep. *J. Agric. Sci. Camb.* 85: 39-43.

MOIR, R.J. and SOMERS, M. (1956). A factor influencing the protozoal population in sheep. *Nature* 178: 1472.

MONTGOMERY, M.J. and BAUMGARDT, B.R. (1965). Regulation of food intake in ruminants 1. Pelleted rations varying in energy concentration. *J. Dairy Sci.* 48: 569-574.

MOORE, J. (1990). Current lot-feeding industry in Australia. In "Lot-feeding and beef production", Postgraduate Committee in Veterinary Science., *Proc.* No. 137., University of Sydney, Sydney. pp. 323-326.

MOORE, J.E. (1969). Procedure for determining voluntary intake and nutrient digestibility of hay with sheep. In "Nutrition Research Techniques for Domestic and Wild Animals (Vol. I)". Lori, E.H. (Ed.). Animal Science Department, Utah State University, Logan, Utah, pp. 5101 (1) - 5101 (3).

MORAN, J.B., SATOTO, K.B. and DAWSON, J.E. (1983). The utilization of rice straw fed to Zebu cattle and swamp buffalo as influenced by alkali treatment and *Leucaena* supplementation. *Aust. J. Agric Res.*, 34:73-84.

MORBEY, A.S.C. and ASHTON, B.L. (1990). Lot-feeding of sheep on Eyre Peninsula during the 1988 drought. Tech. Report. No. 155. Dept. Agric. South Australia. 33p.

MORGAN, K.C., WRIGHT, J.L.C., and SIMPSON, F.J. (1980). Review of chemical constituents of the red alga *Palmaria palmata* (dulse). *Econ. Bot.* 34: 27-50.

MORLEY, F.H.W. (Ed.). (1981a). Grazing animals.(General preface to world animal science; Volume B1). Elsvier Amesterdam. pp. v-vi.

MORLEY, F.H.W.(Ed.). (1981b). Grazing animals.(Preface to volume B1; World animal science). Elsevier Scientific publishing Company,Amsterdam. pp. vii-viii.

MORRISON, F.B. (1957). Feeds and feeding, A handbook for the student and stockman. The Morrison Publishing Company, Ithaca, NY., USA. 1165p.

MOSI, A.K. and BUTTERWORTH, M.H. (1985). The voluntary intake and digestibility of combinations of cereal crop residues and legume hay for sheep. *Anim. Feed Sci. Tech.* 12: 241-251.

MOSS, A.R., GIVENS, D.I. and PHIPPS, R.H. (1992). Digestibility and energy value of combinations of forage mixtures. *Anim. Feed Sci. Tech.* 39: 151-17.

MOULD, F.L. (1988). Associative effects of feeds. In "World Animal Science, B4. Feed science". Ørskov, E.R. (Ed.). Elsevier Sci. Pub. Company. pp. 279-292.

MULLER, Z. and DREVJANY, L. (1967). Feeding and processing of deep litter for feeding purposes. Res. Inst. for biofactors. Prague, 49p

MULLER, Z., DREVJANY, L. and KOZEL, V. (1968). Influences of different materials used for poultry deep litter upon gains and feed conversion and upon final deep litter value as feed for cattle. *3rd Eur. Poult. Conf.*, Jerusaliem, Israel, Sept. pp. 8-13.

MULLER, Z.O. (1980). Feed from animal wastes: State of knowledge, FAO animal production and health, FAO, Rome. 190p.

MURPHY, M.R., DRONE, P.E., JR and WOODFORD, S.T. (1985). Factors stimulating migration of holotrich protozoa into the rumen. *Appl. Environ. Microbiol.* 49: 1329-31.

MUZTAR, A.J. , SLINGER, S.J. and BURTON, J.H. (1978). Chemical composition of aquatic macrophytes: iii. Mineral composition of fresh water macrophytes and their potential for mineral nutrient removal from lake water. *Can. J. Plant Science.* 58: 851-862.

MUZTAR, A.J. ,SLINGER, S.J. and BURTON, J.H. (1976). Nutritive value of aquatic plants for chicks. *Poult. Sci.* 55: 1917.

MYUNG, K.H. and KENNELLY, J. (1990). Effect of alkaline hydrogen peroxide treatment of rice straw on *in sacco* ruminal digestibility. *Asian- Aust. J. Anim. sci.* 3: 1-6.

NAKAMURA, K. and KANEGASAKI, S. (1969). Densities of ruminal protozoa of sheep established under different dietary conditions. *J. Dairy Sci.* 52: 250-5.

NAKASHIMA, Y. and ØRSKOV, E.R. (1992). Rumen degradation of straw: A. Effect of cellulase and ammonia treatment on different varieties of rice straw and their botanical fractions. *Anim. Prod.* 50: 309-317.

NATIONAL RESEARCH COUNCIL (1976). Making aquatic weeds useful: Some perspectives for developing countries. National Academy of Science, Washington, D.C. pp. 1-2

NAVARATNE, H.V.R.G., IBRAHIM, M.N.M. and SCHIERE, J.B. (1987). Validity of some laboratory techniques to predict *in vivo* organic matter digestibility of roughages. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 107-113.

NAYLOR, J. (1976). Production, trade and utilization of seaweeds and seaweed products. FAO technical paper No. 159. Rome, 186p.

NEILSON, M.J. and STONE, B.A. (1987). Chemical composition of fibrous feed for ruminants in relation to digestibility. In "*Proceeding of 4th Asian- Aust.ralian Assosiation of Animal. Production and Animal Scienc Congress.*". AAAP, Hamilton, New Zealand. pp. 63-66.

NELSON, H.W.,LEMON, J.M. (1942). Metabolic studies with algin and Gelatin. *U.S. Fish wild life sers.* 4: 1-5.

NEOG, B.N. and PATHAK, N.N. (1976). Voluntary intake and nutritive value of paddy straw-poultry litter silage. *Indian J. Nutr. Dietet.* 13: 413-415.

NEWELL, R.C. (1965). The role of detritus in the nutrition of two marine deposit feeders, the prosobranch *Hydrobia ulva* and the bivalue Macoma balthica. Proc. Zool. Soc. London. 144: 25-45.

NICOTRI, M.E. (1980). Factors involved in herbivore food performance. *J. Exp. Mar. Biol. Ecol.* 42: 13-26.

NIKOLIC, J.A. (1982). Some factors influencing the effect of alkali treatment on crop residues. *J. Agric. Sci. Camb.* 99: 115-122.

NIVEN, D.R. and ENTWISTLE, K.W. (1983). Supplementary feeding of sheep consuming mulga (*Acacia aneura*) with low levels of molasses and urea under field conditions. *Aust. Rangeland. J.* 5(2) :74.

NOCEK, J.E. (1985). Evaluation of specific variables affecting *in situ* estimates of ruminal dry matter and protein digestion. *J. Anim. Sci.* 60: 1347-1358.

NOCEK, J.E. (1988). *In situ* and other methods to estimate ruminal protein and energy digestibility: A review. *J. Dairy Sci.* 71: 2051-2069.

NOLAND, P.R., FROD, B.F. and RAY, M.L. (1955). The use of ground chicken litter as a source of nitrogen for gestating-lactating ewes and fattening steers. *J. Anim. Sci.* 14: 860-865.

NOUR, A.M., ABOU AKKADA, A.R., EL-SHAZLY, K., NAGA, M.A., BORHAMI, B.E. and ABAZA, M.A. (1979). Effect of increased levels of urea in the diet on ruminal protozoal counts in four ruminant species. *J. Anim. Sci.* 49: 1300-1305.

O'SHEA, J. and BALDWIN, A. (1986). Evaluation of the increase in feeding value of barley straw following mild treatment with sulphur dioxide. *Anim. Feed Sci. Technol.* 15: 13-19.

ODDY, V.H., ROBARDS, G.E. and LOW, S.G. (1983). Prediction of *in vivo* dry matter digestibility from the fibre and nitrogen content of a feed. In "Feed information and animal production". Commonwealth Agricultural Bureaux. UK. pp. 395-398.

ODUM, W.E. (1984). Dual-gradient concept of detritus transport and processing in estuaries. Bull. Mar. Sci. 35: 510-521.

ODUM, W.E., KIRK, P.W. and ZIEMAN, L.C. (1979). Non-protein nitrogen compounds associated with particles of vascular plant detritus. *Oikos* 32: 363-7.

OGDEN, J.C. (1976). some aspects of herbiver- plant relationship on Caribbean reefs and seagrass beds. *Aquatic Bot.* 2: 103-16.

OGDEN, J.C. (1980). Faunal relationships in Caribbean seagrass beds. In "Handbook of seagrass biology- An ecosystem approach". Phillips R.C. and McRoy, C.P. (Eds.). Garland Press, New York. pp. 173-98.

OGIMOTO, K. and IMAI, S. (1981). Atlas of rumen Microbiology. Japan Scientific Society Press. Tokyo, Japan, 311p.

OJI, U.I. and MOWAT, D.N. (1978). Nutritive value of steam-treated corn stover. *Can. J. Anim. Sci.* 58: 771-781.

OLDHAM, J.D. and EMMANS, G.C. (1990). Animal performance as the criterion for feed evaluation. In "Feedstuff evaluation". Wiseman, J. and Cole, D.J.A. (Eds.). Butterworths. UK. pp. 73-90.

OLOLADE, B.G., MOWAT, D.N. and WINCH, J. E. (1970). Effect of processing methods on the *in vitro* digestibility of sodium hydroxide treated roughages. *Can. J. Anim. Sci.* 50: 657-662.

OLTJEN, R.R., GUTIERREZ, J., LEHMANN, R.P. and DAVIS, R.E. (1966). Rumen chemical and microbial characteristics of steers fed a purified and a natural diet. *J. Anim. Sci.* 25: 521-525.

ORPIN, C.G. and LETCHER, A.J. (1978). Some factors controlling the attachment of the rumen holotrich protozoa *Isotricha intestinalis* and *Isotricha Prostoma* to plant particles *in vitro*. *J. Gen. Microbiol.* 106: 33-40.

ØRSKOV, E. R. (1982). Protein nutrition in ruminants. Academic Press, London, 175p.

ØRSKOV, E.R. and DeB HOVEL, F.D. (1978). Rumen digestion of hay (measured with dacron bags) by cattle given sugar cane or pangola hay. *Trop. Anim. Prod.* 3: 9-11.

ØRSKOV, E.R., DeB HOVEL, F.D. and MOULD, F. (1980). The use of the nylon bag technique for the evaluation of feedstuffs. *Trop Anim. Prod.* 5: 195-213.

ØRSKOV, E.R., REID, G.W. and KAY, M. (1988). Prediction of intake by cattle from degradation characteristics of roughages. *Anim. Prod.* 46: 29-34.

OWEN, E. (1976). Farm wastes; straw and other fibrous materials. In "Food production and consumption, the efficiency of human food chains and nutrient cycles". North-Holland. Amsterdam. pp. 299-318.

OWEN, E. (1978). Processing of roughages. In "Recent advances in animal nutrition". Butterworths, London. pp. 127-148.

OWENS, F.N. and Goetsch, A.L. (1988). Ruminal Fermentation. In "The ruminant animal digestive physiology and Nutrition. Churche D.C. (Ed.). Prentice Hall. Englewood Cliff, New Jersey. pp. 145-171.

OXFORD, A.E. (1955). The bacteriology and protozoology of ruminant digestion. *J. Sci. Agric.* 6: 413-418.

PADUANO, D.C., SLOCOMBE, R.F., HOLMES, J.H.G. and DIXON, R.M. (1990). Cow peas and navy beans as supplements for roughage diets for sheep. *Proc. Aust. Soc. Anim. Prod.* 18: 536.

PARIGI-BINI, R. (1969). Utilization of pure dried poultry litter (Toplan) in feeding sheep. Cited in *Nutr. Abstr. Rev.* 40: 3809 (1970).

PARTHASARTHY, M. and PRASAD, D.A. (1976). Effect of processing laying hens litter on the chemical composition, digestibility and rumen metabolic process by cattle. *Indian J. Anim. Sci.* 46: 427-430.

PATERSON, J.A., STOCK, R. and KLOPFENSTEIN, T.J. (1980). Calcium hydroxide treatment. Nebraska Beef Cattle Report EC 80-218, P.21.

PEARCE, G.R. and MOIR, R.J. (1964). Rumination in sheep. 1. The influence of rumination and grinding upon the passage and digestion of food. *Aust. J. Agric. Res.* 15: 635-644.

PEARSON, H.A. (1965). Rumen organisms in white-tailed deer from south Texas. *J. Wildl. Mgmt*. 29: 493-6.

PEARSON, H.A. (1969). Rumen microbial ecology in mule deer. *Appl. Microbiol.* 17: 819-24.

PIDGEN, W.J. and HEANEY, D.P. (1969). Lignocellulose in ruminant nutrition. *Adv. Chem. Ser.* 95: 245-261.

PIENAAR, J.P., ROUX, C.Z. and CRONJE, P.B. (1989). Comparison of *in vivo* and *in sacco* methods to estimate mean retention time of fermentable organic matter in the rumen. *S. Afr. Anim. Sci.* 19: 71-75.

PIRC H. (1989). Seasonal changes in soluble carbohydrates, starch and energy content in Mediterranean seagrasses. *Mar. Ecol.* 10(2): 97-105.

PIRC, H. (1985). Growth dynamics in *Posidonia oceanica.* I. Seasonal changes of soluble carbohydrates, starch, free amino acids, nitrogen and organic anions in different parts of plant. *Mar. Ecol.* 6: 141-65.

PIRIE, N.W. (1980). Water weed uses. *Water Spectrum.* 12: 43-49.

PLAYNE, M.J., KHUMNUALTHONG, W. and ECHEVARRIA, M.G. (1978). Factors affecting the digestion of oesophageal fistula samples and hay samples in nylon bags in the rumen of cattle. *J. Agric. Sci. Camb.* 90: 193-204.

POLLARD, P.C. and KOGURE, K. (1993). Bacterial decomposition of detritus in a tropical seagrass (*Syringodium isoetifolium*) ecosystem. measured with [methyl-3 H] thymidine. *Aust. J. Mar. Freshwater Res.* 44: 155-72.

POMEROY, L.R. (1980). Detritus and its role as a food source. In"Fundamentals of aquatic ecosystems. Barnes, R.S. and Mann, K.H. (Eds.). Blackwell, Oxford, England. pp. 84-102.

POTTER, E.L. and DEHORITY, B.A. (1973). Effects of changes in feed level, starvation, and level of feed after starvation upon the concentration of rumen protozoa in the bovin. *Appl. Microbiol.* 26: 692-8.

PRATLEY, J.E. (1991). Pasture management. In "Australian sheep and wool hand book". Cottle, D.J. (Ed.). Intaka press, Melbourne. pp. 267-285.

PRICE, I.R. (1980). Plants of the marine environment. In " Marine botany an australian perspective". Clayton, M.N. and King, R.J. (Eds.). Longman Cheshire. 307p.

PRINS, R.A. (1977). Biochemical activities of gut micro-organisms. In "Microbial ecology of the gut". Clarke, R.T.J. and Bauchop, T. (Ed.). Academic Press, New York, pp. 73-183.

PRINS, R.A. and VAN DEN VORSTENBOSCH, C.J. (1975). Interrelationship between rumen microorganism. *Misc. Pap.* Landbouwhogesch. Wageningen, The Netherlands. 10: 15-24.

PRITCHARD, D.A., STOCKS, D.L., O'SULLIVAN, B.M., MARTIN, P.R., HURWOOD, I.S. and O'ROUKE, P.K. (1988). The effect of Poly-Ethylene Glycol (PEG) on wool growth and live weight of sheep consuming a mulga (*Acacia aneura*) diet. *Proc. Aust. Soc. Anim. Prod.* 17: 290-293.

PRITCHARD, G. I., PIDGEN, W.J. and MINSON, D.J. (1962). Effect of gamma irradiation on the utilisation of wheat straw by rumen microorganisms. *Can. J. Anim. Sci.* 42: 215-217.

PROMMA, S. (1988). Urea treatment of roughages: A review of present technology and adoption. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 27-35.

PROMMA, S., TUIKUMPEE, S., RATNAVANIJA, A., VIDHYAKORN, N. and FROEMERT, R.W. (1985). The effects of urea-treated rice straw on growth and milk production of crossbred Holstein Friesian dairy cattle. In "The utilisation of fibrous agricultural residues as animal feeds". Doyle, P.T. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 88-93.

PUNIA, B.S., LEIBHOLZ, J. and FAICHNEY, G.J. (1987). The role of rumen protozoa in the utilization of paspalum (*Paspalum dilatum)* hay by cattle. *Brit. J. nutri.* 57: 395-406.

PURSER, D.B. (1961). A diurnal cycle for holotrich protozoa of the rumen. *Nature* 190: 831-2.

PURSER, D.B. and MOIR, R.J. (1959). Ruminal flora studies in the sheep. IX. The effect of pH on the ciliate population of the rumen *in vivo*. *Aust. J. Agric. Res.* 10: 555-64.

PURSER, D.B. and MOIR, R.J. (1966). Dietary effect upon concentration of protozoa in the rumen. *J. Anim. Sci.* 25: 668-674.

QUIN, J.I., VANDER WATH, J.G. and MYBURGH, S. (1938). Studies in the alimentary tract of Merino sheep in South Africa. 4. Description of experimental techniques. *J. Vet. Sci. Anim. Ind.* 11: 341.

RAHAL, M.S. and NAIK, D.G.(1976). Improvement in the nutritive value of paddy straw by supplementation. In "Improved utilisation of agricultural waste materials and industrial by-products as livestock feed (research progress report (1975-1976)". Pant G.B. (Ed.). University Press, Pantnagar. p. 52.

RALSTON, A.T., CHURCH, D.C. and OLDFIELD, J.F. (1962). Effect of enzymes on digestibility of low-quality roughage. *J. Anim. Sci.* 21: 306-308.

RAMASAMY, K. and VERACHTERT, H. (1979). Fermentation of wheat straw by *Pseudomonas* sp. In "Straw decay and its effect on disposal and utilisation". Grassbard, E. (Ed.). John Wiley & Sons. London. pp. 155-163.

RANDALL, J.E. (1965). Grazing effect on seagrasses for herbivorous reef fishes in the West Indies. *Ecology*. 46: 255-60.

RANDALL, J.E. (1967). Food habits of reef fishes of the West Indies. *Stud. Trop. Oceanogr.* 5: 665-847.

RANDELE, P.F. (1972). A comparison of the digestibility of two complete ration containing either raw or alkali-treated sugarcane bagasse. *J. Agric. Univ. P.R.* 52: 18-25.

RANGNEKAR, D.V., BADVE, V.C., KHARAT, S.T., SOBALE, B.N. and JOSHI, A.L. (1982). Effect of high-pressure steam treatment on chemical composition and digestibility *in vitro* of roughages. *Anim. Feed Sci. Technol.* 7: 61-70.

RANSOM, K. (1991). Dryland lucerne - An under utilised resource. *Proc. 32nd Annu. Conf. Grasslands Soc.*, Victoria 80-3.

RAO, B.N., RANJHAN, S.K. and KRISHNAMOHAN, D.V.G. (1977). Effect of feeding poultry droppings-maize silage on growth rate and nutrient utilization in crossbred calves. *Indian J. Anim. Sci.* 67: 613-616.

REHN, B. (1988). Overcoming the feedlot phobia. *Stock Journal, Adelaide, Aug. 18*, p. 17.

REICHL, J. (1961). Relationship of the occurence of protozoa in the sheep rumen to feed utilization. In "*Proceedings of the First International Conference on Protozool .Prague*". pp. 537-542.

REID, J. and SMITH, H.C. (1919). An investigation of the 'marine fibre' of *Posidonia australis*. *Comm. Aust. Inst. Sci. Ind. Bull.* 14: 1-60.

REXEN, F. and THOMSON, K.V. (1976). The effect on digestibility of a new technique for alkali treatment of straw. *Anim. Feed Sci. Technol.* 1: 73-83.

REYNOLDS, P. J. and LINDAHL, I. L. (1960). Effect of pelleting on the digestibility of hay by sheep. *J. Anim. Sci.* 19: 873-880.

RODDA, Q. (1992). Flexibility in management - feedlotting. *Pasture Symposium,* No. 5. The University of Adelaide. pp. 46-48.

RONALD, M.T. and PETER J.W. (1982). Use of congo red - polysaccharide intractions in enumeration and characterization of cellulolytic bacteria from the bovine rumen, *Appl. Environ. Microbiol.* April: 777-780.

ROUNDS, W., KLOPFENSTEIN, T.J., WALLER, J. and MESSERSMITH, T. (1976). Influence of alkali treatments of corn cobs on *in vitro* dry matter disappearance and lamb performance. *J. Anim. Sci.* 43: 478-482.

ROWE, J.B., DAVIES, A. and BROOME, A.W.J. (1985). Quantitative effects of defaunation on rumen fermentation and digestion in sheep. *Brit. J. Nutr.* 54: 105-119.

ROXAS, D.B., LAPITAN, R.M., ROXAS, E., del BaRRIO, A.N., MOMONGAN, V.G. and RANJHAN, S.K. (1987). Effects of soaking rice straw on growth performance of Philippine Carabaos. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 173-175.

RUSSELL, J. B. (1984). Factors influencing completion and composition of the rumen bacterial flora. In "Herbivore nutrition in the subtropics and tropics". Gilchrist, F. M. C. and Mackie, R. I. (Eds.). The science press, South Africa. pp. 313-345

RUSSELL, J.B, SHARP, W.M. and BALDWIN R.L. (1979). The effect of pH on maximal bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. *J. Anim. Sci.* 48: 251-255.

RUSSELL, J.B. and DOMBROWSKI, D.B. (1980). Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. *Appl. Environ. Microbiol.* 39: 604-610.

RUSSLE, A.J.F., DONEY, J.M., GUNN, R.G. (1969). Subjective assessment of body fat in live sheep. *J. Agric. Sci. Camb.* 72: 451-454.

SAHNOUNE, S., BESLE, J.M., CHENOST, M., JOUANY, J.P. and COMBES, D. (1991). Treatment of straw with urea. 1. Ureolysis in a low water medium. *Anim. Feed Sci. Technol.* 34: 75-93.

SAND-JENSEN, K. (1975). Biomass, net production and growth dynamics in an eelgrass (*Zostera marina L.*) population in Vellurup Vig., Denmark. *Ophelia.* 14: 185-201.

SATTER, L.D. and SLYTER, L.L., (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro. Br. J. Nutr.* 32: 199-208.

SAVVANT, D., CHAPOUTOT, P. and LAPIERRE, O. (1983). A general model of diet least cost formulation for lactating and growing cattle. In "Feed information and animal production". Robards, G.E. and Packham, R.G. (Eds). Commonwealth Agricultural Bureaux, UK. pp. 275-382.

SAWARDEKER J.S., SLONEKKER J.H. and JEANES A. (1965). Quantitative determination of monosaccharides as their alditol acetates by gas liquid choromatography. *Anal. Chem.* 37: 1602.

SCA (Standing Committee on Agriculture). (1990). Feeding standard for Australian livestock. Ruminants. CSIRO. Australia. 266p.

SCHEIFINGER, C.C. and WOLLIN, M.J. (1973). Propionate formation from cellulose and soluble sugars by combined cultures of *Bacteroides succinogenes* and *Selenomonas ruminantium. Appl. Microbiol.* 26: 789-795.

SCHIERE, J.B., IBRAHIM, M.N.M., SEWALT, V.J.H. and ZEMMELINK, G. (1988). Effect of urea-molasses lick block supplementation on intake and digestibility of rice straw fed to growing animals. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 205-212.

SCHNEIDER, B.H. and FLATT, W.P. (1975). The evaluation of feeds through digestibility experiments. The University of Georgia Press, Athens. USA. 423p.

SCHOPF, J.J.M., FISHER, J.B. and SMITH, C.A.F. (1978). Is the marine latidudinal diversity gradient merely another example of the species area curve? In "Marine organism". Battaghia, B. and Beardmore, J.A. (Eds.). Plenum Press, New York. pp. 365-86.

SCHWARTZ, C.C, NAGY, J.G. and STREETER, C.L., (1973). Pesticide effect on rumen microbial function. *J. Anim. Sci.* 37: 821-826.

SCULTHORPE, C.D. (1967). The biology of aquatic vascular plants. Edward Arnold, London. 610pp.

SELVENDRAN, R.R., MARCH, J.F. and RING, S.G. (1979). Determination of aldoses and uronic acid content of vegetable fibre. *Anal. Biochem.* 96: 282-292.

SEOANE,J.R., COTE, M. and VISSER, S.A. (1982). The relationship between voluntary intake and the physical properties of forages. *Can. J. Anim. Sci.* 62: 473-480.

SETÄLÄ, J. (1985). Aspects on the determination of ruminal feed protein degradation and the quality of the undegradable feed protein *in sacco. Acta Agric. Scand. Suppl.* 25: 98-102.

SHAW, F.D. and THORNTON, R.F. (1974). Technical notes, linear programming techniques for the formulation of least cost feedlot ration. *J. Aust. Inst. Agric. Sci.* Dec., 1974: 295-298.

SHEEHY, E.J. (1955). Animal Nutrition. MacMillan & Co. Ltd. London. 732p.

SHEEHY, E.J., BROPHY, J., DILLON, J. and O'MUINEACHIN, P. (1942). *Econ. Proc. R. Dublin Soc.* 3: 150.

SIEBERT, B.D. (1974). The treatment of a tropical roughages with alkali, nitrogen and sulphur in relation to the nutritional limitations of pastures in northern Australia. *Proc. Aust. Soc. Anim. Prod.* 10: 86-90.

SINGH, M. and JACKSON, M.G. (1971). The effects of different levels of sodium hydroxide spray treatment of wheat straw consumption and digestibility by cattle. *J. Agric. Sci. Camb.* 77: 5-10.

SLYTER, L.L. (1976). Influence of acidosis on rumen function. *J. Anim. Sci.* 43: 910-929.

SLYTER, L.L., KERN, D.L., WEAVER, J.M., OLTJEN, R.R. and WILSON, R.L. (1971). Influence of starch and nitrogen sources on ruminal microorganisms of steers fed high fibre purified diets. *J. Nutri.* 101: 847-853.

SMITH, L.W. (1973). Nutritive evaluation of animal manures. Sympossium: processing agricultural and municipal wastes. George, E. (Ed.). Avi. Publ. Co., Westport, CT. 123p.

SMITH, L.W. and WHEELER, W.E. (1979). Nutritional and economic value of animal excreta. *J. Anim. Sci.* 48: 144-156.

SMITH, R.H., (1979). Synthesis of microbial nitrogen compounds in the rumen and their subsequent digestion. *J. Anim. Sci.* 49: 1604-1614.

SMITH, T. and BROSTER, W.H. (1977). The use of poor-quality fibrous source of energy by young cattle. *Wld. Revi. Anim. Prod.* 13(1): 49-58.

SMITH, W.R., YU, I. and HUNGATE, R.E. (1973). Factors affecting cellulolysis by *Ruminococcus albus*. *J. Bacteriol*. 114: 729-737.

SNEDECOR, G.W. and COCHRAN, W.G. (1971). Statistical Methods. The Iowa State University Press, Ames, Iowa, USA. 593p.

SOLOMON, R., MIRON, J., RUBINSTEIN, A. and BEN-GHEDALIA, D. (1992). Ozone-treated cotton stalks as a component of a ration for growing lambs. *Anim. Feed Sci. Technol.* 37: 185-192.

SOUTH AUSTRALIA DEPARTMENT of AGRICULTURE. (1991). Agriculture in South Australia: a profile. Dep. Agric. South Australia. Adelaide. 23p.

SPEDDING, C.R. (1971). Grassland Ecology. Claredon Press, Oxford. 221p.

SQUELLA, F. (1992). The ecological significance of seed size in Mediterranean annual pasture legumes. Ph. D. Thesis, The University of Adelaide, 466p.

SQUIRES, V.R. (1981) Livestock management in the arid zone. Inkata Press. Melbourne. pp. 3-13.

SQUIRES, V.R. (1991). Extensive grazing systems. In "Australian Sheep and Wool Handbook. Cottle, D.J. (Ed.). Inkata Press. Melbourne. pp. 369-379.

STEPHENS, E.L., EASLEY, J.F. , SHIRLEY, R.L. and HENTGES, J.F., Jr. (1973). availability of nutrient mineral elements and potential toxicants in aquatic plant diets fed to steers, *soil crop soc. Fla.* 32: 30-32.

STEPHENSON, W.A. (1974). Seaweed in agriculture and horticulture, 3rd ed., Rateaver, california. 241pp.

STERN, M.D. and HINKSON, R.S. (1974). Effect of defaunation and faunation on intraruminal factors. *J. Anim. sci.* 39: 253.

STERN, M.D. and HOOVER, W.H. (1979). Methods for determining and factors affecting rumen microbial protein synthesis: a review. *J. Anim. Sci.* 49: 1590-1603.

STEWART, C.S. (1977). Factors affecting the cellulolytic activity of rumen contents. *Appl. Environ. Microbiol.*. 33: 497-502.

SUBERKROPP, K.F., GODSHALK, G.L., KLUG, M.J. (1976). Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology*. 57: 720.

SUNDSTØL, F. (1984). Ammonia treatment of straw: Methods for treatment and feeding experience in Norway. *Anim. Feed. Sci. Technol.* 10: 173-187.

SUNDSTØL, F. (1988). Improving of poor-quality forages and roughages. In "World Animal Science, B4. Feed science". Ørskov, E.R. (Ed.). Elsevier, Oxford, pp. 257-277.

SUNDSTØL, F. SAID, A.N. and ARNASON, J. (1979). Factors influencing the effect of chemical treatment on the nutritive value of straw. *Acta Agric. Scand.* 29: 179-190.

SUNDSTØL, F., COXWORTH, E. and MOWAT, D.N. (1978). Improving the nutritive value of straw and other low-quality roughages by treatment with ammonia. *World Anim. Rev.*. 26: 13-21.

SUNDSTØL, F., SAID, A.N. and ARNASON, J. (1979). Factors influencing the effect of chemical treatment on the nutritive value of straw. *Acta Agric. Scandinavia.* 29: 179-90.

SURIYAJANTRATONG, W. and WILAIPON, B. (1985). Supplementing rice straw with Verano stylo (*Stylosanthes hamata cv.* Verano) for native cattle. In "The utilization of fibrous agricultural residues as animal feeds". Doyle, P.T. (Ed.). International development program of Australian Universities and Colleges Ltd, (IDP), Canberra. pp. 149-153.

SUSMEL, P., STEFANO, B., MILLS, C.R. and SPANGHERO, M. (1990). Rumen degradability of organic matter, nitrogen and fibre fractions in forages. *Anim. Prod.* 51: 515- 526.

SUTTON, J.D. (1979). Carbohydrate fermentation in the rumen: variations on a theme. *Proc. Nutr. Soc.* 38: 275-281.

TAGARI, H., LEVY, D., HOLZER, Z. and IAN, D. (1976). Poultry litter for intensive beef production. *Anim. Prod.* 23: 317-320.

TAKECHI, T., SHIBATA, Y. (1978). Microbiological and chemical characteristics of the rumen ingesta in sheep fed on purified diets. *Japn. J. Ecol.* 28: 85-96.

TALMADGE, K.W., KEEGSTRA, K., BAUER, W.D. and ALBERSHEEIM, P. (1973). The structure of plant cell walls. *Plant Physiol.* 51: 158.

TAMMINGA, S. (1978). Measurement of microbial protein synthesis in the rumen. In "Ruminant digestion and feed evaluation". Osbourn, D.F., Beever,D.E and Thomson, D.J. (Eds.). A.R.C., London. pp. 5.1-5.13.

TANNENBAUM, S.R. and PACE, G.W. (1976). Food from waste: an over-view. In Food from waste. ASP Ltd., London. pp. 8-22.

TARKOW, H. and FIEST, W.C. (1969). Cellulases and their applications. In "American Chemical Society". Gould, R.F. (Ed.). Series 95. Washington, 211p..

TARO, V.A. (1981). Studies on the utilization of poultry litter as a feed in the ration of ruminants. Ph.D. Thesis, Kurukshetra, Haryana, India, 239p.

THEATHER, R.M., MAHADEVAN, S., ERFLE, J.D. and SAUER, F.D. (1984). Negative correlation between protozoal and bacterial levels in rumen samples and its relation to the determination of dietary effects on the rumen microbial population. *Appl. Environ. Microbiol.* 47: 566-70.

THOMAS, C. and CHAMBERLAIN, D.G. (1990). Evaluation and prediction of the nutritive value of pastures and forages. In "Feedstuff evaluation". Wiseman, J. and Cole, D.J.A. (Eds). Butterworths. UK. pp. 319-336.

THOMAS, C. and THOMAS, P.C. (1985). Factors affecting the nutritive value of grass silage. In: "Recent advances in animal nutrition". Haresign, W. and Cole, D.J.A. (Ed.). Butterworths, London. pp. 223-256.

THOMAS, I. R. (1985). Livestock- sheep, cattle, goats and deer. In "A manual of Australia agriculture". Reid, R. L. (Ed.). William Heinemann, Melbourne. pp. 385-454.

THOMSON, D.J., BEEVER, D.E., LATHERN, M.J., SHARPE, M.E. and TERRY, R.A. (1978). The effect of inclusion of mineral salts in the diet on dilution rate, the pattern of rumen fermentation and the composition of the rumen microflora. *J. Agric. Sci., Camb.* 91: 1-7.

THOMSON, D.J. (1972). Physical form of the diet in relation to rumen fermentation. *Proc. Nutr. Soc.* 31: 127-134.

THOMSON, I.J.M. and POOLE, N.J. (1979). Improving the nutritional value of straw. In "Straw decay and its effect on disposal and utilisation". Grassbard, E. (Ed.). John Wiley & Sons. London. p.261.

THORLEY, C.M., SHARPE, M.E. and BRYANT, M.P. (1968). Modification of the rumen bacterial flora by feeding cattle ground and pelleted roughage as determined with culture media with and without rumen fluid. *J. Dairy Sci.* 51: 1811-16.

TILLEY, J.M.A., and TERRY, R.A. (1963). A two-stage technique for the *in vitro* digestion of forage. *J. Br. Grassld. Soc.* 18: 104-111.

TINNIMIT, P. (1978). Dried poultry waste as an animal feed. Cited from Nutr. Abstr. Rev. 51(B): 2985.

TINNIMIT, P., YU, Y., McGREFFY, K. and THOMAS, J.W. (1972). Dried animal waste as a protein supplement for sheep. *J. Anim. Sci.* 35: 431-435.

TUCKER, R.W., WATTS, P.J., LOTT, S.L. and JUKES, P. (1991). Lot-feeding in Australia, a survey of the Australian lot-feeding industry. Qld. Dept. Prim. Indus. 143p.

TYLER, C. (1966). Animal Nutrition. Chapman and Hall. London. UK. 253p.

UDEN, P. and VAN SOEST, P.J. (1984). Investigation of the *in situ* bag technique and a comparison of the fermentation in heifers, sheep, ponies and rabbits. *J. Anim. Sci.* 58: 213-221.

UDEN,P., PARRA, R. and VAN SOEST P.J. (1974). Factors influencing reliability of the nylon bag technique. *J. Dairy Sci.* 57: 622 A.

USHIDA, K. and JOUARY, J.-P. (1985). Effect of protozoa on rumen protein degradation in sheep. *Reproducti. Nutri. Develop.* 25: 1075-1081.

USHIDA, K., JOUANY, J.-P. and THIVEND, P. (1986). Role of protozoa in nitrogen digestion in sheep given two isonitrogenous diets. *Brit. J. Nutr.* 56: 407-419.

VALDEZ, R.E., ALAVAREZ, F.J., FERREIRO, H.M., GUERRA, F., LOPEZ, J., PRIEGO, A., BLACKBURN, T.H., LENG, R.A. and PRESTON, T.R. (1977). Rumen function in cattle given sugar cane. *Trop. Anim. Produc.* 32: 260-272.

VALIZADEH, R. (1994). Summer nutrition of sheep based on residues of annual crops and medic pastures. Ph.D. Thesis, The University of Adelaide, Australia. 291p.

VAN der HONING, Y. and STEG, A. (1990). Comparison of energy evaluation systems of feeds for ruminants. In "Feedstuff evaluation". Wiseman, J. and Cole, D.J.A. (Eds.). Butterworths. UK. pp. 1-19.

VAN der KOELEN, C.J., GOEDHART, P.W., VAN VUUREN, A.M. and SAVOINI, G. (1992). Sources of variation of the *in situ* nylon bag technique. *Anim. Feed Sci. Technol.* 38: 35-42.

VAN der WATH, J.G. and MYBURG, S.J. (1941). Studies on the alimentary tract of merino sheep in South Africa. VI. The role of infusoria in ruminal digestion with some remarks on ruminal bacteria. *Onderspoort J. Veter. Sci. Anim. Indust.* 17: 61-88.

VAN HOVEN, W. (1978). Development and seasonal changes in the rumen protozoan population in young blesbok (*Damaliscus dorcas phillipsi* Harper 1939). *S. Afr. J. Wildl. Res.* 8: 127-30.

VAN KEULEN, J. and YOUNG, B.A. (1977). Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44: 282-287.

VAN KEUREN, R.W. and HEINEMANN, W.W. (1962). Study of a nylon bag technique for *in vivo* estimation of forage digestibility. *J. Anim. Sci.* 21: 340-345.

VAN NEVEL, C.J. and DEMEYER, D.I. (1977a). Determination of rumen microbial growth *in vitro* from P- labelled phosphate incorporation. *Br. J. Nutr.* 38: 101-114.

VAN NEVEL, C.J., and DEMEYER, D.I. (1988). Mainipulation of rumen fermentation. In "The Rumen Microbial Ecosystem". Hobson, P.N. (Ed.). Elsevier Applied Science, London, PP. 387-443.

VAN NIEKERK, A.I., GREENHALGH, J.F.D. and REID, G.W. (1973). Importance of palatability in determining the feed intake of sheep offered chopped and pelleted hay. *Br. J. Nutr.* 30: 95-105.

VAN SOEST, P.J. (1965). Symposium on factors influencing the voluntary intake of herbage by ruminants. Voluntary intake in relation to chemical composition and digestibility. *J. Anim. Sci.* 24: 834-843.

VAN SOEST, P.J. (1967). Development of a comprehensive system of feed analysis and its application to forages. *J. Anim. Sci.* 26: 119-128.

VAN SOEST, P.J. (1969). Newer knowledge on the composition and methods of analysis of feedingstuffs . In "Nutrition of animals of agricultural importance, part 1: The science of nutrition of farm livestock. Cuthbertson, D. (Ed.). Pergamon Press. UK. pp. 38-58.

VAN SOEST, P.J. (1977). Plant fibre and its role in herbivore nutrition. *Cornell Vet.* 67: 307-326.

VAN SOEST, P.J. (1981). Limiting factors in plant residues of low biodegradability. *Agric. Environ.* 6: 135-143.

VAN SOEST, P.J. (1982). Nutritional ecology of the ruminant. O and B Books, Corvallis, Oregon. 374 pp.

VAN SOEST, P.J. and WINE, R. H. (1967). Use of detergents in the analysis of fibrous feed: 4. The determination of plant cell- wall constituents. *J. Assoc. Offic. Anal. Chem.* 50: 50-55.

VANCE, R.D., PRESTON, R.L., KLOSTERMAN, E.W. and CAHILL, V.R. (1972). Utilization of whole shelled and crimped corn grain with varying proportions of corn silage by growing-fishining steers. *J. Anim. Sci.* 35: 598-605.

VEARASLIP, T. (1981). Digestibility of rice straw rations supplemented with *Leucaena leucocephala* and *Gliricidia maculata.*. *Thai J. Agric. Sci.* 14: 259-264.

VEIRA, D.M. (1986). The role of ciliate protozoa in nutrition of the ruminant. *J. Anim. Sci.* 63: 1547-60.

VEIRA, D.M., IVAN, M. and JUI. P.Y. (1983). Rumen ciliate protozoa: effect on digestion in the stomach of sheep. *J. Dairy Sci.* 66: 1015-1022.

VERMA, M.L. (1983). Practical aspects of treatment of crop residues. In "The utilisation of fibrous agricultural residues". Pearce, G.R. (Ed.). Australian Development Assistance Bureau, Canberra, Australia. pp. 85-99.

VERMA, M.L., AGARWAL, I.S., JAISWAL, R.S. and SINGH, R. (1982). Effect of chemical treatment of crop residues on animal performance. In "Maximum livestock production from minimum land". Preston, T.R., Davis, C.H., Dolberg, F., Mozammel, H. and Saadullah, M. (Eds.). pp. 135-140. (Cited by Doyle *et al.* 1986).

VIK-MO, L. and LINDBERG, J.E. (1985) *In sacco* degradability of protein (N) and dry matter in samples of individual feeds or combinations tested with diets medium or high in protein. *Acta Agric. Scand.* 35: 117-128.

VON KEYSERLINGK, M.A.G. and MATHISON, G.W. (1989). Use of the *in situ* technique and passage rate constant predicting voluntary intake and apparent digestibility forages by steers. *Can. J. Anim. Sci.* 69: 973-987.

WAISS, Jr., A.C., GUGGOLZ, J., KOHLER, G.O., WALKER, Jr., H.G. and GARRETT, W.N. (1972). Improving digestibility of straws for ruminant feed by aqueous ammonia. *J. Anim. Sci.* 35: 109-112.

WALKER, D.I. and McCOMB, A.J. (1985). Decomposition of leaves from *Amphibolis anarctica* and *Posidonia australis*, the major seagrass species of Shark Bay, Western Australia. *Bot. Mar.* 28: 407-13.

WALKER, D.I. and PRINCE, R.I.T. (1987). Distribution and biogeography of seagrass species on the northwest coast of Australia. *Aquat. Bot.* 29: 19-32.

WALKER, D.J. and MONK, P.R., (1971). Fate of carbon passing through the glucose pool of rumen digesta. *Appl. Microbiol.* 22: 741-747.

WALLACE, J.D., RALEIGH, R.J. and SOWYER, W.A. (1961). Utilisation of chopped, wafered and pelleted native meadow hay by weaned Hereford calves. *J. Anim. Sci.* 20: 778-781.

WALLACE, R.J. and McPHERSON, C.A. (1987). Factors affecting the rate of breakdown of bacterial protein in rumen fluid. *Brit. J. Nutri.* 58: 313-323.

WALLER, J.C. and KLOPFENSTEIN, T.J. (1975). Hydroxides for treating crop residues. *J. Anim. Sci.* 41: 424 A.

WALSH, F. (1974). Cheap feed - clutching at straw. *Dairy Farmer.* May: 29.

WANAPAT, M. (1987). Effect of concentration of urea, addition of salt and form of urea-treated rice straw on intake and digestibility. In "Ruminant feeding systems utilising fibrous agricultural residues". Dixon, R.M. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 177-179.

WANAPAT, M., SUNDSTØL, F. and GARMO, T.H. (1985). Comparison of different alkali treatments applications to barley straw. In "The utilisation of fibrous agricultural residues as animal feeds". Doyle, P.T. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 103-109.

WARNER, A.C.I. (1962a). Enumeration of rumen microorganisms. *J. Gen. Microbiol.* 8: 119-128.

WARNER, A.C.I. (1962b). Some factors influencing the rumen microbial population. *J Gen. Microbiol.* 28: 129-148.

WARNER, A.C.I. (1966a). Diurnal changes in the concentrations of microorganisms in the rumen of sheep fed limited diets once daily. *J. Gen. Microbiol.* 45: 213-35.

WARNER, A.C.I. (1966b). Periodic changes in the concentrations of microorganisms in the rumen of a sheep fed a limited ration every three hours. *J. Gen. Microbiol.* 45: 237-42.

WARNER, A.C.I. (1966c). Diurnal changes in the concentrations of microorganisms in the rumen of sheep fed to appetite in pens or pasture. *J. Gen. Microbiol.* 45: 243-51.

WEAKLEY , D.C., STERN, M.D. and SATTER. L.D. (1983). Factors affecting disappearance of feedstuffs from bags suspended in the rumen. *J. Anim. Sci.* 56: 493-507.

WEAKLEY, D.C. and OWENS, F.N. (1975). Ozone delignification. *J. Anim. Sci.* 41: 425A.

WEBSTER'S DICTIONARY. (1988). Budget books Pty. Ltd. Melbourne Australia. p. 322.

WEDEKIND, k.J., MUNTIFERING, R.B. and BARKER, K.B. (1986). Effect of diet concentrate level and sodium bicarbonate on site and extent of forage fibre digestion in the gastrointestinal tract of wethers. *J. Anim. Sci.* 62: 1388-95.

WEINSTEIN, M.P. and HECK, K.L. (1979). Ichthyofauna of seagrass meadows along the Caribbean coast of Panama and in the Gulf of Mexico: composition, structure and community ecology. *Mar. Biol.* 50: 97-107.

WEIR, W.C., MEYER, J.H., GARRETT, W.N. and LOFGREEN, G.P. (1959). Pelleted rations compared to similar rations fed chopped or ground for steers and lambs. *J. Anim. Sci.* 18: 805-814.

WELLER, R.A., GRAY, F.V. and PILGRIM, A.F. (1958). The conversion of plant nitrogen to microbial nitrogen in the rumen of sheep. *Brit. J. Nutr.* 12: 421-429.

WEST, R.J. and LARKUM, A.W.D. (1979). Leaf productivity of the seagrass *Posidonia australis* in eastern Australia waters. *Aquat. Bot.* 7: 57-65.

WESTERILING, B. (1970). Rumen ciliate fauna of semi-domestic reindeer (*Rangifer tarandus L.*) in Finland: composition, volume and some seasonal variations. *Acta Zoologica Fennica*:. 127: 1-76.

WESTLAKE, D.F. (1963). Comparisons of plant productivity. *Biolog. rev. Camb. philosop. Soc.* 28: 385-425.

WESTON, R.H. (1966). Factors limiting the intake of feed by sheep. 1. The significance of palatability, the capacity of the alimentary tract to handle digesta and the supply of glycogenic substrate. *Aust. J. Agric. Res.* 17: 939-954.

WESTON, R.H. (1967). Factors limiting the intake of feed by sheep. II Studies with wheaten hay. *Aust. J. Agric. Res.* 18: 983-1002.

WESTON, R.H. (1979). Feed intake regulation in sheep. In ""Physiological and environmental limitations to wool growth". Black, J.L. and Reis, D.J. (Eds). University of New England Publishing Unit. Armidale, NSW. pp. 163-177.

WESTON, R.H. (1981). Animal factors affecting feed intake. In "Nutritional limits to animal production from pastures". Hacker, J.B. (Ed.). CAB (Commonwealth Agricultural Bureaux), Farnham Royal, UK. pp. 183-198.

WESTON, R.H. (1985). The regulation of feed intake in herbage-fed ruminants. *Proc. Nutr. Soc. Aust.* 10: 55-62.

WHEELER, J.L. and FREER, M. (1986). Pasture and forage: The feed base for pastoral industries. In "The Pastoral Industries of Australia". Alexander, G. and Williams, O.B. (Eds). Sydney University Press, Australia. pp. 165-182.

WIILLIAMS, O. B. (1981). Evolution of grazing systems. pp. 1-12. In ".Grazing animals (World animal science; volume B 1)". Morley, F. H. W. (Ed.). Elsevier scientific publishing company, Amsterdam.

WILKINS, R.J. (1981). Improving forage quality by processing. In "Nutritional limits to animal production from pastures". Hacker, J.B. (Ed.). CAB (Commonwealth Agricultural Bureaux), Farnham Royal, UK. pp. 389-408.

WILKINSON, J.M. and GONZALEZ SANTILLANA, R. (1978). Ensiled alkali-treated straw. I. Effect of level and type of alkali on the composition and digestibility *in vitro* of ensiled barley straw. *Anim. Feed Sci. Technol.* 3: 117-132.

WILLIAMS, A.G. and COLEMAN,G.S. (1992). The rumen protozoa. Springer-Verlag, New York Inc. 441pp.

WILLIAMS, O. B. (1981). Evolution of grazing systems. In "Grazing animals (World animal science; volume B 1)". Morley, F. H. W. (Ed.). Elsevier scientific publishing company, Amsterdam. pp. 1-12.

WILLIAMS, O.B. and CHAPMAN, R.E. (1966). Additional information on the dye-banding technique of wool growth measurement. *J. Aust. Inst. Agric. Sci.* 32: 298-300.

WILLIS, C.M., STALLCUP, O.T. and KREIDER, D.L. (1980). Influence of sodium hydroxide and enzyme additions on nutritive values of rice straw. *J. Anim. Sci.* 50: 303-308.

WILSON, R.K. and PIGDEN, W.J. (1964). Effect of a sodium hydroxide treatment on the utilisation of wheat straw and poplar wood by ruminant micro organisms. *Can. J. Anim. Sci.* 44: 122-123.

WINTERBOTTOM, D.C. (1917). Marine fibre. *Dept. Chem. Bull.* 4: 1-36.

WISE, J.J. and SILVESTRI, A.J. (1976). Newsletter. *Oil and Gas J.* 22: 1140-1142.

WOLIN, M.J. (1990). Rumen fermentation: biochemical interactions between the populations of the microbial community. In "Microbial and plant opportunities to improve lignocellulose utilization by ruminants". Akin, D. E. , Ljungdahl, L.G., Wilson, J.R., Harris, P. J., Elsevier. pp. 237-251.

WOMERSLEY, H.B.S. (1959). The marine algae of Australia. Bot. Rev. 25: 545-614.

WOMERSLEY, H.B.S. (1980). Biogeography of Australian marine algae. In "Marine botany an australian perspective". Clayton, M.N. and King, R.J. (Eds.). Longman Cheshire. pp.294-307.

WONGSRIKEAO, W. and WANAPAT, M. (1985). The effects of urea treatment of rice straw on the feed intake and live weight gain of buffaloes. In "The utilisation of fibrous agricultural residues as animal feeds". Doyle, P.T. (Ed.). International Development Program of Australian Universities and Colleges, Canberra, Australia. pp. 81-84.

YODER, R.D., TRENKLE, A. and BURROUGHS, W., (1966). Influence of rumen protozoa and bacteria upon cellulose digestion in vitro. *J. Anim. Sci.* 25: 609-612.

YOUSSEF, F.G. and ALLEN, D.M. (1968). Part played by ciliate protozoa in rumen function. *Natu.* 217: 777-778.

YU, Y., THOMAS, J.W. and EMERY, R.S. (1971). Treatment of straw with chlorine compounds and radiation. *J. Anim. Sci.* 33: 1155 A.

YU, Y., THOMAS, J.W. and EMERY, R.S. (1975). Estimated nutritive value of treated forages for ruminants. *J. Anim. Sci.* 41: 1742-1751.

ZADRAZIL, F. (1979). Screening of *Basidiomycetes* for optimal utilisation of straw (production of fruiting bodies and feed). In "Straw decay and its effect on disposal and utilisation". Grassbard, E. (Ed.). John Wiley & Sons. London. pp. 139-146.

ZARCINAS, B.A., CARTWRIGHT, B. and SPOUNCER, L.R. (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun Soil Sci. Plant Anal.* 18:131-146.

ZIEMAN, J.C. (1975). Quantitative and dynamic aspects of the ecology of turtule grass, *Thalassia testudinum.* In "Estuarine research Vol. 1: Chemistry, biology and the estuarine system". Cronin, L.E. (Ed.). Academic Press, New York. pp. 541-62.