OPTIMISING THE USE OF GLIRICIDIA, CALLIANDRA LEAF AND KENAF SEED PROTEIN IN RUMINANT FEEDING.

Thesis submitted by

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School of Veterinary and Biomedical Sciences

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DECLARATION

I declare that this is my own work and has not been submitted in any form for another degree or diploma at any university or institution of higher learning. All information derived from published or unpublished work of others has been acknowledged in text and a list of references is given.

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STATEMENT OF CONTRIBUTION OF OTHERS

Associate Professor Esala Teleni and Professor Phillip Summers supervised the research that is reported in this thesis, provided advice and assistance with the preparation of the thesis. Esala Teleni was a co-author on all papers resulting from this thesis.

A stipend support was provided by the Australian Agency for International Development (AusAID) for the duration of the research candidature. Project costs were met from IRA accounts held by Associate Professor Esala Teleni.

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ABSTRACT

A major constraint to ruminant animal production in the tropics and sub-tropics is the inadequate supply of good quality feeds particularly during the dry season. During such a time, only fibrous crop residues such as maize stover, wheat and rice straws, or low quality grass or hay (H) may be available. These feedstuffs are invariably low in nitrogen (N) and are unable to support normal microbial activity in the rumen. Research in the recent past therefore had focused much attention on the nutritional potentials of shrub plants since these generally are more tolerant to drier conditions and a number, particularly the legumes, have a high N content. In the current study, the leaves of two shrub legumes, Gliricidia sepium and Calliandra calothyrsus, and the seeds of a non-leguminous shrub plant, Kenaf (Hibiscus cannabinus), were examined for their nutritional potential as supplements to H in ruminant animal feeding.

Of the seven experiments undertaken, three involved the evaluation of Gliricidia leaves, two of Calliandra leaves, one of a mixture of Gliricidia and Calliandra leaves and one of Kenaf seeds. Except for the number of animals used and dietary treatments examined, the materials and methods employed across the seven experiments were the same. The experimental animals were kept in metabolism cages where the feed and water intake and faeces and urine excreted were recorded daily. Samples of feedstuffs offered to the animals, feedstuffs refused by the animals, faeces, urine, rumen fluid and blood were taken and analysed where relevant, for dry matter (DM), organic matter (OM), nitrogen (N), urea, ammonia, short chain fatty acids (SCFA) and glucose.

Four even groups of a total of 16 wethers were each allocated at random the following four dietary treatments: basal low quality hay (H) fed with fresh Gliricidia leaves either simultaneously or at two hours or at six hours or at 12 hours after H was fed. Experiment 1 was undertaken to examine the effect of time of offering Gliricidia leaves supplement to H after H was fed to sheep, on feed DM digestibility and N utilisation.

Sheep fed the legume supplement simultaneously with or at 12 hours after the H was fed had lower DM digestibility (54%) and N balance compared to those fed the supplement at two or six hours after H was fed.

It was concluded that to better match the ammonia supplied from the Gliricidia supplement with the SCFA produced from the H for microbial synthesis in the rumen, the legume supplement should be offered two to six hours after the H has been fed.

Experiment 3 was undertaken to examine the effect of time of offering the Gliricidia leaves supplement to H after H was fed to sheep, on voluntary feed DM intake. Three even groups of a total of 15 wethers were each allocated at random the following three dietary treatments: H fed with fresh Gliricidia leaves either simultaneously or at two hours or at six hours after H was fed.

No increase in DM intake was observed across the dietary treatments. It was concluded that manipulating the time of feeding of the Gliricidia supplement would not significantly increase the DM intake of a diet based on H.

Experiment 5 was undertaken to examine the effect of time of offering fresh Gliricidia leaves supplement to H either simultaneously or at four hours after H was fed to sheep. Three even groups of a total of 18 wethers were each allocated at random the following three dietary treatments: fed H only; H fed with fresh Gliricidia leaves either simultaneously or at four hours after H was fed. Compared to feeding of the supplement simultaneously with H, feeding the supplement at four hours after H was fed improved DM digestibility by 23%. The molar ratio of ammonia : SCFA was lower at 0.14 and the percentage of N retained (12%) was higher in the latter feeding regimen. It was concluded that offering the Gliricidia leaves supplement to H at four hours after H was fed to sheep would result in the best outcome in terms of dietary DM digestibility and N utilisation.

Experiment 2 was undertaken to examine the effect of time of offering the Calliandra leaves supplement to H after H was fed to sheep, on DM digestibility and N utilisation. Two even groups of a total of 12 wethers were each allocated at random

the following two dietary treatments: H fed simultaneously with fresh Calliandra leaves and H + Calliandra leaves fed at two hours after H was fed.

The animals fed the supplement simultaneously with H excreted more N through faeces (76 % vs 72 %) and retained less N (- 4 % vs 12 % of total N intake).

It was concluded that offering the Calliandra leaves supplement to H at two hours after H was fed to sheep would significantly improve DM digestibility and N utilisation of the diet.

Experiment 6 was undertaken to examine the effect of drying on the utilisation of Calliandra-supplemented diets. Three even groups of a total of 15 wethers were each allocated at random the following three dietary treatments: fed H only, H fed simultaneously with either fresh Calliandra leaves or dried Calliandra leaves
.
The N retained was higher in sheep fed the dried legume leaves than in those fed fresh leaves (42 % vs 27 %). The amount of N excreted through faeces was lower in animals fed the dried leaves (41 % vs 49 % of N intake).

It was concluded that drying Calliandra leaves, while it may not improve the digestibility of the diet when included as a supplement, would improve N retention by the animals.

Experiment 4 was undertaken to examine the effect of a mixture of Gliricidia and Calliandra leaves as a supplement to H. Three even groups of a total of 15 wethers were each allocated at random the following dietary treatments: H fed with a mix of fresh Gliricidia:Calliandra (1:1) leaves either simultaneously or at two hours or at six hours after H was fed.

Improvement in N retention (51 % of total N intake) across the dietary treatments was observed. DM digestibility was relatively high (68 %) in all sheep on the different dietary treatments.

It was concluded that the best use of Gliricidia and Calliandra as supplements to H would be to use the two legumes combined as a supplement.

Experiment 7 was undertaken to examine the effect of Kenaf seed supplement to H after H was fed to sheep on feed DM digestibility and N utilisation. Four even groups of a total of 24 wethers were each allocated at random the following dietary treatments: H; H + fresh Gliricidia leaves fed at four hours after H was fed; H + fresh Gliricidia leaves + milled Kenaf seeds fed simultaneously; and H + milled Kenaf seeds.

The DM digestibility of H + milled Kenaf seeds diet was similar to that of the H diet (50 %). Although the DM digestibility values for the diet containing fresh Gliricidia leaves were slightly higher at 52 – 53 %, the highest N retention (13 % of N intake) was observed in sheep fed the H + milled Kenaf seeds.

It was concluded that Kenaf seeds may be used as a supplement to H to improve N retention in animals.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ANOVA	Analysis of variance
Ca	Calcium
CP	Crude protein
CT	Condensed tannin
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DOM	Digestible organic matter
DOMI	Digestible organic matter intake
HCl	Hydrochloric acid
HT	Hydrolysable tannin
JCU	James Cook University
LW	Live weight
$LW^{0.75}$	Metabolic live weight
ME	Metabolisable energy
N	Nitrogen
NDF	Neutral detergent fibre
NI	Nitrogen intake
OM	Organic matter
OMD	Organic matter digestibility
OMI	Organic matter intake
P	Phosphorous
S	Sulphur
H_2SO_4	Sulphuric acid
SE	Standard error
SCFA	Short chain fatty acid
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

One of the major causes of low productivity of ruminant animals in tropical countries, particularly in the drier regions, is the generally low availability of good quality feedstuffs. Forage quality changes with seasons and it can be too low to maintain an acceptable level of nutrition in grazing ruminant animals during the dry season. This general scenario underpins the increased interest in the nutritional potential of leguminous and non-leguminous shrubs/trees. Such plants cannot only survive in relatively dry conditions an foliage is rich in nitrogen (N) the nutrient that generally is most limiting to ruminant animal productivity, especially during the dry season.

Plants such as shrub legumes are richer in proteins than many other plants. These generally are hardy, require little maintenance and thus can be sustained in most farming systems. It is not surprising therefore that in the last 10 to 20 years of ruminant animal production research much interest has been focused on defining the agronomy and nutritive values of such plants. Interest in shrub plants other than leguminous ones has arisen due to local availability among other reasons. An example of this is the shrub plant, *Hibiscus cannabinus* L (Kenaf) which is developed and cultivated primarily for fibre but has by-products such as seeds, leaves and unwanted stems, that could be used as feedstuffs in rations for ruminant animals.

Of the shrub legumes widely studied, two [*Gliricidia sepium* (Gliricidia) and *Calliandra calothyrsus* (Calliandra)] have emerged as potential substitutes for the well respected *Leucaena leucocephala* (Leucaena) which, in the 1980s, was devastated by psyllids (*Heteropsilla cubana*) worldwide. Studies by Widiawati (2000) at James Cook University (JCU) had shown that while both Gliricidia and Calliandra leaves have relatively high concentrations of proteins, the proteins are degraded at significantly different rates in the digestive tract of the ruminant animal. The Gliricidia protein has a relatively large fraction that is water soluble and

therefore is highly degradable in the rumen while the Calliandra protein is more slowly degradable in the rumen as well as in the lower digestive tract, probably due to the high concentration of condensed tannins. Both these characteristics of the two shrub legumes limit their nutritional potential. Thus, while good productivity responses have been recorded in ruminant animals fed these legumes, their nutritional potentials have yet to be fully exploited.

In tropical Queensland, Australia, Kenaf is an emerging potential alternative crop to sugar cane. Currently, in the Burdekin region of North Queensland, the agronomy and management of Kenaf appropriate to prevailing environmental conditions are being investigated. As mentioned earlier, Kenaf is grown primarily for fibre production. However, there are byproducts from the crop, including seeds, leaves and unused stems, that potentially could be used as feedstuffs in ruminant animal feeding. The seeds of Kenaf have a high protein and fat content [approximately 26 % and 20 % respectively of dry matter (DM); CASCO, 2003]. While the high protein content of the seeds would be an advantage nutritionally, the high fat content could pose some problems in that increased levels of dietary fats have been shown to inhibit rumen microbial activity (Palmquist, 1994).

The experiments described in the current study were undertaken to define more clearly the nutritional potential of Gliricidia and Calliandra leaves and of Kenaf seeds and to suggest ways that may be employed to realize their potential.

CHAPTER 2

LITERATURE REVIEW

This review will examine the quality of proteins in Calliandra, Gliricidia and Kenaf seeds and the factors that may promote synchronization of nitrogen and short chain fatty acids release, for efficient microbial activity in the rumen, thus improving their potential use by ruminant animals as supplements to low quality basal diets.

This review focuses mainly on optimising the use of Calliandra, Gliricidia and Kenaf proteins in ruminant feeding.

2.1 AGRONOMICAL ASPECTS OF GLIRICIDIA AND CALLIANDRA

2.1.1 *Gliricidia sepium* (Jacq)

Gliricidia (Jacq) is a member of the family Fabaceae and the sub-family Papilionoideae and lies within the tribe Robinieae (Simons and Stewart, 1994). It is a leguminous tree, found in tropical and subtropical countries. This legume is fast growing and can grow to a height of 10-15 m and has pinnate leaves. It is one of the most common multipurpose trees in Central America, from where it is believed to have originated. It has been introduced to many tropical and sub-tropical countries in Africa, Asia and tropical America (Simons and Stewart, 1994).

The establishment of Gliricidia can either be from cuttings or seeds. Seed establishment is recommended when used *in situ* because this leads to deep rooting. In a case where the seeds are not fresh, they should be soaked overnight in hot water and then planted immediately (Simons and Stewart, 1994). However the use of mature stakes (cuttings) is also recommended (Smith, 1991). During the early stage of growth, the process is slow, but once established, growth rate is usually very fast, with an average of 3 metres in height per year under adequate rainfall. For forage production it is recommended that pruning be done 9 months after establishment.

Gliricidia may be harvested at 3 month intervals so as to maximize foliage yield (Simons and Stewart, 1994). This legume can be harvested (pruned) 3 - 4 times a year at a height of 30-150 cm. It can withstand pruning, lopping, coppicing and even regular browsing. Pruning is known to stimulate growth (see figure 2.1). The DM yield of Gliricidia has been reported to range from 2-20 tonnes/ha/yr, when planted at a density of 40,000 trees/ha (Simons and Stewart, 1994). The average annual production is approximately 1.5-2.5 kg of fresh leaves per tree, with an estimate of 4.7 tonnes/ha/year of crude protein (CP). The leaves represent between 53 - 63 % of the total edible biomass. For maximum yield, it is advisable to have a spacing of 80 cm between seedlings, this will give approximately 25,000 trees per hectare (Smith, 1991). The yield per tree will depend on rainfall, altitude, soil type and general management. In most tropical farming systems, it is grown in hedgerows in house yards rather than as filled crop.

Figure 2.1 A mature shrub legume *Gliricidia sepium*, (at JCU) showing one month re-growth after pruning.

Gliricidia performs well in warm, wet conditions with optimal temperatures of 22-30 ^{o}C and as little rainfall as 600 mm per annum but will also produce better in

areas of 800-2300 mm of rainfall per annum. It is suited to humid coastal lowlands or lake basins and grows well in a variety of acidic, fertile and low fertility soils. It tolerates water logged soils but does not perform well in regions of low temperatures. If temperatures fall to 15 ^{o}C and below, loss of foliage and poor growth occurs. The maximum altitude for Gliricidia is 1600 m above sea level.

2.1.2 *Calliandra calothyrsus* (Meisn.)

Calliandra (meissner) is a leguminous tree native to Mexico and Central America. It belongs to the Fabacaea family and Mimosaceae sub-family. In most tropical countries it was introduced as a protein supplement to ruminant animals. Together with other species of Calliandra, it is commonly known as Red Powder Puff Tree in Australia.

Seed pretreatment procedure is recommended before setting a seed bed. Seeds should be immersed in hot water, allowed to cool and continue soaking for 12–24 hours before planting. Calliandra seedlings are established in nurseries and transplanted following the onset of rains (Smith, 1991). Calliandra can be grown in the humid and sub-humid zones with minimal rainfall of 700–3000 mm per year and at a maximum altitude of 1860 m above sea level (Palmer *et al.*, 1994). It thrives in light textured types of soils although it can also be grown on a wide range of soils, as long as they are slightly acidic. Calliandra can tolerate infertile and compacted, poorly aerated, soils but will not withstand water logged and alkaline soils (Palmer *et al.*, 1995)

Calliandra is a small tree ranging from 2-14 m in height with a trunk diameter of up to 30 cm. It has a white or red-brown bark and forms a dense canopy. The feathery leaves of Calliandra are bipinnate and alternate. The first pruning after planting can be done after 12 months. Calliandra can be harvested three to five times a year, down to a height of about 80-100 cm. The recommended cutting height is 0.6-1.0 m (MacQueen *et al.*, 2004). The rate of growth of Calliandra is known to be slower during the cold season and therefore the pruning interval during this period should be longer (Palmer *et al.*, 1995). It is necessary to cut the tree back to 30 cm after three to four years, to allow it to shoot again. Cutting Calliandra six months before the dry

season will give maximum yield during the season. The life span of this tree is about 10 -20 years (Palmer *et al.*, 1994).

Figure 2.2 Flowering shrub legume *Calliandra calothyrsus*

A population density of 40,000 trees/ha of Calliandra has been estimated to yield 36 tonnes /ha /year. The average DM yield in Australia and Indonesia has been reported to range between 3.28-11.4 tonnes/ha/yr (Palmer *et al.*, 1994). The best production achieved has been where the rainfall ranged between 1000-3000 mm/yr and soil pH was 5 to 8.

2.2 USES OF GLIRICIDIA AND CALLIANDRA

Gliricidia is a multipurpose tree. It is used for timber, firewood, and has some medicinal properties; it can also be used for charcoal, live fencing, plantation shade and green manure. It has high potential as a supplement in livestock feeding due to its high protein content.

Calliandra, like Gliricidia, has many uses (Simons and Stewart, 1994). It has been widely accepted as a forage by farmers due to its high growth rate, allowing harvests of leafy or woody material over many years. The tree height allows farmers to manipulate it easily (MacQueen *et al.*, 2004). The predominant use of Calliandra is as a feed supplement for ruminant animals, but it is also used in different farming systems for other purposes which include the provision for green manure, fuel wood, shade or support for perennial crops, land rehabilitation and soil erosion control, honey or shellac production (Smith, 1991). Due to the limited area of land per farmer, in the tropics and sub-tropics, it is best when these legumes are carefully incorporated in the landscape rather than planted as mono-culture fodder banks.

The recommendations for growing these trees without interfering with the land needed for crop production are:

Intercropping trees with Napier grass, as this has proved to be more beneficial as fodder yields have been reported to increase other than when the trees are planted as pure stands.

Planting trees on boundaries (as hedges), instead of using fencing material of low value, this strip of land can be better utilised by planting fodder trees.

Depending on the terrain, fodder tress can be planted on the upper or lower side of the soil conservation structures.

2.3 NUTRITIVE VALUE OF GLIRICIDIA AND CALLIANDRA

The nutritive value of a feed is based on the amount of nutrients it contains and on the efficiency of extraction of the nutrients from the feed during digestion by the ruminant animal. The extent at which a feed could promote animal production depends mainly on the voluntary feed intake and its digestibility (Norton and Waterfall, 2000).

During the dry period in the tropics and sub-tropics, the protein content of the native grasses and crop residues are usually low, and this has been found to have a depressing effect on voluntary feed intake by the ruminant animals. The effect of low

dietary protein on intake and digestibility was previously reported by other workers (Davendra, 1991). Feeds that are of high nutritive value will lead to an improved voluntary feed intake, improved digestibility and promotion of live weight gain in growing animals.

Voluntary feed intake is influenced by the preference of animals for particular feed available to them. Such preferences may be due to the physical characteristics of the feed and/or the presence of compounds in the feed that may contribute to its odour and taste (Norton, 2004). Feed intake of ruminant animals consuming fibrous forages is also determined by the effect it has on rumen fill, which directly reflects on the rate of digestion and passage of fibrous particles from the rumen to the abomasum and lower digestive tract. Shrub legumes which are high in soluble protein (Widiawati, 2002) have the potential of improving voluntary feed intake and digestibility when incorporated in ruminant animals diet.

Shrub legumes over the years have been highly valued as feeds or feed supplements for ruminant animals due to their high nutritive value, which is reflected both in their chemical and physical characteristics (Table 2.1). Legumes are distinctive in that the crude protein (CP) content of their leaves is commonly greater than 12 % of DM, and hence they are usually superior to grasses as a protein source. However, tropical shrub legumes particularly may contain secondary compounds, that may significantly affect rumen function and even palatability of the feed (Perez-Maldonado and Norton, 1996; Merkel *et al.*, 1999; Norton and Waterfall, 2000).

The CP and calcium (Ca) in the leaf DM of Gliricidia may be as high as 27 % and 1.2 % respectively (see Table 2.1). These two nutrients are usually found in very low levels in non-leguminous tropical forages. The high level of neutral detergent fibre (NDF) that Gliricidia contains (35-47 %) makes this legume a good roughage source for ruminant animals. Legumes contain sufficient levels of most minerals, except for Phosphorus (P), Zinc (Zn) and Copper (Cu), that could meet tropical livestock requirements, and therefore they could be a valuable feed resource during the dry period when the quality of basal diets is low (Suttie, 2005).

Table 2.1 The crude protein (CP), neutral detergent fibre (NDF), condensed tannins (CT), and dry matter digestibility (DMD), Phosphorous (P) and Sulphur (S) content of Gliricidia and Calliandra as compared to C*hloris gayana*

	Nutrients (%)						
Fodder type	**CP**	**NDF**	**CT**	**DMD**	**S**	**P**	**Ca**
Calliandra[1] *calothyrsus*	17- 28	26 -60	19.4	47 -59	0.19	0.15	1.6
Gliricidia[2] *sepium*	24 -27	35 - 47	4	63 - 79	0.11	0.19	1.2
Chloris[3] *gayana*	5 –6	71 - 76	-	50 – 57	-	-	-

[1] (Salawu *et al.*, 2002), [2] (Chadhokar, 1982), [3] Karda and Dryden (2001).

The nutrient content of Gliricidia varies with age, season and physiological state (i.e. before and after flowering). It has been observed that, in the leaves of older plants after flowering, the protein and Ca content start to decline whereas fibre, P and other minerals increase (Simons and Stewart, 1994).

Digestibility of DM in Gliricidia has been found to be 60 % or higher. This could probably improve the digestibility of poor quality feeds when it is used as a supplement to low quality forage/hay. From previous observation, Gliricidia degradability in nylon-bags (*in situ*) was found to be as high as 62 % DM and 19 % N in 24 hours, as compared to corresponding values of 49 % and 7 % for *Leucaena* (Suttie, 2005).

The CP content of Calliandra is higher than that of most tropical grasses and other leguminous trees, as indicated in Table 2.1. The edible fraction of Calliandra has a CP level of up to 28 %. Incubation of Calliandra over a period of 24 hours *in sacco* resulted in N degradation of 35.9 % when freeze dried and 46.9 % when oven dried (Ahn *et al.*, 1997; Merkel *et al.*, 1999). The cutting interval and the leaf to stem ratio of the offered material has been observed to also influence the degradation rate (Roothaert, 1999). It has NDF levels ranging from 26 to 60 % of DM. Its DM digestibility (DMD) range of 47 to 59 %, this is almost equal to that of grasses. However, the presence of condensed tannins could affect the N utilisation by the

rumen microbes due to protein tannin complexing. High tannins could also be beneficial due to an increase in the amount of proteins bypassing rumen digestion, and being absorbed in the small intestine.

2.4 QUALITY OF PROTEINS IN GLIRICIDIA AND CALLIANDRA

2.4.1 Nature of Protein

Improving productivity of ruminant animals involves providing right nutrients that can meet the requirements for both the rumen microbial fermentation and the animal metabolism in the tissues (Teferedegne, 2000). However, the composition and population of rumen microbes will influence the rate of feed degradation in the rumen, which is highly dependant on the type of diet the ruminant animal is fed.

Microbial growth depends on the availability of N supplied from dietary proteins and energy from the carbohydrate components of a diet (figure 2.3). To improve N efficiency of ruminant animals, it is necessary to balance the protein component with the readily available carbohydrates for efficient microbial activity. A feed that deters the population growth and activity of microbes in the rumen will reduce degradability of fibrous diets, resulting in ineffective supply of N. An example of this type of feed is one which is high in grains or concentrates (Kempton, 1980). Therefore, it is important to balance nutrient requirements of the ruminant animal and ensure that the type of feed offered can effectively stimulate microbial activity in the rumen for maximum degradation, and hence improve animal productivity.

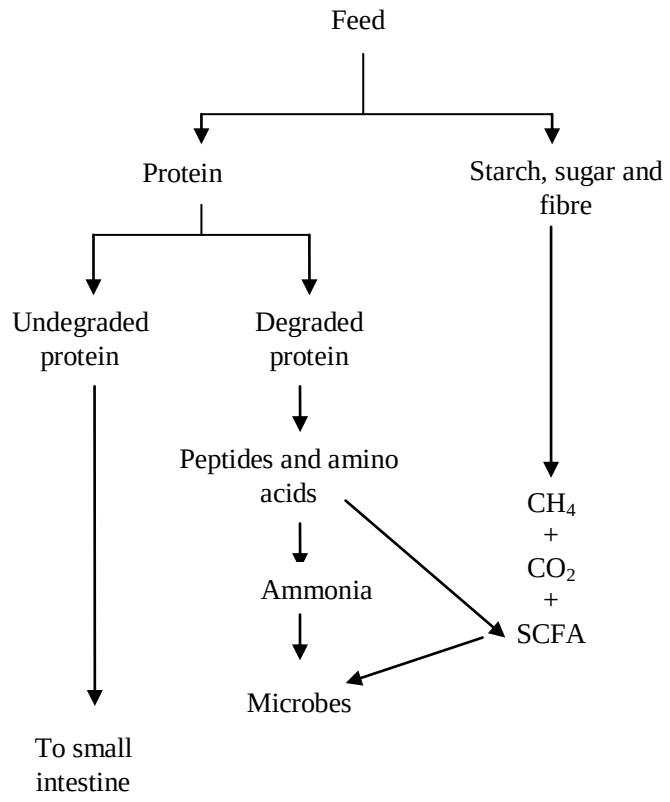

Figure 2.3 An illustration of ruminal degradation of feed. Modified from Kempton *et al* (2004).

Ruminants can convert low quality protein sources to high quality proteins by bacterial action. However, the quantities produced are not sufficient to support normal rumen function. It is necessary to supplement the low quality basal diet with a good quality protein source in order to optimize rumen function. It would be expected that a diet that is low in protein, can be improved if supplemented with leguminous leaves which are high in proteins. Previous work carried out on ruminant feeding observed that supplementation of roughages with adequate quantities of shrub legumes can improve the availability of N in ruminant animals and thus improve ruminal microbial activity (Bonsi *et al.*, 1994).

Microbial protein synthesis in the rumen is important as it provides 50 % or more of all the amino acids required for beef cattle, depending on the concentration of the undegraded CP in the diet (Karsli and Russell, 2002). Ruminal ammonia is the major N source used for protein synthesis by the microbes (Karsli and Russell, 2002). The

microbial population in the rumen requires a minimal level of ammonia of about 80 mg of N/L of rumen fluid, to support optimum rumen activity; lower values have been associated with decreased microbial activity during digestion and a N deficiency (Kempton *et al.*, 2004). Feeds with less than 1.3 % N (8 % CP) are considered to be deficient as they cannot provide the minimum levels of ammonia required by the ruminant animal for effective microbial activity in the rumen. Ammonia produced from low quality basal diets has been reported to range between 25 mg/L – 31 mg/L (Bonsi *et al.*, 1996; Abdulrazak *et al.*, 1997).

With Gliricidia supplementation at the level of 30 % DM to a diet of maize stover, ammonia concentration was reported to increase from 31 mg/L to 101 mg/L (Bonsi *et al.*, 1996). In general, an increase in ammonia concentration from 26 mg N /L to 85 mg N/L has been found to increase microbial protein production by 177 % per day (Bonsi *et al.*, 1996). Therefore, shrub legumes which normally are high in CP are likely to improve the ammonia concentration in the rumen and thus promote microbial activity for improved animal production.

Legume leaves, compared with tropical grasses contain lower NDF and therefore the total organic matter (OM) may be more easily fermented. (Widiawati, 2002). When sugars, cellulose, and other substrates such as N, S and other mineral salts are available as sources of energy, maximum fermentation by the ruminal micro-organisms is likely to be attained. During the fermentation process in the rumen, if one or more of the important nutrients unavailable, fermentation is restricted; resulting in a reduced feed intake and limited nutrient availability to the animal (Norton, 2004). Diets that contain straw and mature grasses, for example, tend to lead to low animal productivity as they are deficient in N and other essential nutrients that are necessary for microbial activity. The evergreen nature of the shrub legume gives them the advantage of a lower fibre content, which can improve digestibility of poor quality tropical forages (Nunez-Hernandez *et al.*, 1989).

The leaves of Calliandra and Gliricidia are able to supply adequate nutrients, which means that they have the potential to promote microbial activity in the rumen when used as supplements to low quality basal diets.

The degradation of CP in the rumen depends on the type of protein and the presence of other secondary compounds. Plant materials have water soluble and non-water soluble proteins. The soluble proteins degrade rapidly in the rumen, and this may have a negative effect on the efficient utilisation of N by ruminal microbes. The non-soluble protein will degrade at a slower rate, and is likely to promote efficient utilisation of N by the rumen microbes.

Soluble and insoluble protein components of legumes provide important sources of N for increased rumen microbial activity and increase the amounts of high quality proteins passing to the lower gastrointestinal tract (GI tract) (Preston and Leng, 1987). Calliandra and Gliricidia are rich in protein, and have significant fractions of water soluble CP. These two legumes have the potential of increasing the digesta flow rate from the rumen, hence resulting in a considerable quantity of high quality protein available digestion and absorption in the small intestine, when supplementing low quality forage (Widiawati, 2002).

2.4.2 Amino Acids

In the small intestines the determining factors for amino acid digestion include digestibility of micro-organisms, rumen undegradable protein (or rumen by-pass protein), endogenous secretions and the rate of digesta flow that is influencing the efficiency of digestion, coupled with the presence of parasites and micro-organisms in the small intestine.

Out of over 200 amino acids that have been identified from biological materials, twenty of these are major common components of proteins. Eight of these have been identified as essential amino acids (EAA) that must be acquired in the diet, and the non-essential amino acids can be synthesised by the body of the animal (McDonald *et al.*, 2002). The actual essential amino acids differ from one species of plant to

another. Some of the essential amino acids that are necessary for the promotion of lean muscle growth are histidine, lysine, methionine and arginine, and these are not readily supplied from microbial protein sources, but are found in relatively high concentrations in Calliandra and Gliricidia (Chadhokar, 1982; Salawu *et al.*, 1997a). This is of particular interest as lysine and methionine are usually the most limiting essential amino acids in forages (Taghizadeh *et al.*, 2005). It could be expected that supplementing the microbial amino acids with those from Calliandra and Gliricidia would promote maximum lean tissue growth in ruminant animals.

An important factor that influences the availability of amino acids from the digestive tract of the ruminant animal is the activity of (Figure 2.4) rumen microbes. The microbes are responsible for determining the availability of fermentable substrates and fermentation processes in the rumen. Their activity also could determine voluntary feed intake and rumen fluid turnover rate and pH levels in the rumen. A unique feature of rumen microbes is that these organisms are able to use N to synthesise their proteins, which are of high biological value (Leng, 1997). Thus protein nutrition of the ruminant animal on a low quality basal forage diet, may be improved to a certain extent by supplementing the diet with N, either from a non-protein N (NPN) or a protein source.

In relation to this, it would be of interest to determine the amino acid availability in the intestine of the ruminant animal fed a basal low quality diet supplemented with shrub legume leaves. Defining the profile and amounts of amino acids made available in the small intestine from such supplementation would be an important prerequisite to optimizing the use of shrub legume leaves in the ruminant animal feeding.

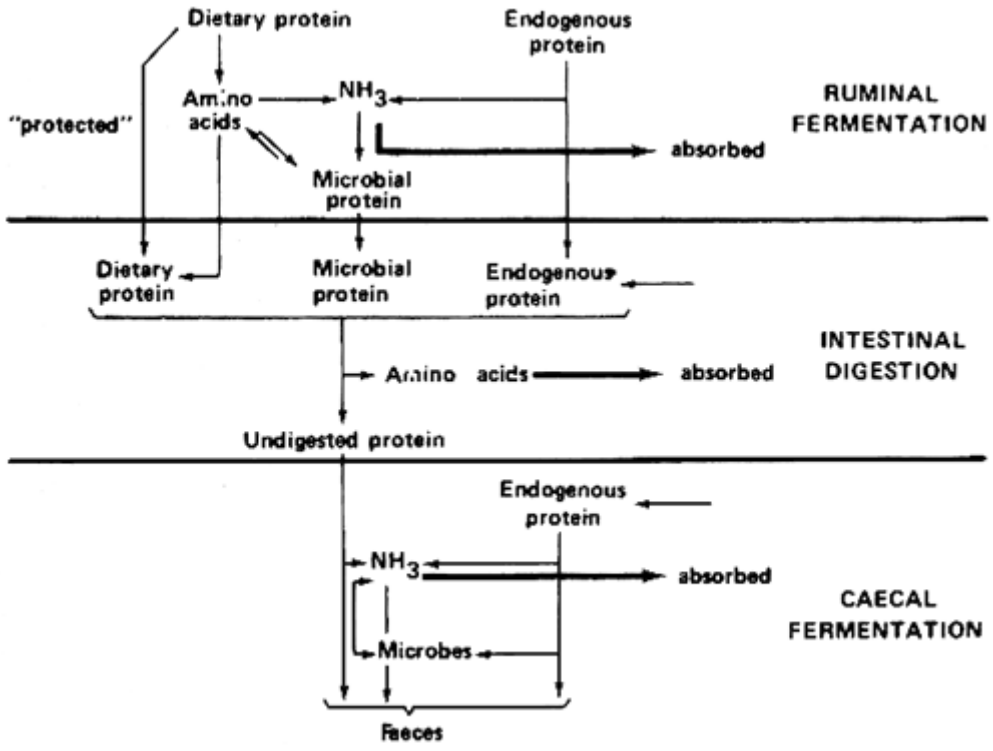

Figure 2.4 Degradation and digestion of dietary protein in the rumen (Kempton *et al.*, 2004).

2.4.3 Protein Degradation

The rate at which proteins are degraded in the rumen depends on microbial protein synthesis which could predominantly be protozoal or bacterial, depending on the conditions within the rumen (Van Soest, 1994). Most of the nutrients required for microbial activity are derived from degradable protein in the rumen (Bohnert *et al.*, 2002). However, when pH is low, the protozoal activity is reduced and that of certain bacteria is stimulated in the rumen (Van Soest, 1994).

Moderate levels of condensed tannins (CT) ranging from 20 to 40 g/kg DM can bind with protein by hydrogen bonding at near neutral pH in the rumen to form CT-protein complexes, which later dissociate and release bound proteins at pH less than 3.5 in the abomasum . Hence CT in plants may protect dietary protein against degradation in the rumen and therefore increase the flow of these proteins to the abomasum and the small intestine, resulting in an improved nutritional status of the animal, and hence improving productivity (Paterson *et al.*, 1999). The degradable

proteins of legumes are the most beneficial supplement to low quality basal diets for ruminant animals (Bohnert *et al.*, 2002).

Calliandra has been reported to have higher undegradable proteins than Gliricidia, which could be related to the high levels of CT (19.4 %) as compared to 4 % in Gliricidia (Davendra, 1991; McSweeney *et al.*, 2000). It is probable that feeding animals with Calliandra could lead to an increased supply of dietary amino acids to the small intestine, thus improving the nutritional status of the animal, as compared to Gliricidia. It has been reported that among the two shrub legumes, Gliricidia has a concentration of water soluble CP fraction of up to19 % higher than Calliandra (Widiawati, 2002).

During degradation, a decreased supply of ammonia will result in a reduction in microbial protein synthesis, leading the rumen micro-organisms to be ammonia deficient and revert to non-protein N.

The degradation and digestion process of dietary proteins in ruminant animals (Figure 2.4) is dependant on the rate of growth and production as influenced by the metabolisable energy intake, the genetic make of the ruminant animal, the proportions of different amino acids absorbed, the microbial protein production, patterns of ruminal fermentation which directly affect the production and availability of short chain fatty acids (SCFA) in the rumen which are glucogenic and subsequent requirements for energy supply for the ruminant animal (Kempton *et al.*, 2004).

It is probable that Calliandra and Gliricidia that are high in CP could satisfy most of the above mentioned nutritional requirements when used as supplements to a low quality basal diet in ruminant feeding.

2.4.4 Protein Requirements of Ruminant Animals

The protein requirements for ruminant animals are based on the digestible CP during degradation. Ruminant animals obtain their essential amino acids from microbial protein produced in the rumen from ammonia. An increase in undegraded protein will increase the amount of amino acids entering the small intestines. The degradability of dietary proteins by ruminal micro-organisms results in the production of amino acids and peptides. The effect of increased undegraded protein in the rumen was previously reported by Volden (1999). The peptides and amino acids in the rumen are responsible for microbial growth, fermentation and ammonia production (Armstead and Ling, 1993).

Generally the modern protein evaluation systems for ruminant animals are based on the supply and requirements of true protein that can be absorbed from the small intestines (Taghizadeh *et al.*, 2005). Thus the protein requirements of ruminants will vary in relation to changing productive status or physiological state the animal is in, as indicated in Figure 2.5.

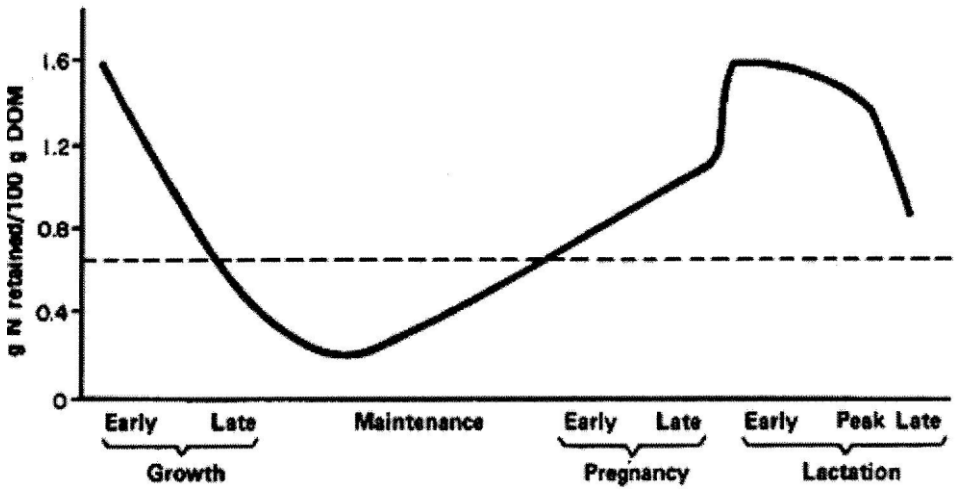

Figure 2.5 Effect of physiological state on potential retention of nitrogen in relation to digestible organic matter intake (Ørskov, 1970; Norton and Ahn, 1997).

Dietary protein of low rumen degradability results in a greater post ruminal digestion in ruminant animals. At the same time, the amounts required to meet the needs of the rumen micro-organisms and the net amino acid N made available to the host animal will increase, leading to an improved protein intake for animal production (Ørskov, 1977; Norton and Ahn, 1997).

The use of legumes as supplements to low quality basal diets at a level of 30 % to 35 % DM may not affect fibrous feed intake, but could lead to an increase in dry matter intake (DMI). It is probable that the increased DMI could be enhanced by an increase in the rate of legume degradation due to the highly soluble proteins resulting in a higher rate of digesta passage through the GI tract of the ruminant animal. The effect of legume supplementation to low quality forage was previously reported by Simbaya (2002).

The minimum dietary protein concentration required to satisfy a ruminant animal's needs is said to be 14 % CP. However the utilisation of the proteins will depend on their digestibility and rate of absorption in the rumen and the amount that bypass rumen digestibility (Ørskov, 1977; Norton and Ahn, 1997). It is therefore necessary to describe the protein degradation of Calliandra and Gliricidia as supplements to different basal diets, as supplements to ruminant animal nutrition, and identify factors that will promote their potential use by these animals for improved production.

The accretion of N in sheep fed a diet of low protein (LP), medium protein (MP) or high protein (HP) concentration between days 110 and 140 of pregnancy was evaluated by McNeill *et al* (1997). The amounts of CP were; 79 g CP/kg DM for LP; 116 g kg/DM for MP and 157 g CP/kg DM for HP, each with an estimated metabolisable energy (ME) of 2.7 Mcal/kg DM. Variation in dietary supply of proteins was found to affect the capacity of the maternal carcass tissue to mobilize or deposit amino acids. The higher CP content had a greater effect than the diet with low CP. In comparison, Calliandra and Gliricidia which have been found to have

high levels of 280 g and 270 g CP/kg DM respectively have the potential to satisfy the protein requirements of pregnant animals.

2.4.5 Dried Calliandra Leaves Supplement

It is probable that the drying of Calliandra leaves would denature potentially toxic substances present and /or modify the biological activity of the CT, hence increasing post ruminal digestibility of CP and hence improving the N balance in ruminant animals. Drying has been linked with a possible change in the chemical composition of Calliandra by increasing the cell wall thickness and reducing the CT concentration (Ahn *et al.*, 1997). However, a number of contradictory observations have been reported on the effect of drying on DM digestibility (Palmer *et al.*, 2000).

Previously, drying of the legume leaves was thought to have some negative effects on N metabolism in ruminant animals, as it was speculated to denature soluble plant proteins. However, McSweenet *et al.* (2000) reported that fresh Calliandra leaves had some inhibitory effect on the growth of predominant rumen bacteria due to the high CT concentrations.

Previous observations by other workers on the effect of supplementation of dried Calliandra to barley straw in sheep, showed an increase in voluntary feed intake of barley straw from 58 % to 78 % and a marked improvement in dry matter digestibility from 40 to 60 % (Ahn *et al.*, 1997). It was concluded that CT in fresh Calliandra depressed feed intake and that this could be reversed by drying. Under normal farming systems, it would appear that fresh Calliandra fed to ruminant animals is more acceptable than dried. However, N retention has been found to decrease when fresh Calliandra (compared to dried leaves) is offered to sheep. Faecal N losses have also been reported to decrease when dried Calliandra is used, thus improving N balance in sheep (Ahn *et al.*, 1997). Similar observations were reported by Salawu *et al.* (1997a). Fresh Calliandra leaves fed as a supplement to a level of 35 % DMI, increased total DMD (30 %) but decreased N digestibility (50 %) (Norton and Waterfall, 2000).

Dried Gliricidia leaves were found to be unpalatable when supplemented in goats and sheep basal diets (Karda *et al.*, 1998), whereas dried Calliandra did not have any negative effect on acceptability by sheep. However, dried Calliandra was reported to lower voluntary feed intake, thus reducing *in sacco* digestibility (Norton and Ahn, 1997; Merkel *et al.*, 1999). Drying not only decreased intake of both the total phenolics and CT, but also seemed to shift the site of digestion of these compounds. However, the drying effect was reported to vary depending on the nature of the tannins and the drying method (Norton and Ahn, 1997).

It is possible that drying facilitates tannin binding to both soluble cell proteins and the cell wall, thus leading to decreased digestion in the rumen, and increased post ruminal digestibility allowing more absorption of the protein in the small intestines. The increased post-ruminal protein absorption has the potential to improve animal productivity (Annison and Lewis, 1959).

2.4.6 Effects of Polyphenols

Polyphenols have been identified as anti-nutritive factors that limit the effective utilisation of legumes as protein supplements to ruminant animals. These substances include compounds such as tannins, saponins, cyanogens, mimosine, and coumarins. The detrimental effects of these anti-nutritive compounds have been linked to reduced animal productivity, neurological effects and increased mortality rates. Tannins especially, can have an adverse effect on microbial and enzyme activities, and have been found to interfere with nutrient absorption and utilisation by ruminant animals and in some instances can cause toxicity, leading to death (Balogun *et al.*, 1998). The toxicity effects of these anti-nutritive compounds depend on their concentration level in specific leguminous species and quantities of intake by the ruminant animal. Condensed tannins and hydrolysable tannins (HT) are the most common in some legumes such as Calliandra and to a lesser extent Gliricidia. Hydrolysable tannins have been reported to be toxic to animals, whereas low levels of CT in a diet have been found to be beneficial to the ruminant animal (Perez-Maldonado and Norton, 1996).

The detrimental effects of the tannins have been linked to the suppression of DMI and digestibility due to their ability to bind to proteins forming a protein-tannin complex. This protein bound complex has been found to be resistant to rumen microbial fermentation, thus promoting the loss of endogenous protein and affecting the total N metabolism of the animal (Waghorn, 1986; Kumar and D'Mello, 1995). However, low levels of 2-4 % of tannins in a diet can be beneficial to ruminants, where they have been found to suppress bloat and reduce protein degradation in the rumen, making it available in the small intestine for absorption (Simbaya, 2002).

The toxic effects of these legumes are rarely exhibited since they are never used as sole diets for ruminant animals. However, most ruminant animals are capable of adapting to anti-nutritive compounds in legumes. There is a possibility that these anti-nutritive compounds could be made harmless to the animal by the rumen microbes during degradation or they could be inactivated by endogenous secretions and detoxified by the liver (Simbaya, 2002).

Although Calliandra has been found to have significant potential as a protein supplement when included at the rate of 30 % of the total DM offered (Perez-Maldonado and Norton, 1996) high levels of CT of about 19-27 % (Table 2.1) have the potential to limit its nutritive value.

Some of the beneficial effects of CT - protein complexes are the reduction of microbial protein degradation in the rumen and subsequent dissociation in the small intestines for absorption. Effects of CT on animal performance were previously reported by other workers (Perez-Maldonado and Norton, 1996). One of the positive effects was that of reduced fatness in growing lambs when legumes were supplemented at concentrations of 20 g/kg DM of CT, whereas, high levels of dietary CT highly decreased ammonia concentrations in the rumen (Waghorn and Shelton, 1997). This was due to the efficient utilisation of N by ruminal microbes during degradation in the rumen. Crude protein degradability in the rumen was found to decline by 39 % when forages containing more than 10 % CT were fed to sheep, but declined by 18 % when the forages contained less than 7 % tannin (Norton, 1994).

Other observations indicate that tannins may reduce fibre digestibility in the rumen when a concentration of 9 % DM or higher of tannins are consumed (Seresinhe and Iben, 2003).

Generally, tannin-protein complexes dissociate in the low pH environment of the abomasums, facilitating dietary protein to be made available for digestion and absorption in the small intestine of a ruminant animal. However, this is not always the case as some reports suggest that the tannins protect the proteins from digestion even in the small intestines (Perez-Maldonado and Norton, 1996).

However, the relative effects of these polyphenolics on rumen function and supply of microbial proteins have not received much attention. Therefore the effect of polyphenolics on the nutritional quality of the proteins in Calliandra and Gliricidia need further investigation.

2.4.7 Anthelmintic Effect of Gliricidia and Calliandra

Condensed tannins in Calliandra and to a lesser extent in Gliricidia have been found to have an effect on the control of gastro-intestinal parasites. Condensed tannins are believed to directly interfere with the development of parasites or to have an indirect effect through increasing host resistance and resilience (Min and Hart, 2003). However, the exact mechanism by which CT exert their effects on nematodes has not been well established.

2.4.8 Animal Performance

Shrub legume supplementation of low quality basal diets of ruminant animals has the potential to increase the productive capacity of ruminant animals. Incorporation of Gliricidia to a diet of low quality maize bran plus maize Stover offered to cattle at two different levels (15 % or 30 %) of dietary DMI, showed an increase in live weight gain of up to five and nine times, respectively (Abdulrazak *et al.*, 1996; Abdulrazak *et al.*, 1997). In another study, daily weight gain of 717g/d was attained when Gliricidia was supplemented at the level of 10 % DM to a fibrous crop residue

basal feed in beef animals in Zambia (Simbaya, 2002). Gliricidia supplementation to a Rhodes grass basal diet in sheep increased liveweight gain by 1.7 times compared to the control group (Widiawati, 2002).

Observations made on Calliandra supplementation in sheep at the rate of 30 %, increased DMI from 435 g/d to 572 g/d (Davendra, 1991) and a daily weight gain of 11 g/d in goats in Kenya was observed (Ondiek *et al.*, 2000). Goats in Zambia were reported to have gained weight at an average of 24 g/d when poor quality pasture was supplemented with Calliandra at a rate of 149 g DM /d (MacQueen *et al.*, 2004). A study in North Queensland on steers at a stocking rate of five steers/ha grazing Calliandra and *Brachiarira decumbens*, observed an increase in live weight gain of up to 900 g/head/d (Palmer and Ibrahim, 1996). In Kenya Calliandra supplementation had the potential to replace commercial supplements in lactating dairy cows, where 3 kg of Calliandra replaced 1 kg of dairy meal, and an improvement of 10 % butter fat content in milk was observed (Paterson *et al.*, 1999).

The positive animal response reported when legumes are fed as supplements is an indication of the high potential they have in improving animal productivity. These performance indicators suggest that legumes have great potential as supplements to low quality basal diets in any farming system. However, a more detailed knowledge is required to optimize the use of these valuable supplements.

It is probable that the improvement in live weight gain that occurred when shrub legumes were supplementing low quality basal diets were related to the improvement of ruminal microbial activity, resulting in an improvement in N utilisation by the ruminant animal. This is due to the potential the shrub legumes have in supplying the required nutrients for ruminal microbial activity, and hence improving nutrient utilisation by the ruminant animal. It is speculated that the efficacy of the utilisation of N from the shrub legumes and the energy yielding substrates from the low quality hay could be improved further if they were readily available synchronously.

The efficiency by which an animal utilises feed nutrients has a significant impact on its productive performance. Investigations on tropical legumes as supplements for ruminant animals highlight the potential benefits they have on increasing metabolisable energy, N utilisation and feed efficiency, thus increasing ruminant animal productivity.

For this information to be translated to useful feeding strategies for ruminant animals, investigations need to be carried out on Calliandra and Gliricidia in relation to their rate of digestibility *in vivo* as feed supplements. It will also be necessary to evaluate the release of various nutrients in the rumen, when the basal diets of ruminants are supplemented with Calliandra and Gliricidia. The challenge is to understand the best time to feed the shrub legumes in order to gain maximum response in terms of production from the ruminant animals. If investigations show that their foliage can improve ruminant production when incorporated in low quality basal diets at a specific time, farmers in tropical and sub-tropical regions may be advised to grow the legumes along side their crops as a good source of protein supplement for low quality basal diets during the dry period.

2.4.9 Short Chain Fatty Acid Production

The amounts of SCFA produced in the rumen of sheep over a period of 24 hours have been reported to be in the of range 300-400g (Bondi, 1987) and 70-150 mmol/L (McDonald *et al.*, 2002). These values could vary depending on the type of diet the ruminant animal was fed and the time that had lapsed since the ruminant animal had the last feed. Previous work on sheep fed a diet of chopped Lucerne hay, showed the concentration of SCFA in the rumen to be 113 mmol/L, whereas when sheep were fed on ground hay or young rye grass herbage the concentration reduced to 105 mmol/L and 107mmol/L respectively (McDonald *et al.*, 2002). In similar studies where diets were examined for the effect on SCFA production in sheep that were fed a mixed diet of 50 % wheaten hay and 50 % Lucerne hay, the mean value per unit weight of fodder was found to be 5 moles/Kg dry weight (Gray *et al.*, 1966). In a case where the sole diet was hay fed to sheep, SCFA production was observed to range between 3-5.5 mmol/kg DMI which was equivalent to 7.5-11.7 mmol/kg

digestible organic matter intake (DOM) (Weller *et al.*, 1967; Nozière *et al.*, 2000).The ratio between DOM:SCFA in the rumen is usually around 1:10 when animals are on a hay or grass diet (Martin *et al.*, 2001).

The effect of feed intake on SCFA production was reported by Gray *et al*. (1951). This review has highlighted the importance of synchronizing the availability of SCFA and the energy yielding substrates for ruminal microbial activity.

To understand the potential of Gliricidia and Calliandra N as supplements to a low quality basal diet, it is necessary to determine the utilisation of N in the rumen from the shrub legume supplementation in the presence of readily available SCFA.

2.5 KENAF SEEDS (*Hibiscus cannabinus L.*)

It often can be more beneficial for producers to grow forages for livestock feeding than to purchase protein supplements (Phillips *et al.*, 2002). One novel feed that can be used to overcome low CP during a time when its supply is limiting in ruminant diets is Kenaf seeds. Kenaf is grown in many countries mainly for fibre supply (Phillips *et al.*, 2002). However, it is a multipurpose tree with diverse properties and has been in existence for over 6000 years (Webber and Bledsoe, 2002). The origin of this plant is not clear, although there are speculations that it was first introduced to India from Africa (Cheng *et al.*, 2004). Kenaf is commercially cultivated in more than 20 countries, with China, India and Thailand leading in productivity (Cheng *et al.*, 2004).

Kenaf is a warm season annual fibre crop very similar to cotton, okra, and hibiscus. Kenaf is planted after the frost period in the United States of America and is localised in the southern states. In Australia it is grown as a summer crop in some tropical regions for fibre (DPI, 2007), but since the drop in price of sugar in 1999 it is being introduced as an alternative crop to sugar cane as a livestock feed. The novelty of Kenaf seed cannot be ignored as it has high potential as a protein and energy

supplement in livestock nutrition. In India, China and Thailand it is incorporated in various farming systems.

Kenaf can be grown in a variety of soil types, which range from high organic peat soils to sandy desert soils (Webber *et al.*, 2002). However it thrives in well drained fertile soils with a neutral pH. Kenaf has been known to withstand late season flooding in the United States of America and can thrive in low soil fertility and a wide rage of soil pH values (Webber *et al.*, 2002). It is drought tolerant, which makes it a suitable crop in many subtropical and tropical farming systems.

For maximum yield, N fertilizer application is recommended, although different agro-ecological zones will respond differently to fertilizers (Webber *et al.*, 2002). Seed yield has been reported to range between 997 kg/ha in Mexico to 3819 kg/ha in Florida (Webber *et al.*, 2002).

Immature Kenaf plant is leafy and contains a high level of N that can be used in animal feeding. Although Kenaf is mostly grown for fibre, the entire plant, stalk and leaves can be used for livestock feeding (William *et al.*, 1996). The CP content of the various components of the plant is high. The leaves of Kenaf contain about 14 % to 34 % CP, the stalk between 2 % to 12 % and the whole plant CP ranges from 6 % to 23 % (Webber *et al.*, 2002). The chemical composition of Kenaf is summarised in Table 2.2. The total CP of Kenaf seed ranges from 26 % to 35 % and 15 % - 20 % fat (Webber *et al.*, 2002; CASCO, 2003).

2.5.1 Nutritive Value

An important aspect of Kenaf seeds as a potential supplementary source for ruminant animal feeding is its high nutritive value which includes its chemical composition (Table 2.2) and physical characteristics.

Table 2.2 Chemical composition of Kenaf seed (CASCO, 2003)

Seed composition	% in DM
Moisture	11
Crude protein	26
Ash	4
Crude fibre	12-26
NFE	13
Calcium	0.2
Phosphorus	0.6

Kenaf seeds are rich in particular amino acids which are limiting in natural forages and grasses but are similar to those found in Calliandra and Gliricidia (see Section 2.4.2). These are histidine, tyrosine, lysine and arginine (Charles *et al.*, 2002).

Table 2.3 Fatty acids composition of Kenaf and other common oil seeds

	Fatty acid (% in DM)						
Seed type	**Oleic**	**Linoleic**	**Alpha-Linoleic**	**Archidonic**	**Stearic**	**Palmitic**	**Palmitoleic**
Kenaf[1]	28	44.9	0.5	-	6	19.1	1.6
Cotton[2]	31	44	-	2.5	2	19	-
Sunflower[2]	18.7	69.4	0.3	-	4.2	6	-
Rape[2]	13.5	17	7.5	56.3	2	3.5	0.2
Tallow[2]	40.7	2	-	2.5	24.1	27	2
Peanuts[2]	52	27	-	-	4.5	7	1.5
Soybean[2]	26	51	5	-	2.5	7.8	0.4

[1](CASCO, 2003), [2] (Almad and Lele, 2007).

The DMD of feeds eaten by ruminant animals is highly affected by feed composition. For example, the fat content of a supplement offered to animals on a basal forage diet could affect significantly the nutritive value of the particular diet (Kucuk *et al.*, 2003). A high fat content in ruminant diets may reduce feed intake, rates of fibre digestion and intestinal absorption of nutrients, and also may increase

the molar ratio of propionate to acetate in the rumen (Michel and Chilliard, 1997). The major detrimental effect of high dietary fat in ruminant animal diets is on ruminal fermentation. A feed that has the potential to interfere with the normal function of the rumen microbes is likely to depress digestibility, inhibit nutrient accretion and therefore result in nutrient losses that could otherwise be beneficial in improving animal productivity.

Fats coat feeds and hence inhibit microbial attachment and enzymatic activity during ruminal fermentation. Fats also have been found to exhibit cytotoxic effects on cell membranes which interfere with energy metabolism. Digestibility of saturated fatty acids (e.g palmitic and stearic acids) decreases as the chain length increases whereas unsaturated fatty acids (e.g oleic, linoleic, linolenic and arachadonic acids) have been reported to improve digestibility in ruminant animals (Palmquist, 1994; Volden, 1999). The latter acids are found in relative concentrations in Kenaf seed. Therefore, it is likely that within defined inclusion limits, Kenaf seed supplement added to low quality forage could improve digestibility of the diet. The supplement of Kenaf seeds also would act as an energy source due to its fat content.

Ruminal ammonia concentration is reported to be affected by an inclusion of 6 % of DM as dietary fat when Soybean oil was supplementing brome-grass hay basal diet in ewes (Kucuk *et al.*, 2003). The fat content of Kenaf seed is about 20 % so it is probable that the high fat content in Kenaf seed could have a similar effect on ruminant feed digestibility. In contrast, the high soluble protein present in Kenaf seed could promote microbial protein synthesis and improve digestibility.

Inhibition of microbial activity and reduced digestibility was observed when ruminant animals were offered 2-3 % dietary fat and in steers, a dietary fat of 5 % in feeds composed of equal proportions of forage and concentrate resulted in a reduced total tract digestibility of organic matter (Elliot *et al.*, 1997; Kucuk *et al.*, 2003). Similar observations were made when 6 % supplementary fat was fed to steers, ruminal digestibility of OM, NDF, starch and feed N and total tract OM digestibility were reduced (Kucuk *et al.*, 2003). Therefore utilisation of Kenaf seeds as a feed

supplement will need to be evaluated to determine the effect of the high fat content on digestibility and nutrient accretion in ruminant animals.

Despite the detrimental effects of fat on digestibility, there are beneficial effects such as the ability to increase the energy density of a feed in ruminant animal diets, an increase in the absorption of fat soluble nutrients and a reduction in dustiness of a feed (NRC, 2001).

Fat is a general term used to describe compounds that are high in long chain fatty acids (FAs). The FAs are the energy rich entities of fat. Oil seeds are mostly made up of triglycerides that are rich in unsaturated fats (Palmquist, 1994). During ruminal digestion, the etherified FAs, mainly the triglycerides, are rapidly hydrolyzed by the lipolytic microorganisms to a free form (Elliot *et al.*, 1997; NRC, 2001). The hydrolysis of dietary fatty acids in the rumen was earlier reported by other workers (Michel and Chilliard, 1997).

Kenaf seed is likely to promote the production of SCFA when supplemented to low quality hay. The high protein content in Kenaf seeds would produce higher SCFA than feeds that are low in proteins. The proportions of SCFA production per unit of protein and carbohydrates will depend on the rate of fermentability. Results of studies reported by Blummel and Bullerdieck (1997) indicate that the rate of SCFA production per unit of protein fermented in the rumen is lower than per unit of carbohydrate. It would be interesting to investigate the effect of the Kenaf proteins on production and utilization of SCFA by the rumen microbes.

Little or no work has been carried out on the potential of Kenaf seeds as a feed supplement to ruminant animals. It is speculated that Kenaf supplementation could have similar effects to sunflower or tallow supplementation (Markus *et al.*, 1996). Part of this project will investigate the potential of Kenaf seed as a supplementary source of protein to low quality hay and the effect of the milled seed supplement on digestibility of the diet.

2.6 METHODS OF EXAMINING NITROGEN UTILISATION IN THE RUMEN

In the current study the N content in feeds, feed residues, faeces and urine was determined by the use of the Kjeldahl method (AOAC., 1995). This method has been used for a long time to examine N balance in nutritional trials. During the experimental period, the main parameters that would affect the accuracy of the method were addressed, and these are outlined below.

Sampling

The animals were fed on their respective dietary treatments and all samples were collected and measured after every 24 hrs. To avoid any inconsistency in data collection, feeding and sample collection were carried out at the same time every day during the experimental period.

Ammonia production in the rumen

Satter and Slyter (1974) indicated that 50 mg/L of ammonia in the rumen was the minimum concentration required to promote maximum microbial growth. Observations from investigations carried out by Mehrez *et al.* (1977) showed that ammonia concentration in the rumen at levels as high as 235 mg/L were necessary for efficient microbial activity which could result in maximum digestion of fibre. Supplementation of Calliandra and Gliricidia to a low quality basal diet could elevate the ammonia concentration in the rumen and promote microbial activity. However, the rate at which ruminal micro-organisms utilise ammonia does not correspond to the rate of ammonia production during fermentation. Ammonia production has been observed to be higher than the rate of utilisation (Russell *et al.*, 1992). An excessive concentration of ammonia at a given time in the rumen does not indicate inefficient utilisation by the ruminal microbes.

2.6.1 Blood Metabolites

Blood and urine urea

Excess rumen ammonia is absorbed into the blood, transferred to the liver where it is converted to urea and about 80 % is excreted through the kidney in urine (Bondi, 1981). The ammonia concentration in the rumen will influence the rate at which urea enters the rumen. A change of diet that is likely to cause an increase in the quantities of ammonia concentration in the rumen could also cause an increase in plasma urea concentration, decreasing the amount of urea entering the rumen (Bohnert *et al.*, 2002). Approximately 10 - 42 % of the dietary N enters the blood as urea (Huntington and Archibeque, 1999). An increase in N intake through supplementation with Calliandra and Gliricidia in a low quality diet could reduce the rate of urea being transferred to the rumen.

Glucose concentration in the blood

The plasma glucose concentration in sheep normally ranges between 2.8 mM-4.4 mM or 40-70 mg/100 mL (Bondi, 1987). The rate at which glucose is synthesized in animals is dependant on the production status of the animal and the availability of the glucose precursors for gluconeogenesis. Growing, lactating and pregnant animals would require a higher level of glucose than animals that are on normal maintenance. Therefore, inclusion of Calliandra and Gliricidia as supplements to a low quality basal diet, could improve the gluconeogenesis process in ruminant animals.

CHAPTER 3

EXPERIMENTS 1-4

EFFECT OF FEEDING TIME ON THE UTILISATION OF *CALLIANDRA CALOTHYRSUS* AND *GLIRICIDIA SEPIUM* PROTEINS IN SHEEP

3.1 INTRODUCTION

Gliricidia and Calliandra have significant potential as protein supplements in diets of ruminant animals. The legumes may contain up to 28 % crude protein (CP) in their dry matter (DM) (see Table 2.1). In addition, the CP in these legumes contains a relatively high concentration of the essential amino acids lysine and methionine; which generally are limiting in forages (see section 2.4.2).

Results of previous studies in this laboratory (Widiawati, 2002) indicate that the proteins in Gliricidia and Calliandra are degraded at different rates in the rumen of sheep. Gliricidia is degraded at a faster rate in the rumen compared to Calliandra. These findings suggest that supplementing Gliricidia or Calliandra at the same time hay is fed could result in significant amounts of protein losses as ammonia through faeces and urea through urine. These protein N wastages could be reduced if supplementation of the legumes was offered when a large quantity of energy yielding substrates were present in the rumen.

The effective DM degradability of Gliricidia, Calliandra and hay during incubation in the rumen at six hours was 45 %, 29 % and 23 % respectively (Widiawati, 2002). These results indicate that feeding any of the two legumes simultaneously with hay is likely to result in N losses due to the slow rate at which the energy yielding substrates from hay are likely to be made available for N utilisation by the rumen microbes. It is probable that for the N from the legumes to be optimally utilised, supplementation time of the two legumes should be delayed. It would also mean that Gliricidia leaves should be supplemented much later than Calliandra leaves. It would therefore be necessary to match up the availability of SCFA from the low quality

basal hay diet in the rumen with the N from the legumes. Therefore, supplementation should probably be offered at 6 hours for Gliricidia and 2 hours for Calliandra after hay has been fed.

However, investigations on the synchronization of N from Gliricidia and Calliandra with the SCFA from low quality basal feed for ruminant animals have not been carried out. It would be necessary to investigate both the effect of changing the feeding pattern of the shrub legumes to ruminant animals, and the promotion of efficient utilisation of N by the rumen microbes from the two shrub legumes when used as supplements to low quality basal diets.

Three hypotheses were therefore proposed in relation to supplementation of a low quality forage basal feedstuff and examined in Experiments 1-4.

i. Gliricidia proteins would be utilised most efficiently if Gliricidia were to be fed to a ruminant animal six hours after the basal feedstuff had been fed.
ii. Calliandra would be utilised most efficiently if Calliandra were to be fed to a ruminant animal two hours after the basal feedstuff had been fed.
iii. Calliandra and Gliricidia proteins will be utilised most efficiently if Calliandra and Gliricidia were to be fed to a ruminant animal two hours after the basal feed had been fed.

3.2 EXPERIMENT 1

THE EFFECTS OF TIME OF FEEDING OF FRESH GLIRICIDIA LEAVES SUPPLEMENT ON DIETARY PROTEIN UTILISATION IN SHEEP

The aim of Experiment 1 was to compare the effect of different times of feeding fresh Gliricidia leaves on the utilisation of its CP in sheep on a low quality basal feed.

3.2.1 Materials and Methods

As the materials and methods used in Experiments 1 to 4 were common to all, details of these will be presented in this section. The experiments were carried out in the Nutritional Physiology and Metabolism Unit at the School of Veterinary and Biomedical Sciences, James Cook University (JCU). The experiments were undertaken with the approval of the JCU Human and Animal Experimentation Ethics Committee.

Because of ease of handling and relatively low cost of feeding, sheep were used as the ruminant experimental model.

a. Animals and management

Tropical Merino wethers, six months of age and weighing approximately 16 kg were obtained from a commercial farm in Western Queensland. On arrival at JCU, the animals were transferred into three concrete floor pens (each 3.88 m x 3.65 m) at eight wethers per pen. The animals were ear tagged with identification numbers, weighed and drenched with Ivomec broad spectrum oral anthelmintic (Merial Ltd., Australia). Each was vaccinated with Ultravac 5 in 1 vaccine (CSL Ltd., Australia) against enterotoxaemia, tetanus, black disease, malignant oedema, black leg and swelled head in rams.

The animals were fed mixed grass hay daily at 0800 and at 1600 hours.

After one week in the concrete floor pens, the animals were moved to individual slatted floor pens (1.2 m x 0.6m) and introduced to their experimental basal hay diet. At the start of each experiment each animal was transferred to a metabolism cage (0.6 m x 1.2 m) where it was introduced to its complete experimental diet.

b. Experimental design

Sixteen wethers were used in Experiment 1. Based on live weight the animals were divided into four balanced groups of four animals per group. Each group was allocated at random to either of the four dietary treatments:

i. low quality grass hay (H) plus Gliricidia fed at the same time as H (HGO)

ii. Gliricidia leaves fed 2 hours after the H was offered (HG2)

iii. Gliricidia leaves fed 6 hours after the H was offered (HG6)

iv. Gliricidia leaves fed 12 hours after the H was offered (HG12)

As a matter of routine practice, where relevant, pens and cages were cleaned each morning before the resident animals were fed their respective diets. All animals always had free access to mineralised stock blocks (Ridley Agri- Products Pty Ltd. Australia) (see Table 3.1) and clean drinking water. All animals were inspected twice daily for signs of ill health and when required, were administered appropriate veterinary treatment.

The animals were weighed (before feeding) on the morning of the same day of each week.

The animals were kept in these pens in between measurement periods for Experiment 1-4.

Table 3.1 Composition of mineralised stock block offered *ad libitum* to the animals.

Mineral type	**Quantities**
Salt (Nacl)	75 – 85 %
Min. Calcium (Ca)	4.9 %
Min. Phosphorus (P)	1 %
Min. Sulphur (S)	2 %
Min. Copper (Cu)	600 mg/Kg
Min, Cobalt (Co)	60mg/Kg
Min. Iodine (I)	60 mg/Kg
Min. Zinc (Zn)	1000 mg/Kg
Min. Ferrous Iron (Fe^{++})	1100 mg/Kg
Min. Selenium (Se)	5 mg/Kg

c. Experimental procedures

On transfer to the metabolism cages (Section 3.2.1a) the wethers underwent an adaptation period of seven days during which the animals were expected to adapt to the experimental environment and diet. This period was followed by a five-day measurement period during which feed and water consumption, and faecal and urinary excretions were recorded and blood and rumen liquor samples were taken.

Each animal in the metabolism cages was fitted with a urine collector (a moulded silicon cup) joined to polyethylene tubing with an outer diameter (OD) of 10 mm and an internal diameter (ID) of 8 mm. The collector was fitted around the penis of the wether and secured in place by a harness. The urine was then diverted through the polyethylene tube into a 2.5 L Winchester bottle placed in a plastic bucket to prevent any urine loss that could occur through overflow or accidental leakages (Figure 3.1). An aliquot of 20 mL of 9 M hydrochloric acid (HCl) was dispensed into the Winchester bottle to prevent any microbial growth in the urine during collection, by maintaining a pH value below 4. A plastic bag was attached to the separator under the cage (Figure 3.1) to collect excreted faeces from the animals.

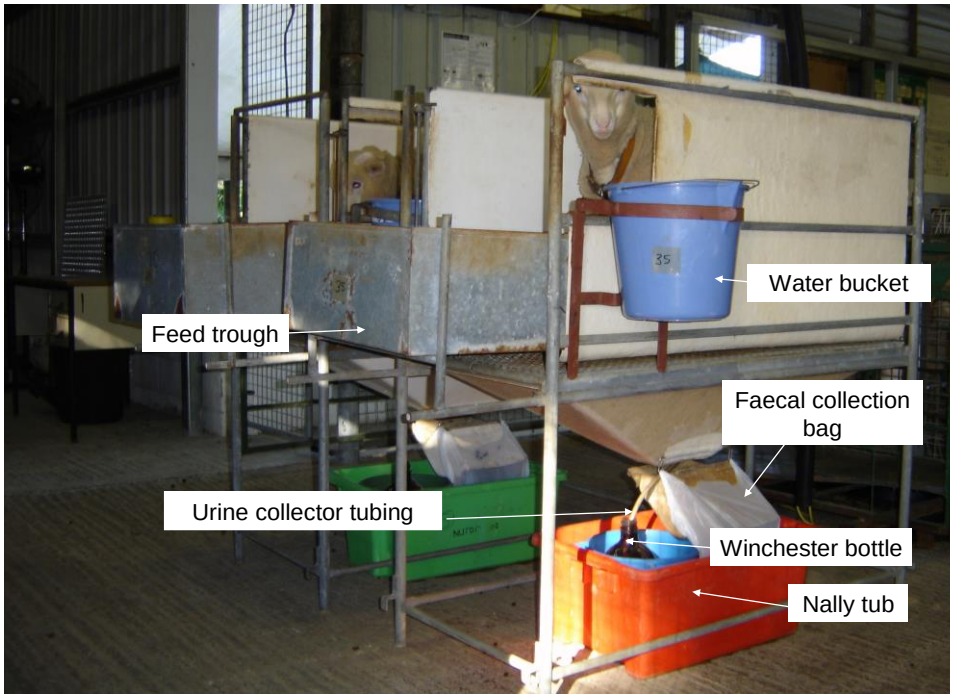

Figure 3.1 Sheep in a metabolic cage fitted with a urine collector and faecal collection bag, in the Metabolism Unit, JCU.

Feeds and feeding

During the experimental period the animals were fed their respective diets as outlined in Section 3.2.1b. The low quality hay was purchased from a local commercial supplier while the Gliricidia leaves were harvested daily from the JCU legume plots. The leaves were harvested from plants that were cut six months previously. Leaves were harvested at 1600 hours each day, kept at 23 ^{o}C overnight in the temperature-controlled room of the Metabolism Unit and fed to animals the next day.

The amount of DM offered to each animal (equivalent to 2.5 % live weight) was designed to be eaten completely by the animal. The composition of the daily ration fed to each animal each day was 70 % hay and 30 % Gliricidia on DM basis. Hay was fed at 0800 hours and the Gliricidia leaves were offered according to times outlined in Section 3.2.1b.

Liveweight

In addition to the routine weekly weighing (see Section 3.2.1a) sheep involved in the experiment also were weighed at the start and end of the experimental period.

Feed samples

Before the sheep received their daily ration at 0800 hours, feed residue from the previous feeding remaining in each feed bin was collected, weighed and recorded. A 20 % sub-sample of each was taken and dried in a fan-forced oven (Wessberg Martin, Wessberg and Martin Pty. Ltd. Australia) for 24 hours at 100 ^{o}C to determine its DM content. A sub-sample (200 g) of each feedstuff offered to the animals was taken each day and dried as described. The dried feed sub-samples were pooled while the dried feed residues were pooled for each sheep. Each pooled sample was thoroughly mixed and ground using a Cross Beater Mill (Retsch, type KG SK1, West Germany) with a 1 mm bottom sieve. A 100 g ground sample was stored in a 125 mL air-tight clear glass jar (COSPACK, Australia) at room temperature (24 ^{o}C) pending laboratory analysis.

Faecal samples

Each day at 0800 hours, during the five-day measurement period, the faeces excreted by each sheep were weighed and a 20 % sub-sample dried in an aluminium tray in the drying oven. The dried sample was ground and stored as described for the feed and feed residue.

Urine samples

During the five-day measurement period urine samples were collected as described earlier (Section 3.2.1c). The Winchester bottle containing excreted urine under each metabolism cage was exchanged with a clean bottle each day at 0800 hours. The urine was filtered through organza into a graduated measuring cylinder and its volume recorded. For each sheep, 20 % of urine excreted was added to the pooled urine sample for that animal. The pooled sample was stored at -20 ^{o}C pending analysis.

Rumen fluid

The day after the five-day measurement period, rumen fluid from the relevant sheep was collected three hours after the legume supplement was offered. Approximately 30 mL of rumen fluid was withdrawn from each sheep via a polyvinyl stomach tube attached to a vacuum pump (Dynavac Model D4, Vacuum Equipment, Australia). The rumen fluid was transferred to a 30 mL McCartney bottle and 0.2 mL of concentrated sulphuric (H_2SO_4) acid was added to prevent any further microbial activity. The acidified sample was then decanted into three 10 mL flat bottom screw cap polypropylene vials and centrifuged at 3000 g for 20 minutes. The supernatant was decanted into a clean set of 10 mL polypropylene vials and stored at -20 ^{o}C pending analysis.

Blood samples

A few minutes before the rumen fluid was taken from the animal, a 10 mL blood sample was obtained by jugular venipuncture from each animal, using a 10 mL vacutainer with heparin as an anticoagulant (Becton Dickinson Vacutainer Systems, U.K). Blood samples were centrifuged at 3000 g for 20 minutes and the plasma transferred by pipettes into 5 mL polypropylene vials and stored at -20 ^{o}C pending laboratory analysis.

d. Laboratory analysis

Dry matter

Determination of DM for use in laboratory analysis was carried out on the feed, feed residue and faecal samples. A 0.2 g sub-sample from either the feed, feed residue or faecal sample was weighed in duplicate into 20 mL glass vials and dried in an oven (Qualtex Model OG36T, Watson Victor Ltd. Australia) at 100^{o}C for 24 hours as described in Section 3.2.1c. The vials plus the sub-sample were transferred to a desiccator to cool to room temperature before they were weighed.

Organic Matter

Determination of OM was carried out by weighing a 0.5 g sub-sample (in duplicate) of feed, feed residue or faecal sample into 30 mL crucibles and burning the sample in a muffle furnace (Ceramic Engineering Furnace, Sydney, Australia) at 600 ^{o}C for 8 hours. The crucible containing the ash was transferred into a desiccator to cool to room temperature after which it was weighed.

Nitrogen

Analysis of N in feeds, feed residues, urine and faeces was undertaken using the Kjeldahl method (AOAC., 1995). A 0.2 g of the pooled ground samples of feed, feed residue or faeces was weighed in duplicate into digestion tubes. A volume of 1 mL of urine was pipetted in duplicate into the digestion tubes for analysis. A volume of 6 mL of concentrated H_2SO_4 and one kjeltab (3.5 g K_2 SO_4, and 3.5 mg Se) was added to each tube. The digestion tubes with the samples were then placed in a preheated digestion unit (Foss Tector 2040 Digestion System with Exhaust Manifold and 2001 Scrubber Unit) at 420 ^{o}C. The digestion time varied with the type of samples being digested, i.e., feeds for 45-60 minutes; faeces for 60-90 minutes and urine for 45 minutes.

When digestion was completed, the tubes were removed and left to cool to room temperature for about 15 minutes. The samples were then diluted with 30 mL of distilled water each. The tubes were connected to the distilling unit (Foss Tecator System 2100 Distilling Unit) one at a time for distillation. Thirty mL of 40 % (w/v) analytical grade sodium hydroxide (NaOH) (Ajax Chemicals) were dispensed into each digestion tube and distillation (Foss Tecator System 2100 Kjeltec Distillation Unit) commenced for a duration of three minutes. The ammonia produced during distillation was collected into a 250 mL conical flask containing 20 mL of Boric Acid indicator solution [4 % (w/v) of boric acid with 2.5 % of 1 % of both methyl red/bromocresol green]. During distillation, the solution in the conical flask gradually turned green. The solution was then titrated with 0.5 normal hydrochloric acid (HCl) using a digital titrater (Eppendorf Top Buret Model M/H). At the end point of titration, the green solution turned pink. The volume of HCl used to reach

the end point was recorded and used for the calculation of the amount of N in the sample.

Urea, ammonia, glucose

The Cobas Mira Analysis Systems (F. Hoffmann- La Roche Co. Ltd. Diagnostica and Basle, Switzerland) were used to analyse urinary urea, plasma glucose and urea, and rumen ammonia. The Cobas Mira analyser is a discrete random access analyser whose reagents are uniquely formulated to fit the specific quantitative analysis of urea, glucose and ammonia in plasma, ruminal fluid or urine. The analyser consists of a filter photometer with six interference filters which allow measurement at different wave lengths within a run. The analyser chamber is temperature controlled by the air-bath principle which ensures rapid heat transfer is at equilibrium with the reaction mixtures.

The method employed is based on an enzymatic reagent, where standards for specific analysis are reconstituted as per recommendations detailed in the product summary document that is purchased with the reagents. Once the reagents are prepared, calibration is carried out by the use of an aqueous standard based calibrator with an assigned value traceable to a primary standard. Each specific analysis has a calibrated standard which is used as the control (Tiffany *et al.*, 1972).

3.2.2 Calculations

Dry matter content was determined using the following equation:

$$\text{DM (\%)} = \frac{\text{Dry wt of sample x 100}}{\text{Fresh wt of sample}} \qquad \textit{Equation 1}$$

The Ash content was determined using the following equation:

$$\text{\% Ash (in DM)} = \frac{\text{Dry wt of ash x 100}}{\text{Fresh wt of sample x \% DM}} \qquad \textit{Equation 2}$$

Organic matter (%) was determined as follows:

$$\% \text{ OM (in DM)} = 100 - \% \text{ Ash (in DM)} \qquad \textit{Equation 3}$$

The N content of the samples was calculated using the following equation:

$$\% \text{ g N} = \frac{\text{Normality of acid x (mL sample titrant – mL blank titrant x 14.01}}{\text{Dry wt of sample (g) x 10}} \qquad \textit{Equation 4}$$

Where 14.01 is the molecular weight (g) of N and 10 is a constant used as a factor to change value from g per 100 mL to g per 1000 mL

For urine N calculation, the N value of the sample weight in the formula is replaced by one mL, which was the amount of urine sample digested.

Crude protein was calculated as follows:

$$\text{CP (\%)} = \% \text{ N x } 6.25 \qquad \textit{Equation 5}$$

Dry matter intake (DMI) was calculated as follows:

$$\text{DMI (g)} = \text{DMO} - \text{DMR} \qquad \textit{Equation 6}$$

Where DMO is the total DM offered (g); and DMR is the total DM refusal (g).

Dry matter digestibility (DMD) was derived using the following equation:

$$\text{DMD} = \frac{\text{DMI - DMF x 100}}{\text{DM}} \qquad \textit{Equation 7}$$

Where DMF is the faecal DM excreted.

Nitrogen retained (NR) was calculated as follows:

$$\text{NR (g)} = \text{NI} - \text{NF} - \text{NU} \qquad \textit{Equation 8}$$

Where NI is the total N intake by the animal (g); NF is the total N in faeces (g) and NU is the total N in urine (g)

Digestible organic matter (DOM) was calculated using:

$$DOM\ (\%) = \frac{OMI - Faecal\ OM \times 100}{OMI} \qquad \textit{Equation 9}$$

DOM % in DM (DOMDM) was calculated using the following equation:

$$DOMDM\ (\%) = \frac{OMI \times DOM\% \times 100}{DM} \qquad \textit{Equation 10}$$

3.2.3 Statistical Analysis

Tabulation of data was carried out by the use of Microsoft® office Excel 2003 for Windows XP 2003 (Microsoft Corporation, USA). Graphs were produced by the use of Microsoft® office Excel 2003 for Windows XP 2003 (Microsoft Corporation, USA).

To show the significant effects of the different dietary treatments analysis of variance (ANOVA-Cohen, 1988) was used. The differences in the mean values were compared by the least significant differences (LSD) test (Steel and Torrie, 1980). The statistical software package, SPSS 14 for Windows (SPSS Inc., Chicago, Illinois, USA) was used to undertake the analysis. Raw data was tabulated using Microsoft® Excel for Windows 2003 (Microsoft Corporation, USA).

3.2.4 Results

Table 3.2 The liveweight (LW) intakes of dry matter (DM), organic matter (OM) and water, digestibilities, concentrations of metabolites in blood plasma, urine and rumen fluid, nitrogen (N) balance and ratio of N intake (NI) to digestible OM intake (DOMI) in wethers fed a basal diet of low quality hay (H) supplemented with Gliricidia leaves offered either immediately with H (HG0), two hours after H was fed (HG2), six hours after H was fed (HG6) or 12 hours after H was fed (HG12).
Within rows, means with different superscripts are significantly different ($P < 0.05$). Values are means ± standard errors (± s.e).

	Dietary Treatments					
Variables	**HG0**	**HG2**	**HG6**	**HG12**	**± s.e.**	**P**
LW (kg)	16.6	16.7	15.1	15.8	0.34	>0.05
Intake (g/d)						
DM	398[a]	443[b]	482[c]	397[a]	10	<0.05
DM (g/kgLW$^{0.75}$)	50[a]	52[c]	60[c]	51[a]	2	<0.05
OM	400[a]	435[c]	476[b]	371[b]	13	<0.05
OM (g/kgLW$^{0.75}$)	47[a]	51[a]	59[c]	48[a]	2	<0.05
Water	1451	1612	917	905	125	>0.05
Digestibility (%)						
DM	53	58	59	54	1.6	>0.05
OM	56	59	61	55	1.9	>0.05
Metabolites in						
Plasma (mM)						
Glucose	3.05[a]	5.32[b]	5.12[b]	5.51[b]	0.32	<0.05
Urea	4.95	5.33	5.13	5.51	0.22	>0.05
Urine						
Urea N (mg/L)	2[a]	2[a]	2[a]	3.6[b]	0.28	<0.05
Rumen fluid (mg/L)						
Ammonia	143[a]	114[b]	110[b]	143[a]	10	<0.05
Nitrogen balance (g/d)						
N intake	6.7[a]	7.2[b]	6.4[c]	6.3[c]	0.14	<0.05
Faecal N	3.2	3.1	3.0	3.0	0.10	>0.05
Urinary N	1.5[a]	1.6[a]	1.2[a]	3.6[c]	0.52	<0.05
N retained	2.0[a]	2.5[a]	2.0[a]	-0.3[b]	0.35	<0.05
N retained as % N intake	30[a]	35[a]	34[a]	- 5[b]	0.36	<0.05
Urine (mL/d)						
Output	1057	869	549	695	82	>0.05
NI:DOMI ratio	0.033[a]	0.035[a]	0.029[b]	0.041[c]	0.01	<0.05

Intake

Data on the intakes of DM and OM, and estimated metabolisable energy (ME) ratios of NI to DOMI, are presented in Table 3.2.

Intake of DM and OM by sheep on the dietary treatments HG2 and HG6 were significantly higher than by animals on HG0 and HG12 diets ($P < 0.05$). The highest intakes were observed in sheep on the HG6 dietary treatment. Intakes of DM and OM expressed per unit of metabolic live weight were highest in sheep on the HG6 treatment. These animals also had the highest N intake ($P < 0.05$).

Water intake and urine output

Animals on HG0 and HG2 dietary treatments were observed to have the highest water intake (Table 3.2). The amounts of urine excreted by the animals were also higher.

Urinary urea

Sheep on the HG12 treatment had a significantly higher ($P < 0.05$) amount of urea excreted through urine than those animals on the other three dietary treatments.

Rumen ammonia

Sheep on the HG0 and HG12 dietary treatments had significantly higher rumen ammonia concentrations ($P < 0.05$) than sheep on HG2 and HG6 treatments.

Blood plasma metabolites

Glucose

Plasma glucose concentration in sheep on the HG0 diet was significantly lower than values observed in animals on the other treatments (Table 3.2).

Urea

No significant differences among the groups were observed for plasma urea concentration ($P > 0.05$).

Nitrogen balance

While there appeared to be no differences among treatment groups in N loss through faeces, a significant urinary N loss was observed in animals on the HG12 diet (Table 3.2). The highest N retention was observed in sheep on the HG2 diet ($P < 0.05$).

Ratios of intake of N and DOM

There was significant difference observed among the treatment groups in the ratio of intake of N and DOM.

3.2.5 Discussion

The hay that was used in Experiment 1 was of low quality with a DM of 90 %. Its crude protein content of 5.4 % was lower than the limiting level of 8 % that is needed to support normal microbial activity in the rumen. Supplementary proteins from Gliricidia leaves would have been sufficient to promote efficient microbial function. The amount and type of feed offered to the animals on the basis of live weight was the same across dietary treatments to minimise variations that could be caused by variation in feed intake. Only the times that Gliricidia leaves were offered to the animals were different.

It was anticipated that an altered supplementation time of Gliricidia leaves could result in enhanced microbial activity in the rumens of the fed sheep.

Although the amounts of feed offered to the animals across treatment groups were similar, the intakes of DM were different among the four treatment groups (Table 3.2). Such differences in intake probably reflect differences in the efficiency at which the different diets were utilised in the rumens of the experimental animals. Expressed on the basis of metabolic live weight the mean DMI of HG2 and HG6 sheep were significantly higher than the mean DM intakes of the HG0 and HG12 sheep. Consistent with this DMI difference between groups was the difference in mean DM digestibility in which the mean values observed in the HG2 and HG6 sheep were

greater than those in the HG0 and HG12 sheep. Since the basal low quality hay diet was low in N, the supply of N by the Gliricidia leaves supplement might be expected to improve the utilisation or digestibility of the basal hay diet. However, this only may be suggested as no control group of animals fed the hay only was included in the comparison. The DMD values of 53-54 % observed in the HG0 and HG12 groups are similar to values observed in sheep fed low quality hay and Gliricidia leaves (Widiawati, 2002). In her study Widiawati (2002) observed a (Table 3.2) lower digestibility value (49 %) for such low quality hay diet.

In the current experiment, feeding the Gliricidia leaves supplement two hours and six hours after the basal hay was offered, improved the DMD values of the diets by four to six percent. As the composition of the diets was the same across dietary treatments, it is suggested that the difference in digestibility values observed would have been due to the difference in the timing of offering the supplement of Gliricidia leaves to the animals. From previous studies (McDonald *et al.*, 2002) it was observed that the breakdown of dry forages in the rumen of sheep or cattle would result in a slow increase in the concentration of energy yielding substrates, short chain fatty acids (SCFA) in rumen liquor. The concentration peaks at four to six hours from the start of feeding. It is possible that feeding the Gliricidia leaves between two to six hours after the basal hay was fed in the current experiment, resulted in better utilisation of the available SCFA in the rumens of those sheep.

Feeding Gliricidia leaves immediately with the basal hay diet or at 12 hours after the hay was offered to the animals probably resulted in a greater wastage of the legume N which was released into rumen liquor of the HG0 sheep much earlier than the SCFA from the hay or the HG12 sheep much later than the SCFA from the hay. Indeed, this is consistent with the observations that the concentrations of rumen ammonia in the HG0 and HG12 sheep were significantly higher than those observed in the HG2 and HG6 sheep (Table 3.2). Increased rumen ammonia concentration in the rumen would result in leakage of ammonia across the rumen wall and into the bloodstream of the animal. The liver of the animal would detoxify the incoming ammonia by converting it to non-toxic urea. The urea formed could be recycled to

the rumen via saliva or excreted in urine. Taking into account the concentrations of urea in urine and the volume excreted by the experimental animals (Table 3.2) it may be calculated that more urinary urea was excreted by the HG0 and HG12 animals compared with the HG2 and HG6 animals.

The volume of urine excreted by the experimental sheep tended to correspond with the volume of water drunk by the animals. It is known that a major determinant of water intake is DMI. In the current experiment, this is evident in the HG2 animals in particular. The amount of water consumed by the HG0 animals was higher than that consumed by the HG6 animals, although the mean values of DMI for these two groups of animals were not statistically different. It is probable that the difference between the groups in water intake was due to the greater need for HG0 animals to excrete urea in urine. With regards to the HG12 animals, a different physiological strategy for excreting urea evidently was employed. These animals appeared to have concentrated urea in their urine (Table 3.2) and had drunk and excreted water and urine respectively in amounts similar to those for the HG6 sheep. The plasma glucose concentration values of 3.05 mM to 3.68 mM are within the normal range of 2.8 mM to 4.4 mM reported by Kaneko *et al.*, (1997).

Consistent with the HG2 DMI and diet digestibility values the glucose concentration in the HG2 sheep was highest at 3.68 mM. The similar value to this in the HG6 sheep might be explained by the high digestibility of the diet consumed by these animals. Similarly, the lower glucose concentration in the HG0 sheep might be explained by their DMI and the lower digestibility of the HG0 diet. However, the relatively high plasma glucose concentration in the HG12 sheep is difficult to explain as comparatively (see Table 3.2) the mean DMI of the HG12 animals and the digestibility of the HG12 diet was low. It is possible that the greater gap (8-12 hours) in the availability of SCFA and legume N in the rumens of these animals might have resulted in increased rate of gluconeogenesis and thus glucose concentration in the blood plasma of animals.

The dietary NI of 6.3 g/d - 7.2 g/d by animals in the current experiment (Table 3.2) were sufficient to supply the daily N requirement of maintenance of growing sheep (ARC, 1980). The amount of N retained by sheep across dietary treatment groups was in the main low and similar. This observation might be expected, given the restricted feeding employed and the smaller number of animals used in the comparison. The N balance method may not be sensitive enough to detect differences between small numbers of animals subject to restricted feeding.

It is clear from the results of the current experiment that the timing of offering Gliricidia leaves as a supplement to a low quality basal hay diet could significantly affect the extent to which the diet would be utilised by sheep. The improvements in DMI and digestibility of the diets in the HG2 and HG6 sheep are evidence of this. Offering the legume supplement either at the same time the basal hay is fed or 12 hours after the hay is fed would lead to greater wastage of the legume N. It appears that offering the supplement two to six hours after the basal hay diet is fed would improve the efficiency of utilisation of N in the diet by ruminant animals.

Since there were indications of an effect of the timing of supplementation on DMI, it would be of interest to investigate the effect of timing of the two and six hours on the DMI by sheep offered the basal hay diet *ad libitum*.

3.3 EXPERIMENT 2

THE EFFECT OF DIFFERENT TIMES OF FEEDING OF FRESH CALLIANDRA LEAVES SUPPLEMENT ON DIETARY PROTEIN UTILISATION IN SHEEP

The aim of Experiment 2 was to examine the effect of feeding fresh Calliandra leaves immediately with or two hours after a basal feedstuff, hay, was fed on the utilisation of the ration.

3.3.1 Materials and Methods

The animals used, location, their housing and routine feeding and health management were the same as described in Experiment 1 (section 3.2.1).

a. Experimental design

Twelve sheep were stratified for live weight, divided into two balanced groups of six animals per group and allocated to either of two dietary treatments (6 sheep group) as follows:

i. Low quality hay (H) plus Calliandra immediately with H (HC0) and

ii. H plus Calliandra at two hours after H was fed (HC2). The ration of H and Calliandra leaves were fed in the ratio 70:30 (H:Calliandra) on DM basis.

b. Experimental procedures

Except for the number of animals used and dietary treatments examined, the experimental procedures with regards to adaptation and measurement periods, recordings, sample collection and laboratory analysis, calculations and statistical analysis were the same as described in Experiment 1 and therefore are not being detailed here.

3.3.2. Results

Data on live weights of the sheep, intakes and digestibilities of diets, concentrations of metabolites in blood plasma, urine and rumen and N balance are presented in Table 3.3.

There was no significant difference in DMI and OMI in sheep on the two dietary treatments whether the values were expressed as per animal or per unit of metabolic live weight.

There also was no significant difference in the water intake of sheep between the two dietary treatments (Table 3.3).

With regards to digestibility values, there was no difference between means of the two diets.

Offering Calliandra leaves two hours after the hay was offered resulted in significantly higher ruminal ammonia in those animals compared with the rumen ammonia concentration in animals that received their legume supplement at the same time as the basal hay diet.

Although blood plasma mean concentration values of glucose in the HC2 sheep appeared higher than that in the HC0 sheep, the difference was not significant (Table 3.3).

Table 3.3 The liveweight (LW) intakes of dry matter (DM), organic matter (OM) and water, digestibilities, concentrations of metabolites in blood plasma, urine and rumen fluid, nitrogen (N) balance and ratio of N intake (NI) to digestible OM intake (DOMI) in wethers fed a basal diet of low quality hay (H) supplemented with Calliandra leaves offered either immediately with H (HC0) or two hours after H was fed (HC2).

	Dietary Treatments			
Variables	**HC0**	**HC2**	**± s.e.**	**P**
LW (kg)	18.0	17.0	0.48	> 0.05
Intake (g/d)				
DM	402	417	8	> 0.05
DM (g/kgLW$^{0.75}$)	46	50	2	> 0.05
OM	379	392	7	> 0.05
OM (g/kgLW$^{0.75}$)	43	47	2	> 0.05
Water	1574	1315	130	< 0.05
Digestibility (%)				
DM	50	51	0.5	> 0.05
OM	51	52	2.0	> 0.05
Metabolites				
Plasma (mM)				
Glucose	2.92	3.28	0.18	> 0.05
Urea	4.38 [a]	7.38 [b]	1.50	< 0.05
Urine (mg/L)				
Urea N	1.99	1.79	0.10	> 0.05
Rumen fluid (mg/L)				
Ammonia	119 [a]	139 [b]	10.06	< 0.05
N balance (g/d)				
N intake	6.7	6.8	0.06	> 0.05
N faeces	5.1	4.9	0.12	> 0.05
N urine	1.9 [a]	1.1 [b]	0.40	< 0.05
N retained	-0.3 [a]	0.8 [b]	0.50	< 0.05
N retained as % N intake	-4 [a]	12 [b]	6	< 0.05
Urine (ml/d)				
Output	1074 [a]	877 [b]	98	< 0.05
NI:DOMI ratio	0.034	0.033	0.001	> 0.05

The concentration of plasma urea was higher in the HC2 than the HC0 sheep.

There was no difference in urinary urea concentration between the HC0 and HC2 groups of animals. However, there was a greater volume of urine excreted by the HC0 animals compared with the HC2 sheep.

The animals in the two treatment groups consumed similar amounts of N, but those in the HC0 groups excreted more N in urine and retained significantly less N (Table 3.3). The HC0 animals appeared to excrete more N in the faeces but the differences in the amounts of faecal N between the two treatments groups were not significant.

No significant differences in NI:DOMI ratio were observed between the two treatments.

3.3.3 Discussion

Although the total dietary DM offered to the sheep in the current experiment was restricted to an amount equivalent to 2.8 % of the live weight of the animal, there appeared to be a slight reduction in DMI by sheep on the HC0 dietary treatment (Table 3.3). The apparent difference in DMI between the two treatment groups might have been due to the apparent differences in digestibility values of the two diets. The mean digestibility of the HC2 diet although slightly higher than that of the HC0 diet was not significantly higher. The digestibility values observed for the HC0 and HC2 diets were lower than values observed for the Gliricidia leaves supplemented diets described in Experiment 1 (Section 3.2). This suggests that the Calliandra leaves supplement may not be as effective as Gliricidia leaves supplement in improving the utilisation of a low quality basal hay diet. The fact that the plasma glucose concentration in sheep on the HC0 and HC2 dietary treatments were lower than corresponding values in sheep on Gliricidia leaves supplement observed in Experiment 1 (Section 3.2) is consistent with the above proposition.

It would appear that feeding the Calliandra leaves supplement at two hours after the basal hay diet was offered would result in a higher rumen ammonia concentration (Table 3.3). The higher rumen ammonia concentration is reflected in a higher plasma urea concentration in HC2 sheep. It is probable that the higher rumen ammonia concentration in the HC2 animals would have contributed to a better utilisation of the HC2 diet compared with the HCO diet. This suggestion is consistent with the observation that animals on the HC2 diet tended to consume more DM and the HC2

diet tended to have a higher digestibility value. Furthermore the HC2 animals retained significantly more N than did those animals on the HC0 diet (Table 3.3).

The differences in water intake and subsequently in the amount of urine excreted are difficult to explain. It is possible that the higher amounts observed in the HC0 animals might have been due to a relatively slow rate of passage of digesta through the digestive tract of those animals.

It is concluded from results of the current experiment that feeding Calliandra leaves supplement at two hours after a low quality basal hay diet was offered could improve further the utilisation of the diet by sheep. Much of this improvement was due to the increase in the proportion of N intake that was retained by the animal although the DM and OM digestibility of the diet was not significantly different from the HC0 diet.

3.4 EXPERIMENT 3

THE EFFECT OF DIFFERENT TIMES OF FEEDING GLIRICIDIA LEAVES SUPPLEMENT ON VOLUNTARY FEED INTAKE BY SHEEP

The aim of Experiment 3 was to examine the effects of feeding Gliricidia leaves fed either immediately with a basal diet feedstuff, hay, two hours after or six hours after hay was fed.

3.4.1 Materials and Methods

The animals used, location, their housing and routine feeding and health management were the same as described in Experiment 1 (Section 3.2.1).

a. Experimental design

Fifteen sheep, stratified for liveweight, were divided into three balanced groups of five animals per group and were allocated at random to one of the three dietary treatments:

(i) low quality hay fed at a level equal to 125 % of the animals hay DM intake on the previous day (H) plus Gliricidia offered immediately with H (HG0)

(ii) H plus Gliricidia offered at two hours after H was offered (HG2) and

(iii) H plus Gliricidia offered six hours after H was offered (HG6).

b. Experimental procedures

Except for the number of animals used and the dietary treatments examined, the experimental procedures with regards to adaptation and measurement periods, recordings, sample collections and statistical analysis were the same as described in Experiment 1. These therefore are not described here.

3.4.2 Results

Data on live weights of the sheep, intakes and digestibilities of diets, concentrations of metabolites in blood plasma, urine and rumen and N balance are presented in Table 3.4.

Table 3.4 The liveweight (LW) intakes of dry matter (DM), organic matter (OM) and water, digestibilities, concentrations of metabolites in blood plasma, urine and rumen fluid, nitrogen (N) balance and ratio of N intake (NI) to digestible OM intake (DOMI) in wethers fed a basal diet of low quality hay (H) supplemented with Gliricidia leaves offered either immediately with H (HG0), two hours after H was fed (HG2) or six hours after H was fed (HG6).
Within rows, means with different superscripts are significantly different ($P < 0.05$). Values are means ± standard errors (± s.e).

	Dietary Treatments				
Variables	**HG0**	**HG2**	**HG6**	**± s.e.**	**P**
LW (kg)	16.8	15.9	16.9	0.31	>0.05
Intake (g/d)					
DM	466	407	463	19	>0.05
DM (g/kgLW$^{0.75}$)	53	48	54	1.9	>0.05
OM	441	379	439	20	>0.05
OM (g/kgLW$^{0.75}$)	53	48	53	0.3	>0.05
Water	1152	1052	1138	31	>0.05
Digestibility (%)					
DM	59	51	53	7	<0.05
OM	59	52	55	7	>0.05
Metabolites in					
Plasma (mM)					
Glucose	3.58 ^{a}	2.16^{b}	4.86^{c}	0.78	<0.05
Urea	2.40	1.90	2.68	0.23	>0.05
Urine (mg/L)					
Urea N	1	1	1	0.01	>0.05
Rumen fluid (mg/L)					
Ammonia	177 ^{a}	125 ^{b}	136 ^{c}	15.82	<0.05
N balance (g/d)					
N intake	6.8	6.2	6.8	0.19	>0.05
N faeces	3.7	3.9	4.2	0.13	>0.05
N urine	2.3 ^{a}	1.9 ^{b}	1.6 ^{b}	0.19	<0.05
N retained	0.9	0.5	1.1 ^{a}	0.18	>0.05
N retained as %N intake	13 ^{a}	8 ^{b}	16 ^{a}	2.31	<0.05
Urine (ml/d)					
Output	734	712	698	10	>0.05
NI:DOMI ratio	0.026	0.032	0.028	0.001	>0.05

The lowest intakes of DM and OM were observed in sheep on the HG2 diet. No difference in DMI and OMI was observed between the HG0 and HG6 animals. Water intake reflected the differences among the dietary treatment groups (Table 3.4). Water intake was the lowest in the HG2 animals with no difference between the HG0 and HG6 being observed. The urine output corresponded to the total water intake.

The mean concentration of plasma glucose in the HG2 sheep was significantly lower than those in the HG0 and HG6 sheep. The latter group had the highest mean glucose concentration (Table 3.4). The HG2 group also had the lowest plasma urea concentration with no differences in the mean values of plasma urea between the HG0 and HG6 groups.

Rumen ammonia concentrations were significantly different among the three dietary treatments. The highest concentration was observed in the HG0 (Table 3.4).

Sheep on the HG0 diet excreted the highest amount of N in urine. Those animals on the HG6 diet excreted the lowest amount of urine (Table 3.4). While no significant differences were observed among the treatment groups, the percentage of N retained as % NI in sheep on the HG6 was the highest (Table 3.4).

The ratio of NI to DOM intake were similar across treatment groups.

3.4.3 Discussion

In Experiment 3, each animal in the three dietary treatment groups was offered daily the basal low quality hay at a level equal to 125 % of its hay DM intake recorded for the previous day. This was to allow the effect of the timing of feeding the Gliricidia leaves supplement to be expressed more clearly in DMI and OMI of the diet. The DMI of the animals in the current experiment, contrary to those in Experiment 1, were not significantly different among the treatment groups. The mean intake

expressed per unit of metabolic weight was not much higher than those from corresponding treatment groups in Experiment 1. It is not clear why the Gliricidia leaves did not increase DMI as previously observed, for example, by sheep on the HG6 diet (see Table 3.2). It is probable that the low quality hay, at 5.6 % CP content (see Table 3.4) used in the experiments had limiting factors to its nutritive value other than low N content. That is, while addition of N to the basal hay diet might improve dietary DMI and DMD values, further improvement to dietary utilisation might be achieved by overcoming other limiting factors such as lignified cell walls in the hay.

The acid detergent fibre (ADF) of this feedstuff was not determined but it is likely to be greater than 40 % on DM basis (Van Soest *et al.*, 1991). Thus the breakdown rate of the hay in the rumen would be expected to be relatively low. Accordingly the digestibility and intake of the hay would be limited to the level observed in the current and previous (Section 3.2) experiments.

The fact that the HG6 sheep had a significantly higher concentration of plasma glucose than the sheep in the HG0 and HG2 groups (Table 3.4.1) would suggest that the HG6 animals most probably had the highest ME intake (Teleni *et al.*, 1989). This suggestion also is consistent with the observation that the HG6 animals had the highest rate of N retention among the three groups of animals. Such a result suggests that the HG6 diet was the best utilised of the three examined. However, this was not so clearly demonstrated from the data on DMI and DMD although there was a tendency for the HG6 dietary DM to be consumed in a greater amount by the animals in this group. It would appear that feeding Gliricidia leaves at the same time as the hay was offered led to a higher rumen ammonia concentration than that offered at six hours after the hay was offered. As observed in Experiment 1 (Section 3.2) it is probable that in the HG6 sheep, the N released from the legume leaves in the rumen probably was better utilised by rumen bacteria due to the higher availability of SCFA from the breakdown of hay, however slow it might have been. The higher ammonia in the rumen fluid of sheep on the HG0 diet was reflected in the greater proportion of

N (34 %) excreted through urine compared with the proportion of 24 % excreted by sheep fed the HG6 diet.

It might be suggested from the results of the current experiment that offering Gliricidia leaves supplement at six hours after a low quality basal hay diet was offered to sheep would lead to a better utilisation of the diet than if the legume leaves supplement were offered simultaneously with hay. In addition, it also might be suggested that supplementing a low quality basal hay (such as was used in the current experiment) with Gliricidia leaves would increase DMI by sheep probably to an upper level that would be equivalent to 2.7-2.8 % of its live weight.

3.5 EXPERIMENT 4

THE EFFECT OF DIFFERENT TIMES OF FEEDING OF A MIXTURE OF FRESH GLIRICIDIA AND CALLIANDRA LEAVES SUPPLEMENT ON THE UTILISATION OF DIETARY PROTEIN IN SHEEP

3.5.1 Introduction

It appears from the results presented in Experiments 1, 2 and 3, (Section 3.2, 3.3 and 3.4 respectively) that supplementing a basal low quality hay diet with either Calliandra leaves offered at two hours after the hay was offered or Gliricidia leaves offered at six hours after the hay was offered would significantly improve the utilisation of dietary protein in sheep.

The aim of this experiment was to examine whether a mixture of Gliricidia and Calliandra leaves offered to sheep at either two or six hours after the basal low quality hay was offered would further improve dietary protein utilisation.

3.5.2 Materials and Methods

The animals used, location, their housing and routine feeding and health management were the same as described in Experiment 1 (Section 3.2.1).

a. Experimental design

Fifteen sheep, stratified for live weight, were divided into three balanced groups of five sheep per group. Each group was allocated at random to either of the three dietary treatments: low quality hay (H) plus a supplement of:

i. Fresh leaves of Gliricidia:Calliandra (1:1 on DM basis) offered either immediately with H (HGC0)

ii. at two hours after the H was offered (HGC2) or

iii. at six hours after the hay was offered (HGC6).

The legume leaf mix contributed 30 % of DM to the diet.

b. Experimental procedures

Except for the number of animals used and the dietary treatments examined, the experimental procedures with regards to adaptation and measurement periods, recordings, sample collection and laboratory analysis, calculations and statistical analysis were the same as described in Experiment 1. Therefore these are not detailed here.

3.5.3 Results

Data on live weights of the sheep, intakes and digestibilities of diets, concentrations of metabolites in blood plasma, urine and rumen and N balance are presented in Table 3.5.

No significant differences in intakes of DM and OM were observed among the three treatment groups. Water intake, however, was higher in animals on the HGC6 diet (Table 3.5.1). Sheep in the HGC0 and HGC6 compared with those in the HGC2 treatment groups excreted significantly higher quantities of urine.

The blood plasma glucose concentration was similar in the different dietary treatments.

Significantly higher plasma urea concentrations were observed in the HGC0 and HGC2.

Rumen ammonia concentration was significantly different ($P< 0.05$) among the dietary treatments, with the HGC0 animals having the highest rumen ammonia concentration.

Table 3.5 The liveweight (LW) intakes of dry matter (DM), organic matter (OM) and water, digestibilities, concentrations of metabolites in blood plasma, urine and rumen fluid and nitrogen (N) balance and ratio of N intake (NI) to digestible OM intake (DOMI) Gliricidia and Calliandra leaves offered either immediately with H (HGC0), two hours after H was fed (HGC2) or six hours after H was fed (HGC6).

	Dietary Treatments				
Variables	**HGC0**	**HGC2**	**HGC6**	**± s.e.**	**P**
LW (kg)	16.7	16.6	16.6	0.28	>0.05
Intake (g/d)					
DM	430	425	436	9	>0.05
DM (g/kgLW$^{0.75}$)	49	49	50	0.69	>0.05
OM	403	399	409	9	>0.05
DM (g/kgLW$^{0.75}$)	52	52	53	0.3	>0.05
Water	897	928	1192	101	>0.05
Digestibility (%)					
DM	68	67	68	0.41	>0.05
OM	69	68	69	0.42	>0.05
Metabolites in *Plasma* (mM)					
Glucose	4.40	3.90	4.26	0.49	>0.05
Urea	4	4	2	0.43	>0.05
Urine (mg/L)					
Urea N	1.05	1.14	1.08	0.10	>0.05
Rumen fluid (mg/L)					
Ammonia	125	85	91	8.22	>0.05
N balance (g/d)					
N intake	6.47	6.32	6.38	0.12	>0.05
N faeces	2.35	2.53	2.24	0.09	>0.05
N urine	0.72	0.95	0.60	0.12	>0.05
N retained	3.4	2.8	3.5	0.16	>0.05
N retained as % N intake	53	45	56	2.18	>0.05
Urine (mL/d)					
Output	798	536	787	72	>0.05
NI:DOMI ratio	0.022	0.022	0.025	0.01	>0.05

The highest N retention was observed in animals on the HGC6 dietary treatments.

There was no significant difference observed among the treatment groups in the ratios of NI to DOMI.

3.5.4 Discussion

The results of Experiment 4 when compared to those of Experiments 1, 2 and 3, clearly demonstrate the superiority of the mixtures of Gliricidia and Calliandra leaves over Gliricidia leaves alone or Calliandra leaves alone as a supplement to a low quality basal hay diet. If the animals were offered their diets *ad libitum* their respective mean DMI might have been higher than the equivalent of 2.4 - 2.5 % of live weight that were recorded. Certainly, the extent of utilisation of ingested DM by the animals in the three treatment groups was significantly greater than for the Gliricidia supplemented diet (section 3.2 and 3.4) and for the Calliandra supplemented diet (Section 3.3). In the mixed legume supplement the digestibility values of the three diets were close to 70 %; a level of digestibility value that ranks amongst the best ruminant animal feed observed in other experiments (Widiawati, 2002).

The relatively high digestibility values of diets across treatments were reflected in the concentrations of plasma glucose which were on the higher side of the normal range of 2.8 mM-4.4 mM (Kaneko *et al.*, 1997).

Also reflecting the better utilisation of the mixed legume supplemented diet was the significant improvement in the amounts of N retained by the animals. Across treatment groups no differences were observed in all the variables examined (Table 3.5) suggesting that the times of offering the mixed legume leaves supplement to the animals did not make any significant difference to the efficiency with which the diets respectively were utilised.

There was an indication that the HGC0 diet tended to yield more rumen ammonia than the other two diets. The higher yield of ammonia in the rumens of the HGC0 animals, however, did not result in higher rates of urinary excretion of N. Indeed the amounts of urinary N excreted daily by sheep on the three dietary treatments were lower than those excreted by sheep fed the Gliricidia leaves supplement. The rumen ammonia concentrations in sheep on the three diets, although lower than values recorded for sheep on the Gliricidia supplemented diets in Experiment 1, were within

the optimum range of 50 mg/L (Satter and Slyter, 1974) and 190 mg/L (Miller, 1973) considered to be optimal for minimal rate of fermentation. However, given the observation that the NI:DOMI ratio for the three treatment groups ranged from 0.022 to 0.025 (Table 3.5) largely due to the high digestibility of OM of the diets, there is a case for an increase in rumen degradable protein in diets to give a more balanced NI:DOMI ratio of 0.030-0.035 for optimum utilisation of the available OM in the rumen (Hogan and Weston, 1974).

It is concluded from the results of the current experiment that the readily available N from Gliricidia proteins in the rumen stimulated rumen microbial activity, resulting in an increase in the rate of breakdown of OM, particularly from the Calliandra. This together with the more slowly degrading hay OM provided animals on the mixed Gliricidia and Calliandra supplemented diets a higher DOM, which probably was more than enough for the optimum utilisation of ruminally available N. Thus the diets probably would require increases in rumen degradable proteins for further improvement in utilisation.

CHAPTER 4

EXPERIMENT 5

THE EFFECT OF GLIRICIDIA LEAVES SUPPLEMENTATION TIME ON RATION UTILISATION BY SHEEP FED LOW QUALITY HAY

4.1 INTRODUCTION

The rapid degradation of Gliricidia proteins in the rumen of sheep results in a significant proportion of its proteins being wasted as ammonia and subsequently urea through urine (Widiawati, 2002). Such wastage of protein N may be curtailed if the rumen were to contain large amounts of energy-yielding substrates (SCFA) when the legume is fed to the ruminant animal. This may be achieved with the inclusion of readily fermentable carbohydrates such as starch or molasses. However, where ruminant animals are fed basal low quality forages, a practical alternative might be to introduce Gliricidia leaves supplement to the animals at a time when the availability of SCFA in their rumen is nearing its peak. The peak concentration of SCFA in the rumen fluid of sheep fed a wheat hay diet, for example, is approximately six hours from the start of feeding of the diet. It might be expected that such a profile of SCFA in the rumen of sheep fed low quality forages would be similar.

In Experiment 1 sections 3.2 and 3.4 respectively, the effect of feeding Gliricidia either immediately as the low quality hay was offered, or two hours, six hours or 12 hours after hay was offered, was examined. It was observed in these experiments that general improvement in dietary DM and N utilisation occurred particularly at times of two and six hours after the basal low quality hay diet was offered. However, offering the legume supplement at two hours after the basal diet was fed probably would still result in wastage of a significant proportion of Gliricidia N as the peak release of N would be unlikely to coincide with the peak SCFA concentration in rumen fluid as a result of hay degradation. On the other hand, offering the legume at six hours after the hay was fed also would result in some losses of Gliricidia N as peak concentration in rumen liquor would have occurred when the concentration of SCFA from hay was already on the decline. It might be hypothesised therefore that

Gliricidia N would be utilised efficiently if the legume were to be offered to sheep four hours after low quality hay has been fed.

The aim of the current experiment was to test this hypothesis by examining the effect of offering a supplement of Gliricidia leaves to sheep at four hours after the animals had been fed a basal low quality hay.

4.2 MATERIALS AND METHODS

Thirty Tropical Merino wethers, 12 months of age, were purchased from a commercial property in Western Queensland for the experiment. The animals were handled and routinely managed as described in detail in Section 3.2.1.

4.2.1 Experimental Design

Owing to a limited supply of legume leaves, only three dietary treatments were evaluated: Low quality hay (H) as the control; H + Gliricidia leaves fed at the same time (HG0); and H + Gliricidia leaves fed four hours after the hay was fed (HG4). Eighteen sheep of similar live weights were selected from the 30 and divided into three balanced groups of six sheep per group. A dietary treatment was allocated at random to each group. The experiment was replicated in time so that in Period 1 (20 days), nine sheep were used at three sheep per treatment and in Period 2, the next nine sheep were used.

4.2.2 Experimental Procedures

During each experimental period, the animals were transferred (day 1) to individual metabolism crates (0.57m x 1.23m) to adapt to the experimental conditions and their respective diets for 10 days adaptation period. Feeds and feeding were described in Section 3.2.1c, except that the dietary treatments were different (see Section 4.2.1).

Immediately following their adaptation period was the measurement period (seven days) during which intakes of feed DM and water, and amounts of faeces and urine excreted as described in Section 3.2.1c occurred. Feed, faecal and urine samples

were taken and treated as described in section 3.2.1c. On day 17, a chronic indwelling catheter (polyethylene catheter, 1.0mm ID x 1.5 mm OD, Critchley Electrical Products Pty Ltd, NSW, Australia) was installed in the right external jugular vein of each animal. Each catheter was filled with a sterile physiological saline solution (Baxter, Vaiflex, Baxter, Health Care Pty Ltd, Toongabbie, NSW. Australia) containing 125 IU heparin per mL (Fison Pty, Sydney, Australia) and 2 mg Benzylpenicillin/mL (CSL Limited, Parkville Vic. Australia). A protective foam collar (15 cm wide and 1 cm thick) was fitted over the catheters and around the neck of the animals. On day 18, hourly and some half hourly blood samples (5 - 7mL/sample) were taken from each animal, via its catheter, for 12 hours after start of feeding of H. The blood samples were treated as described in Section 3.2.1c. On day 20, a rumen fluid sample was taken from each animal via a stomach tube and treated as described in Section 3.2.1c. The animals were transferred to group pens on day 20.

4.2.3 Laboratory Analysis

Laboratory analyses were carried out as described in Experiment 1 Section 3.2.1c.

4.2.4 Calculations

Details of calculations for the determination of DM, OM, FI, CP and N are presented in Section 3.2.2.

4.2.5 Statistical Analysis

To examine the effects of the different dietary treatments analysis of variance (ANOVA-Cohen, 1988) was used. The differences in the mean values were compared by the use of the least significant differences (LSD) test (Steel and Torrie, 1980). The statistical software package, SPSS 14 for Windows (SPSS Inc., Chicago, Illinois, USA) was used to undertake the analysis. Raw data was tabulated using Microsoft® Excel for Windows 2003 (Microsoft Corporation, USA).

4.3 RESULTS

Dietary NI in the supplemented sheep was almost twice that of sheep in the control group. The data on the apparent digestibility values of the diets and intakes of DM, OM and N by sheep fed the three dietary treatments are presented in Table 4.1. There were no significant differences observed between treatments in intakes of DM and OM as all the sheep were fed at a restricted level. However, due to differences in dietary composition, the N intakes of the legume supplemented sheep were higher than that of the control sheep ($P < 0.05$).

The times at which the Gliricidia leaves were offered to sheep had a significant effect on the digestibility of DM and OM (Table 4.1). The animals that were supplemented at four hours after the basal hay diet was fed had the highest digestibility values for DM and OM.

The daily urine output of the HGO sheep was significantly higher ($P< 0.05$) than corresponding values observed in the H and HG4 sheep. Daily water intake was much higher in the control group ($P < 0.05$) than in the groups that received the legume supplement.

The rumen ammonia concentration values were significantly different between the three dietary treatments ($P< 0.05$). The highest ammonia concentration was observed in the HG0 sheep and the lowest concentration was observed in the H sheep (Table 4.1).

Significantly more urea was excreted in the urine of the HG0 and HG4 sheep than in the urine of H sheep.

There was no significant dietary effect on plasma glucose concentration (Table 4.1).

Table 4.1 The liveweight (LW) intakes of dry matter (DM), organic matter (OM) and water, digestibilities, concentrations of metabolites in blood plasma, urine and rumen fluid, nitrogen (N) balance and ratio of N intake (NI) to digestible OM intake (DOMI) in wethers fed a basal diet of low quality hay (H) Gliricidia leaves offered either immediately with H (HG0) or four hours after H was fed (HG4).
Within rows, means with different superscripts are significantly different ($P < 0.05$). Values are means ± standard errors (± s.e)

	Dietary Treatments				
Variables	**H**	**HG0**	**HG4**	**± s.e**	**P**
LW (kg)	24.8	24.3	24.3	0.17	>0.05
Intake (g/d)					
DM	587	596	599	9	>0.05
DM (g/kgLW$^{0.75}$)	52	54	54	0.4	>0.05
OM	536	583	584	10	>0.05
OM (g/kgLW$^{0.75}$)	48^{b}	53^{a}	53^{a}	0.7	<0.05
Water	1574	1375	1378	85	>0.05
Digestibility (%)					
DM	47^{a}	51^{c}	58^{b}	1.18	<0.05
OM	53^{a}	57^{b}	63^{c}	1.91	<0.05
Metabolites in					
Plasma (mM)					
Glucose	3.44	3.48	3.52	0.02	>0.05
Urea	4.29^{b}	6.14^{a}	6.47^{a}	0.59	<0.05
Ammonia	0.17	0.21	0.18	0.01	>0.05
Urine (mg/L)					
Urea N	0.25	0.35	0.26	0.03	>0.05
Rumen fluid (mg/L)					
Ammonia	141^{a}	255^{b}	197^{c}	16,67	<0.05
Short chain fatty acid (mmol/L)	60^{a}	75^{b}	79^{b}	3.92	<0.05
N balance (g/d)					
N intake	7.6^{b}	11.5^{a}	11.5^{a}	0.47	<0.05
N faeces	4.7^{a}	5.1^{b}	4.4^{a}	0.11	<0.05
N urine	3.6^{b}	5.6^{a}	5.9^{a}	0.30	<0.05
N retained	-0.3^{a}	1.2^{b}	1.4^{b}	0.23	<0.05
N retained as % N intake	-4^{b}	10^{a}	12^{a}	2	<0.05
Urine (ml/d)					
Output	704^{a}	956^{b}	916^{b}	78	<0.05
NI:DOMI ratio	0.028^{a}	0.035^{b}	0.031^{c}	0.01	<0.05

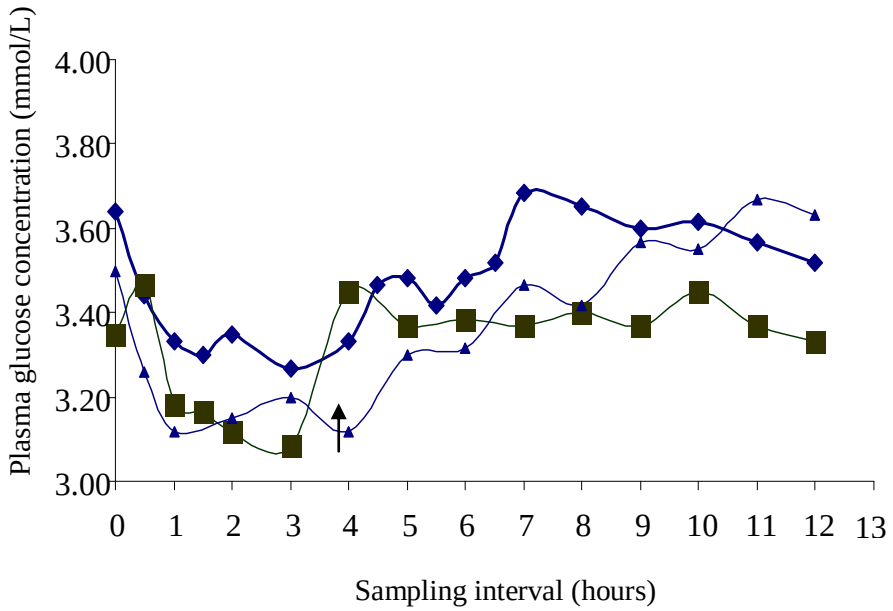

Figure 4.1 Plasma glucose concentration in sheep fed either low quality hay (H) (▲), H plus Gliricidia fed simultaneously (HG0) (■) or Gliricidia supplementation at four hours after the H was fed (HG4) (♦). The hay and the legume in HG0 were offered at zero hour while the legume in HG4 was offered at 4 hours.

Higher plasma urea concentrations were observed in the supplemented animals compared with values in the control animals (Table 4.1). With regards to the profiles of plasma urea concentrations in the three groups over the 12-hour observation period (see Figure 4.2), there were no significant variations within groups. The HG4 sheep exhibited higher plasma urea concentrations during the first 6 hours of sampling with slightly lower urea concentrations on the HG0 sheep. The animals that were not supplemented maintained lower plasma urea concentrations during the 12 hour observation period. Across the treatment groups there was a trend of decreasing concentration over time.

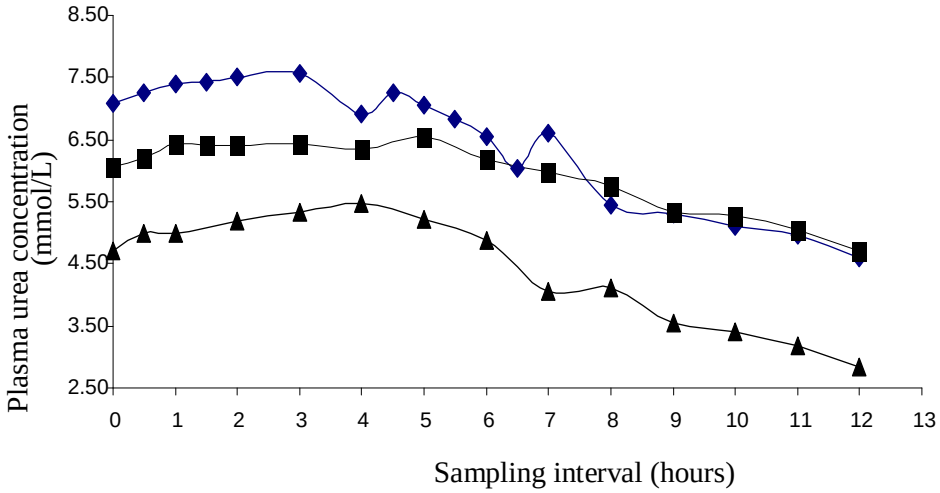

Figure 4.2 Plasma urea concentrations in sheep fed either low quality hay (H) (▲), H plus Gliricidia fed simultaneously (HG0) (■) or Gliricidia supplementation at four hours after H was fed (HG4) (♦). The hay and the legume in HG0 were offered at zero hour while the legume in HG4 was offered at 4 hours.

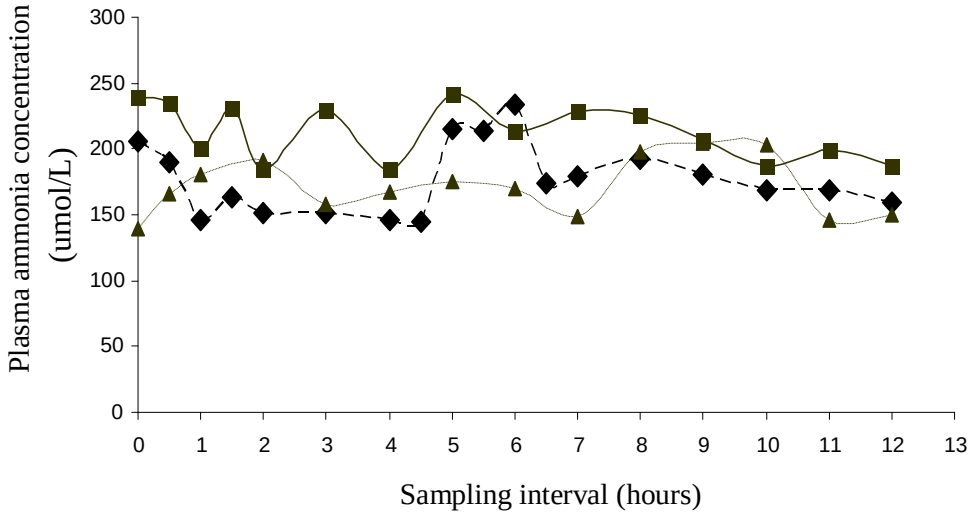

Figure 4.3 Plasma ammonia concentration of sheep fed either low quality hay (H) (▲), H plus Gliricidia fed simultaneously (HG0) (■) or H plus Gliricidia supplemented 4 hours after H was fed (HG4) (♦). The hay and the legume in HG0 were offered at zero hour while the legume in HG4 was offered at 4 hours.

The plasma ammonia concentrations in sheep on the three dietary treatments were not significantly different ($P > 0.05$).

The ammonia concentrations in HG4 animals however were slightly lower before the legume was offered, increased significantly by 30 minutes after the legume was offered (from 123 to 277 mg/L) but started to decline after two hours and remained constant with no future fluctuations thereafter (Figure 4.3).

The effect of supplementation times on rumen SCFA concentration was significant (Table 4.2). The HG4 sheep had the highest mean SCFA concentration ($P < 0.05$).

Table 4.2 Mean of the composition of short chain fatty acids (SCFA) for sheep fed on low quality hay (H), H plus Gliricidia supplemented simultaneously with H (HG0), H plus Gliricidia supplemented fed four hours after H was fed (HG4).
Within rows, means with different superscripts are significantly different ($P < 0.05$). Values are means ± standard errors (± s.e).

	Treatments				
SCFA (mmol/L)	**H**	**HG0**	**HG4**	**± s.e.**	**P**
Acetate	44.82 [a]	54.9 [b]	57.33 [b]	3.83	< 0.05
Propionate	9.76 [a]	14.96 [b]	16.17 [b]	2.00	< 0.05
Isobutyric	0.48 [a]	0.56 [b]	0.55 [b]	0.03	< 0.05
n-Butyric	3.76	3.4	4.32	0.27	> 0.05
Isovaleric	0.54	0.6	0.57	0.02	< 0.05
n-Valeric	0.5 [a]	0.7 [b]	0.48 [a]	0.07	< 0.05

NI: DOMI ratio

The amount of N that passed through the rumen did not exceed N intake.

4.4 DISCUSSION

A positive effect of Gliricidia supplementation to low grass hay fed to sheep was observed in the current experiment. The findings are in agreement with the hypothesis that Gliricidia N would be utilised efficiently if the legume were to be offered to sheep four hours after low quality hay has been fed. Observations on

improved performance of ruminant animals fed a supplement of Gliricidia at the same time with low quality forage have been reported by others (Osuji and Odenyo, 1997; Alayon *et al.*, 1998; Merkel *et al.*, 1999; Widiawati, 2002). The mean digestibility results observed in the current experiment where sheep were fed a supplement of Gliricidia leaves and low quality hay simultaneously is similar to values reported by others (Archimede *et al.*, 2001). However, the improved digestibility values observed in the current experiment when the Gliricidia leaves supplement was fed at four hours after the low quality hay was fed cannot be compared with results from any other previous work as there is no literature on it.

Gliricidia supplementation has been reported to increase intake of low quality hay from 45 % to 72 % (Alayon *et al.*, 1998) when incorporated in the diet. However, these intake data cannot be compared with those in the current experiment, as the animals in the current experiment were on a restricted level of feeding. It was suggested in Experiment 3 (Section 3.4.3) where sheep were offered the basal low quality hay *ad libitum* that improvement in voluntary DM intake of a diet of low quality hay supplemented Gliricidia leaves may be up to equivalent of 2.8 % of live weight of an animal. With such significant improvement in digestibility (58 %) in the HG4 diet in the current experiment, it might be reasonably expected that a voluntary DM intake of such a diet beyond the equivalent of 2.8 % of live weight is probable.

It is clear from results of the current experiment that manipulating the time at which Gliricidia leaves supplement is offered to sheep fed a low quality dry forage (e.g. hay) could substantially improve the digestibility of the diet. For example, the DM digestibility of the HG4 diet was improved by some 23 % over the H diet. It is probable that the hay, after four hours in the rumen was nearing or had reached its maximum rate of degradation, leading to significantly increased concentration of SCFA in the rumen liquor. The introduction of the Gliricidia leaves supplement at four hours after the hay was fed and the fact that the Gliricidia protein breaks down relatively quickly in the rumen (Widiawati, 2002) would have resulted in an improved utilisation of Gliricidia rumen-degradable N and an overall improvement in rumen microbial activity.

In the current experiment this was manifested in the significant improvement in DMD of the HG4 diet. It can be calculated from data in Table 4.1 that the molar ratio of ammonia to SCFA in the rumen liquor was 0.138; a ratio that is probably optimal or near optimal for rumen microbial activity. Although the molar ratio of ammonia to SCFA in the rumen liquor of animals on the H diet (0.131) was only slightly lower, the absolute amounts of ammonia and SCFA in these animals were significantly lower than those in the rumen liquor of animals on the HG4 diet. The corresponding molar ratio in animals on the HG0 diet was much higher at 0.189 which suggests probable wastage of the available Gliricidia N.

Indeed the observation outlined above is consistent with the data on the ratios of NI to DOMI (Table 4.1) in which the value of 0.031 for the HG4 dietary treatment group is the same as the value suggested by Hogan and Weston, (1974) to be optimal for rumen microbial activity. The corresponding value of 0.035 for the HG0 treatment group suggests a higher than optimal N intake by sheep in that group. In the control group of animals the ratio was significantly lower (Table 4.1) than the optimal value.

The data on N balance in the three groups of sheep in the current experiment highlight the stimulatory effect of the legume leaves supplement on N utilisation by the animals.

The urinary urea excretion rate generally is positively related to the plasma urea concentrations. In the current experiment the plasma urea concentration values were closely associated not only with urine output but also with urinary urea concentration. Thornton and Yates, (1968) reported a reciprocal relationship between renal urea excretion and urea transfer to the rumen via saliva. This reciprocal relationship was dependant more on the supply of nutrients that stimulate microbial activity than on the rate of urine outflow. However, the increased urine output from the HG0 and HG4 animals in the current experiment could have also contributed to the increased urinary urea excretion observed.

Water intake is driven largely by DMI and blood osmolality. In the current experiment, there were differences in DMI among animals in the three treatment groups. Also, there were no significant differences among the treatment groups in their water intake, although the mean water intake of the H sheep appeared to be inexplicably higher than water intakes by the HG0 and HG4 sheep. It appears that in the current experiment the volumes of urine excreted by the animals on the different dietary treatments were largely driven by the concentration of urea and ammonia in the blood circulation.

Although the differences in mean plasma glucose concentration among the three dietary treatment groups were not statistically significant (Table 4.1) the trend in increasing concentration from 3.4 mM in the H animals to 5.5 mM in the HG4 animals is reflective of the improvement in the utilisation of diets improving from the H to the HG4 diet. Gluconeogenesis from propionic acid would have proceeded at a higher rate in the HG4 animals which had a significantly higher concentration of rumen SCFA (Table 4.2).

It might be concluded from the observations made in the current experiment that Gliricidia N would be utilised efficiently if the legume were to be offered to sheep four hours after low quality hay has been fed. The availability of fermentable substrates that are responsible for energy supply to the rumen microbes must be present at the right time and in sufficient quantities before the potential nutritional value of Gliricidia N can be realized.

CHAPTER 5

EXPERIMENT 6

DIGESTIBILITY AND NITROGEN UTILISATION OF OVEN-DRIED CALLIANDRA LEAVES FED TO SHEEP ON A BASAL FEED OF HAY

5.1 INTRODUCTION

Supplementation of Calliandra to low quality basal diets in ruminant feeding has previously received a lot of attention (Norton and Ahn, 1997; Salawu *et al.*, 1999; McSweeney *et al.*, 2000; Stewart *et al.*, 2000). However, controversies on DM and N digestibility have been reported. The legume has high potential as a protein source in ruminant feeding in tropical and sub-tropical areas of the world. The presence of relatively high amounts of condensed tannins in Calliandra however, has been reported to limit its nutritive value by complexing with the dietary proteins and endogenous enzymes which affect the normal microbial activity in the rumen during fermentation. Alternative feeding methods and management of this shrub legume need to be explored in order to overcome the depressive effects that it has on DM and N digestibility which generally is expressed in excessive loss of N through faeces of animals fed the legume.

One method of overcoming the effects of the condensed tannins in Calliandra might be drying the legume. It has been demonstrated (Palmer *et al.*, 2000; Rakhmani *et al.*, 2005) that the process of drying would polymerise condensed tannins and thus reduces the content of this polyphenol in a given diet. However, evidence in literature on the effect of drying on the nutritive value of Calliandra is conflicting. For example, Ahn *et al.*, (1997) reported that drying could improve the palatability and nutritive value of Calliandra. On the other hand, responses to drying of Calliandra in terms of nutritive value were indifferent (e.g. Palmer and Schlink, 1992).

Drying the legume at a lower temperature (e.g. 60 ^{o}C) may be more effective as the drying temperature could polymerise the condensed tannins while maintaining the nutritional value of Calliandra proteins.

It was hypothesised that Calliandra proteins will be utilised by ruminant animals more efficiently if the legume were dried before using it as a supplement to a low quality forage diet.

The aim of the current experiment was to examine the effect of oven drying Calliandra leaves at 60 ^{o}C on DM, OM and N.

5.2 MATERIALS AND METHODS

The location, animal housing and routine feeding and management procedures were as described in detail in Section 3.2.1. The experimental animals were drawn from the pool of animals obtained for the experiment described in Section 4.2.

5.2.1 Experimental Design

A total of 15 Merino wethers were stratified for live weight and divided into three balanced groups of five animals per group. Three dietary treatments were then allocated at random to the groups. The dietary treatments were: Low quality hay (H) as the control group, H + Fresh Calliandra leaves (HFC), and H + Dried Calliandra leaves (HDC). The Calliandra leaves were offered to the animals at the same time as H at the equivalent of 2.8 % live weight on DM basis.

5.2.2 Experimental Procedures

Except for the different dietary treatments (Section 5.2.1) examined in the current experiment, details of experimental procedures were described in Sections 3.2.1c and Sections 4.2.2. The animals were transferred (Day 1) to individual metabolic cages to adapt to the experimental conditions and their respective diets for ten days, immediately after which values for intake of dietary DM and excretion of faeces and

urine by the animals were recorded for seven continuous days (measurement period). On day 18, a rumen fluid sample was taken from each animal via a stomach tube as detailed in section 3.2.1c. Blood samples were also taken via jugular venipuncture as detailed in section 3.2.1c.

5.3 RESULTS

Table 5.1 The liveweight (LW) intakes of dry matter (DM), organic matter (OM) and water, digestibilities, concentrations of metabolites in blood plasma, urine and rumen fluid, nitrogen (N) balance and ratio of N intake (NI) to digestible OM intake (DOMI) in wethers fed a basal diet of low quality hay (H) supplemented with fresh Calliandra leaves offered immediately with H (HFC) or H with dried Calliandra was fed (HDC).
Within rows, means with different superscripts are significantly different ($P < 0.05$). Values are means ± standard errors (± s.e).

	Dietary Treatments				
Variables	**H**	**HFC**	**HDC**	**± s.e.**	**P**
LW (g/d)	25.3	25.3	25.5	0.37	>0.05
Intake (g/d)					
DM	616	632	626	8	>0.05
DM (g/kgLW$^{0.75}$)	55	56	57	1	>0.05
OM	554	573	567	7.31	>0.05
OM (g/kgLW$^{0.75}$)	49	51	52	1	>0.05
Water	2228 [b]	1788 [a]	2117 [b]	72.50	<0.05
Digestibility (%)					
DM	50 [a]	51 [a]	48 [b]	0.4	<0.05
OM	53	55	52	0.8	>0.05
Metabolites in					
Plasma (mM)					
Glucose	3.16 [b]	3.22 [a]	3.58 [c]	0.62	<0.05
Urea	5.42	5.54	4.92	0.25	>0.05
Ammonia	0.05	0.04	0.04	0.003	>0.05
Urine (mg/L)					
Urea N	2	1	2	0.01	>0.05
Rumen fluid (mg/L)					
Ammonia	117 [b]	242 [a]	134 [b]	19.78	<0.05
N Balance (g/d)					
NI	8.3 [b]	13.04 [a]	11.6 [c]	0.55	<0.05
N Faeces	4.8 [a]	6.4 [b]	4.8 [a]	0.24	<0.05
N Urine	2.0 [a]	3.1 [b]	2.0 [a]	0.19	<0.05
N retention	1.4 [b]	3.6 [a]	4.8 [b]	0.45	<0.05
N retained as % N intake (%)	17 [b]	27 [a]	42 [c]	3.45	<0.05
Urine (mL/d)					
Output	704	712	770	44.36	>0.05
NI: DOMI ratio	0.028 [b]	0.042 [a]	0.040 [a]	0.002	<0.05

Data on live weights of sheep, intakes and digestibility of diets, concentrations of metabolites in blood plasma, urine and rumen and N balance are presented in Table 5.1.

There were no differences in DMI among the three groups of sheep (Table 5.1).

The DMD of the HDC diet was lower than those of the H and HFC diets. No differences in OM digestibility were observed. The HDC diet had the highest N digestibility value at approximately 59 % compared with 42 % and 51 % for the H and HFC diets respectively. Animals in the HDC group retained the highest amount of N daily compared with the H and HFC animals (Table 5.1).

Plasma glucose concentration was highest in animals on the HDC diet. The lowest value was observed in animals on the control diet. No differences among the treatment groups were observed for the concentration of urea or ammonia in plasma.

There were significant differences in rumen ammonia concentrations among the three dietary treatments, the highest concentration being observed in animals on the HFC diet (Table 5.1).

5.4 DISCUSSION

The DMI by the sheep supplemented with either fresh or dried Calliandra was equivalent to 2.5 % of live weight and 2.4 % for sheep fed the low quality hay. These levels of DM intake are sufficient for maintenance of growing sheep (ARC, 1980). Drying of Calliandra did not result in higher DMD in the current experiment. The DMD values for the H, HFC and HDC diets are shown in (Table 5.1) and are similar to those reported by others (Palmer and Schlink, 1992; Ahn *et al.*, 1997). However, Hove *et al.* (2003) did not observe any difference in digestibility of either dried or fresh Calliandra. The contradicting reports on the effect of drying Calliandra on dry matter digestibility and N balance might be attributed to a number of factors such as tannin type, drying temperatures and feeding strategy (Ahn *et al.*, 1997; Nherera *et al.*, 1998; Palmer *et al.*, 2000). Feeding the dried Calliandra as a sole diet or as a supplement would result in differences in digestibility.

Drying was previously reported (Ahn *et al.*, 1997) to potentially change the chemical composition of Calliandra by reducing its condensed tannin concentration and thus improving its digestibility. If such a change took place in the current experiment, it did not improve DM digestibility of the dried Calliandra.

The digestibility of dried Calliandra could vary depending on the drying method applied (Stewart *et al.*, 2000). The low DMD of diets supplemented with fresh and dried Calliandra (51 % and 48 % respectively) observed in the current experiment are similar to earlier findings where 52.7 % was reported for freeze dried and 45.9 % for oven-dried *in sacco* (Ahn *et al.*, 1989). Digestibility values of 41 - 55.0 % have also been reported by others (Palmer and Schlink, 1992; Salawu *et al.*, 1997b; Palmer *et al.*, 2000).

The leaves in this experiment were dried at 60 ^{o}C over 24 hours. The most acceptable oven-drying temperature, without adversely affecting the nutritive value of the legume seems to be 60 ^{o}C for 24 hours. Palmer *et al.* (2000) proposed a drying temperature of below 65 ^{o}C in order to accurately quantify N content. Temperatures of around 60 ^{o}C over a 48 hour period have been used previously by other workers (Ahn *et al.*, 1989; Palmer *et al.*, 2000; Hove *et al.*, 2003).

Excretion of faecal N by sheep was observed to be higher in the treatment group on the fresh than on the dried Calliandra diet. Similar findings were previously observed by other workers (Hove *et al.*, 2001; Widiawati, 2002; Rakhmani *et al.*, 2005). A lower amount of N was excreted daily through faeces in sheep supplemented with dried Calliandra. This indicates that the chemical change that probably had occurred in the legume during drying had improved the digestibility of N in animals fed the dried legume. The proposed alteration in condensed tannins content by the process of drying in the current experiment as previously discussed would have made more Calliandra proteins available for digestion in the small intestines of the animals. Thus the improvement in digestibility of N was observed.

It was expected that rumen ammonia concentration would have been lower in animals supplemented with fresh Calliandra due to the high level of binding of condensed tannins to the protein, thus limiting the N availability from the legume for microbial utilisation. This was not the case in the current experiment. Other findings previously observed a significant decrease in ruminal ammonia concentration when Calliandra supplement was included in the diet at a level of 30 % on a DM basis (Waghorn *et al.*, 1987; Perez-Maldonado and Norton, 1996). Condensed tannins in a diet may reduce the cellulolytic bacteria population that are responsible for degradation of carbohydrates in the rumen (McSweeney *et al.*, 2002). The importance of readily available carbohydrates during fermentation was discussed in the earlier experiments (Chapters 3 and 4). Interestingly, tannins have no effect on the population of proteolytic bacteria that is responsible for protein degradation (McSweeney *et al.*, 2000). It is possible that the low population of the cellulolytic bacteria could have resulted in a reduction of readily available energy yielding substrates, inhibiting normal microbial activity and N utilisation from ammonia.

Blood plasma glucose concentrations in the current experiment are consistent with the pattern of N retention by sheep across the treatment group. The HDC animals with the highest retention rate also had the highest plasma glucose concentration.

The balance between protein and energy that were consumed by sheep are expressed in values of the NI:DOMI ratios (Table 5.1). The higher ratio of over 0.04 suggests a higher supply of N and insufficient readily available SCFA in the legume supplement diets. This imbalance will need to be addressed if further improvement in N utilisation is to be achieved.

It is concluded that drying Calliandra could modify and promote efficient utilisation of N by the rumen microbes. However, the drying temperatures need to be observed closely as higher temperatures could denature the proteins and hence reduce the nutritive value of the legume. Drying could be a solution to drought feed management as Calliandra can be harvested when flourishing, dried and stored for dry season feeding.

CHAPTER 6

EXPERIMENT 7

THE POTENTIAL OF MILLED KENAF SEEDS (*HIBISCUS CANABINUS L.*) AS A SUPPLEMENT IN RUMINANT ANIMAL FEEDING

6.1 INTRODUCTION

The potential of a supplementary feedstuff is characterised by its physical and chemical composition. One chemical compound that highlights the potential of a feedstuff in ruminant animal nutrition is CP. Kenaf seeds have an average CP content of 26 % and are also high in fat (Charles et al., 2002).The relatively high amount of CP in Kenaf seeds could supply adequate N for rumen microbial protein synthesis and promote efficient utilisation of available nutrients and subsequently, animal productivity. Ruminant animals depend on rumen microbial protein synthesis and dietary protein that escape digestion in the rumen, for their amino acid supply which is responsible for animal growth. Kenaf is rich in the amino acids histidine, tyrosine, and arginine that are the most limiting amino acids in forages. Thus Kenaf seeds have the potential to promote lean muscle growth and thus improve ruminant animal productivity. Additionally, the protein in immature Kenaf plants generally has a higher soluble fraction than the proteins found in common grasses (Suriyajantratong *et al*., 1973). It is probable that the Kenaf seed protein would be equally soluble and therefore potentially could improve DMD and the utilisation of a low quality forage-based diet such as was observed previously (Chapter 4) in the Gliricidia-supplemented low quality forage-based diet fed to sheep.

The nutritive potential of Kenaf seeds as a supplement in ruminant animal feeding has not been investigated.

It was hypothesised that as a supplement to basal low quality forage, milled Kenaf seeds would improve significantly the nutritional value of the diet.

The aim of the current experiment was to examine the effect of Kenaf seed supplementation to low quality hay on the digestibility and N utilisation in sheep.

6.2 MATERIALS AND METHODS

The animals used, location, their housing and routine feeding and health management were the same as described in Experiment 1 (Section 3.2.1).

6.2.1 Experimental Design

A total of 24 sheep were stratified for live weight and divided into four balanced groups of six animals per group. Each group then was allocated at random to either of the four dietary treatments: low quality hay (H), H plus fresh Gliricidia leaves fed at four hours after hay was fed (HG), HG plus milled Kenaf seeds (HGK) or H plus milled Kenaf seeds (HK). The composition of the daily ration of the HG diet was 70 % hay and 30 % Gliricidia.The ratio of Gliricidia to Kenaf in the HGK ration was 1:1 on DM basis. The experiment was replicated in time so that in Period 1 (9 days) 12 sheep were used at three sheep per dietary treatment and in Period 2, the next 12 sheep were used.

6.2.2 Experimental Procedures

Except for the number of animals used and dietary treatments examined, the experimental procedures with regards to adaptation and measurement periods, recordings, sample collection and laboratory analyses, calculations and statistical analysis were the same as described in Experiment 1 (Section 3.2.1) and therefore are not described in details here. Blood samples were collected before any feed was offered (0800 hours), three hours after H and HK (1100 hours) were offered and three hours after the Gliricidia was offered in the HG and HGK dietary treatment groups (1500 hours).

6.3 RESULTS

Data on live weights of the sheep, intakes and digestibilities of diets, concentrations of metabolites in blood plasma, urine and rumen and N balance are presented in Table 6.1. No significant differences were observed in DMI and OMI by sheep among the dietary treatments. Sheep that were fed the H diet had a lower NI than those fed the HG, HGK or HK diet. Sheep on the H and HK diets had higher water intakes than animals on the HG and HGK diets (Table 6.1).

The mean digestibility values of DM for the HG and HGK diets were significantly higher than the corresponding values for the H and HK diets.

The amounts of N retained were similar among the groups of sheep that received the supplements but significantly lower in animals on the H diet only. As a percentage of NI, N retained was highest in sheep on the HK diet and lowest in animals on the H diet. The ratio of N intake to DOMI was highest for the HGK and HK diets, followed by the HG diet and lowest for the H diet (Table 6.1).

No significant differences were observed among the treatment groups in urine output. The mean concentration of urea-N in urine, however, was highest in the HK sheep followed by the HGK and HG sheep and was lowest in the H animals. These significant differences in urinary urea-N concentrations were paralleled by significant differences in the amounts of N excreted in urine per day by these groups of animals (Table 6.1).

Plasma glucose concentration was highest in the HG and HGK sheep and lowest in the H sheep. The mean concentration of urea in plasma was highest in the HK animals, followed by the HGK animals and lowest in the HG and H groups. A similar trend in ammonia concentration in plasma was observed except that the mean ammonia concentration in the plasma of the HGK sheep was similar to that in the H group.

Table 6.1 The live weight (LW) intakes of dry matter (DM), organic matter (OM) and water, digestibilities, concentrations of metabolites in blood plasma and urine, nitrogen (N) balance and ratio of N intake (NI) to digestible OM intake (DOMI) in wethers fed a basal diet of low quality hay (H), H plus Gliricidia leaves offered four hours after H was fed (HG), HG plus Kenaf seeds (HGK) or H plus Kenaf seeds (HK).
Within rows, means with different superscripts are significantly different ($P < 0.05$). Values are means ± standard errors (± s.e).

	Dietary Treatments					
Variables	**H**	**HG**	**HGK**	**HK**	**± s.e**	**P**
LW (kg)	24.3	24.3	24.9	24.5	0.153	>0.05
Intake (g/d)						
DM	602	580	578	574	8	>0.05
DM (g/kg$LW^{0.75}$)	61[a]	58[a]	56[a]	57[a]	0.8	>0.05
OM	666	637	631	625	11	>0.05
OM (g/kg$LW^{0.75}$)	53	55	53	53	0.5	>0.05
Water	1910[a]	1541[c]	1602[c]	1956[a]	96	<0.05
Digestibility (%)						
DM	50[a]	53[b]	52[b]	50[a]	0.8	<0.05
OM	51	52	47	50	1.3	>0.05
Metabolites in *Urine* (mg/L)						
Urea N	3.8[a]	4.6[b]	6.9[c]	11.3[d]	1.75	<0.05
N Balance (g/d)						
N intake	8.8[a]	12.5[b]	13.6[b]	14.5[b]	1.52	<0.05
Faecal N	5.15	5.38	5.02	4.68	0.12	>0.05
Urinary N	4.1[a]	5.7[b]	7.0[c]	8.0[d]	1.02	<0.05
N retained	-0.4[b]	1.5[a]	1.5[a]	1.9[c]	0.61	<0.05
N retained as % N intake	-4.42[b]	11.8[a]	11.3[a]	12.8[c]	2.22	<0.05
Urine (mL/d)						
Output	774	1014	862	764	26.97	>0.05
NI:DOMI ratio	0.029[a]	0.041[b]	0.050[c]	0.051[c]	0.001	<0.05

Data on the plasma concentrations of glucose, urea and ammonia over a seven hour period are presented in Table 6.2. At 0800 hours, the plasma glucose concentrations in the four treatment groups were not significantly different. Urea concentration however, was highest in the HK group and lowest in the H group. Plasma ammonia concentration was highest in the HGK group followed by the HK, H and HG group respectively.

The concentrations of these three metabolites were observed generally to be at their highest values at three hours after offering H and HK (1100 hours) in the H and HK groups respectively and after offering the Gliricidia supplement (1500 hours) in the HG and HGK groups.

Table 6.2 The concentrations of ammonia, glucose and urea from blood samples collected at 0800 hours (h), 1100h and 1600h from sheep fed either a basal diet of Hay (H), HG plus Kenaf seeds (HGK) or H plus Kenaf seeds (HK).
Within rows, means with different superscripts are significantly different ($P < 0.05$). Values are means ± standard errors (± s.e).

Plasma Metabolites	Time (h)	Dietary Treatment H	HG	HGK	HK	± s.e.	P
Ammonia (µmol/L)							
	0800	85[a]	75[b]	111[c]	98[d]	9.12	< 0.05
	1100	97[b]	70[a]	97[b]	128[c]	14.51	< 0.05
	1500	95[a]	67[b]	87[c]	97[a]	7.64	< 0.05
Glucose (mmol/L)							
	0800	3.03	3.65	3.63	3.47	0.29	> 0.05
	1100	3.10	3.02	3.28	3.42	0.00	> 0.05
	1500	3.10	4.00	3.65	3.75	0.29	> 0.05
Urea (mmol/L)							
	0800	4.30[a]	6.00[b]	5.92[b]	7.70[c]	1.00	< 0.05
	1100	5.63[a]	6.67[b]	7.10[b]	8.55[c]	0.76	< 0.05
	1500	5.08[a]	5.45[b]	7.12[c]	8.00[d]	0.76	< 0.05

6.4 DISCUSSION

Live weights of animals were balanced across the treatment groups and the amounts of dietary DM offered to the animals were set at 2.8 % of the live weight of each animal. Thus no differences among the treatment groups were expected in DMI. However, it was anticipated that any differences among the groups in the efficiencies with which they would utilize their respective diets would be expressed in variables such as digestibility and N retention.

It would seem that in the four dietary treatments, the Gliricidia component of the diet caused an improvement in DMD of the particular diet. Consistent with the results of the experiment presented in Chapter 4, it might be suggested that the rumen degradable fraction of the Gliricidia protein caused the improvement observed although the magnitude of improvement observed in the current experiment is not as

large as that observed in the previous experiment (see Table 4.1). In fact, assessing the dietary treatments by their respective N retention values (Table 6.1) one might suggest that the HK diet was similar if not better in value than the HG and HGK diets.

A high fat content in the diet of ruminant animals has been associated with a reduction in carbohydrate digestibility and total OM digested (Palmquist, 1994). In the current experiment, the high fat content (20 %) of Kenaf seeds in the HK treatment could have inhibited effective digestibility of the carbohydrate component of the diet. High dietary fat intake has the tendency to increase the production of proprionic and decrease acetic and butyric acids (Doreau and Chilliard, 1997). These workers also suggested that if the proportion of fat in the diet of cattle exceeds about 2 % of the total feed, the feed would have toxic effects on the rumen microbes. It would appear that fat reduces the growth rate of, or even kills, certain microorganisms which digest fiber in the rumen. This deleterious effect is particularly true for unsaturated fats (Doreau and Chilliard, 1997).

Assuming that the average fat content of Kenaf seed is 20 % of DM (see Section 2.5.1) it might be calculated that the dietary fat in the HGK and HK diets in the current experiment were 3 % and 6 % respectively. These levels of dietary fat, according to Kucuk *et al*. (2003) probably would reduce rumen microbial activity and thus the digestibility of the diet. In the current experiment, there was no evidence that the levels of dietary fat in the HGK and the HK diets were associated with a reduction in digestibility of the diets (see Table 6.1). The DMD value of the HK diet was the same as that of the H diet and the digestibility value of the HGK diet was even higher than that of the control H diet.

The plasma glucose concentrations in sheep in the current experiment were in accordance with the pattern of N retention by these animals. Those animals that received dietary supplements and thus had higher N retention values also had higher plasma glucose concentration values which suggest that these animals most probably also consumed greater amounts of ME.

The variation in NI among the four treatment groups was reflected in the pattern of concentration of urea in the plasma of the groups of animals. Such a pattern was also reflected in the pattern of N excretion in the urine of these animals; the HK animals having the highest urinary urea excretion rate followed by the HGK, HG and the H animals which had the lowest rate of excretion of urea N through urine. The higher plasma urea concentrations and urinary urea excretion rate in animals on the HGK and HK diets would suggest that a significant fraction of the Kenaf seed was of rumen-degradable protein. This is supportive of the suggestion made in Section 6.1 on the degradability of Kenaf seed protein.

Overall, the animals that received dietary supplements had higher NI:DOMI ratio values. These were due largely to the lower than expected digestibility of the HG, HGK and HK diets. The mean digestibility value observed for the HG diet in the current experiment, for example, was much lower that the corresponding value for the HG4 diet (see Section 4.3) as previously discussed. The significantly higher mean NI:DOMI ratio of 0.05 observed in the HGK and HK dietary treatments also would be due to the higher intake of N by animals in these two treatment groups. A reduction in the dietary fat and an increase in carbohydrate content of these diets should optimize their respective NI:DOMI ratios.

It is concluded from the results of the current experiment that a Kenaf seeds supplement to a basal low quality forage diet may not improve the digestibility of the diet but by virtue of the high protein content of the seeds, most probably would increase the amount of N retained by ruminant animals fed the diet. Although the levels of 3 % - 6 % dietary fat did not appear to impact negatively on the digestibility of the HGK and HK diets, it might be suggested that the nutritive value of these diets might be improved if the fat content of the Kenaf seeds were reduced (e.g., by extracting the oils from these) and feeding the resultant Kenaf meal with an added source of readily fermentable carbohydrate to the ruminant animal fed a low quality forage diet.

CHAPTER 7

GENERAL DISCUSSION

In the context of ruminant animal nutrition in the tropics and sub-tropics, much of the focus in recent years has been on the examination of the potential nutritional values of tropical shrub plants. The study described in this thesis was directed towards defining the nutritional potential of two better known shrub legumes (Gliricidia and Calliandra), and an emerging alternative commercial shrub crop in the sugarcane lands of tropical Australia, (Kenaf) (see Section 2.5). In particular, the leaves of these shrub legumes and the seeds of Kenaf were examined for their potential as supplements to low quality forages and subsequently feeding strategies that might be employed to realise the nutritional potential of these feedstuffs were discussed.

It is well established that rumen microbes require readily available N and SCFA to function at an optimum level. Yet despite this available knowledge, many research studies and field practices involving the evaluation of nutritive value and the feeding of legume leaves supplements respectively, have omitted by design or through simple oversight, to take into account the importance of the synchronization of availability of dietary N and SCFA production in the rumen for optimal rumen microbial activity.

The experiments presented in Chapter 3 of the current study were designed to examine the effects of varying the time of offering supplements of Gliricidia and Calliandra leaves to sheep after a low quality dried forage ration was fed. It was clear from the results of those experiments that delaying the time of offering these legume leaves supplements could have a significant impact on rumen function and therefore on the utilisation of the ration by the sheep. These animals were fed at a restricted level of DMI (2.8 % of live weight) to ensure that all animals ate their respective diets completely or as near completely as possible. This feeding regimen was considered to provide a better platform for examination of changes in the utilisation of the diets that might occur as results of the treatments imposed. There was an

improvement in the utilisation of Gliricidia N when the legume leaves were offered as a supplement to low quality hay at two to six hours after the hay was fed (Experiment 1, Section 3.2.4, Table 3.2). To examine whether these times of offering the legume supplements would also significantly affect the voluntary feed DMI, a subsequent experiment (Experiment 3, Section 3.4) was undertaken using sheep that were fed their diets at a level equal to 125 % of their voluntary DM intake on the previous day. From the results of Experiment 3 it is suggested that on basal forage of the quality described in that experiment, the voluntary DMI by sheep of the forage plus a supplement of Gliricidia leaves might be limited to a maximum of 2.8% of live weight. Also evident from the results of Experiment 3 is the increased probability that offering Gliricidia leaves supplement at six hours (rather than zero or two hours) after a basal low quality forage is fed would most likely result in significant increases in N retention by the animals.

It is evident from the literature (see Section 2.4.9) that the degradation of dry forages, similar in quality to wheaten hay, would result in a pattern of production of SCFA that shows a peak concentration of these acids in the rumen at six hours from the start of feeding of the forage to the ruminant animal. In Chapter 4, it was hypothesised that offering the Gliricidia leaves supplement at four hours after the basal low quality hay was fed to sheep should better synchronize the availability of N released as ammonia from the supplement during fermentation with the SCFA produced from the fermentation of the basal hay. The hypothesis was tested in the experiment described and indeed the results showed molar ratios of ammonia to SCFA in rumen liquor of sheep offered the supplement simultaneously with the hay (HG0) and at four hours after the hay was fed (HG4) to be 0.189 and 0.138 respectively. This data indicates that the amount of ammonia in the rumen was more than the rumen microbes could use with the given level of SCFA. In concentration values, the ammonia in the HG0 sheep (255 mg/L) was much higher than what is considered to be optimal (50-197 mg/L) for rumen microbial activity (Satter and Slyter, 1974). The value of 197 mg/L observed in the HG4 animals was at the top end of the optimal range for rumen ammonia concentration and probably was just ideal for rumen microbial activity. Indeed this was manifest in improved dietary

DMD, blood plasma glucose concentration and N retention in the HG4 sheep (Table 4.1).

Because Calliandra protein would be degraded at a lower rate than Gliricidia protein, the effect of offering a supplement of Calliandra leaves to a basal low quality hay at two hours after the hay was fed (HC2) to sheep, was examined in Experiment 2 (Section 3.3). Compared with the group of sheep offered the Calliandra leaves supplement simultaneously with hay (HC0) the HC2 group retained more dietary N daily although no difference was observed in digestibility values between the two groups. Both the digestibility values were relatively low and at approximately 50 %, were comparable with the corresponding value observed in the HG0 group of sheep used in the experiment described in Chapter 4. It is probable that the low digestibility values observed in sheep given the Calliandra supplement was due to the presence of a high level of condensed tannins in the legume. Tannins also would complex with the dietary proteins and inhibit normal microbial function. This effect was expressed in the high N losses through faeces excreted by the two groups of sheep.

One way of overcoming or reducing the negative impact of tannins in Calliandra on the nutritive value of a diet would be to offer the legume together with another that has a significant proportion of rumen-degradable protein. This possibility was examined in Experiment 4 described in Chapter 3. A substantial improvement in digestibility and overall nutritive value of the diets was observed. In the experiment described in Chapter 5, another way of overcoming or reducing the negative impact of tannins was examined. This involved the drying of the legume leaves in order to alter the structure of condensed tannins and thereby freeing up the dietary proteins for digestion in the rumen and in the small intestine. Although the dried Calliandra diet (HDC; Table 5.1) appeared to have a slightly lower digestibility value compared with the fresh Calliandra diet (HFC), the sheep on the HDC diet retained a significantly higher amount of N and excreted in faeces a lower proportion of their mean dietary NI. These results suggest that drying Calliandra leaves before feeding as a supplement to low quality forage would improve overall the utilisation of N by sheep fed the diet.

The relatively slow rate of degradation of Calliandra proteins and the relatively fast rate of degradation of Gliricidia proteins in the rumen of sheep (Widiawati *et al.*, 2000) suggested that the use of a mix of these legumes as a supplement to low quality forages might result in an increased efficiency of utilisation of N in these animals. This possibility was examined in Experiment 4 described in Chapter 3 (Section 3.5). The times of offering the supplement to sheep did not affect DMD of the diets nor did these affect the utilisation of N by the animals. Irrespective of times of offering, the supplement mix resulted in relatively high digestibility values (compare values in Table 3.5 with Tables 3.2 or 3.3) of diets and relatively high percentage of N retained. It is probable that with the legume mix, Gliricidia readily supplied available N for optimal rumen microbial function and Calliandra supplied the appropriate profile of amino acids at the intestinal level, leading to an improvement in N retention in the animal. However, while there would be overall improvements in the nutritional value of diets including the legume mix, there are indications from the results of Experiment 4 that further improvements might be achieved if the proportion of Gliricidia in the mix were higher; i.e., giving the diets a higher NI:DOMI ratio than the observed average value of 0.022.

As previously mentioned, Kenaf, in different parts of the world, has been grown mainly for fibre. In Australia, the drop in sugar price in the 1990s prompted sugarcane farmers to consider alternative crops to sugar cane. In the Burdekin region of North Queensland, Kenaf is being investigated as a potential alternative crop. Primarily it is grown for fibre but there is great interest in utilizing byproducts from the plant with the view of maximizing profit from a given Kenaf crop. The seeds of this plant are energy and protein-rich (see Section 2.5) giving the feedstuff potentially a very high nutritional value. However, dietary fat which could have an inhibitory effect, at certain levels, on rumen microbial activity, is thought to be a potential disadvantage to using Kenaf as a dietary supplement in ruminant animal feeding. The experiment described in Chapter 6 was undertaken to examine the potential of Kenaf seeds as a supplement to a basal low quality forage diet. The levels of dietary fat of 3 – 6 % of DM, used in that experiment, were those that were considered by other workers (Kucuk *et al.*, 2003) to be inhibitory to rumen microbial activity. In the experiment described in Chapter 6, the Kenaf seeds, while not

improving dietary DMD, certainly did not have a negative impact on this parameter as might have been anticipated. Indeed the animals that received the Kenaf supplement retained the highest amount of N as well as the highest proportion of their NI.

From the preceding discussion and reiteration of results of experiments undertaken in the current study, it might be suggested that:

i. the past practices and designs of nutritional experiments for evaluating the nutritive values of feed supplements, particularly legume leaves supplements, would benefit from re-examination; and

ii. improvements in current practices in feeding supplements to ruminant animals in certain farm systems may be made by considering some of the findings made in the current study.

Most studies in the past may have underestimated the potential nutritional value of legume leaves supplements such as Gliricidia and Calliandra since in these studies generally, the supplements were offered simultaneously with the basal low quality forage examined. In the current study, it is clear that the timing of offering a supplement of Gliricidia leaves, for example, after a basal low quality forage has been fed to a ruminant animal would be important in the evaluation of the nutritional impact that the supplement makes on the diet. In the context of farms, such findings would be most relevant in cut-and-carry systems where animals are not free grazing and may be fed in stalls twice a day. Offering the animals their Gliricidia leaves supplements, at approximately four hours after the grass or straw has been fed would be a sensible feeding strategy that takes advantage of the potential nutritional value of the legume. In some areas where night-corralled animals might be offered some legume leaves before they are let out to graze in the morning it could be possible to reverse the practice and instead offer the legume leaves as the animals are put into their corral or pens for the night.

In feeding systems where animals have free access to pasture feeds continuously, the timing of offering a feed supplement such as Gliricidia leaves may be less of a concern with regards to wastage of N released from the supplement. It is likely in these animals that the availability of SCFA in their rumens would be high (within the limits of the quality of the pasture species ingested) and would allow the rumen microbes to utilize supplemental N to an extent commensurate with the potential of the basal pasture feed to supply SCFA. Generally, ruminant animals tend to graze pasture during the earlier part of the morning and of the afternoon. However, major grazing times of these animals may vary from farm to farm and it could be useful if farmers were to observe the general grazing times of their animals to assist them to determine the time they should offer supplements, such as fresh Gliricidia leaves to their animals. For example, if the animals were to graze from 0600 to 0900 hours, the legume leaves supplement could be offered at 1300 hours; a time perhaps just before the next usual grazing time for the animals.

The dry season is a critical time for farmers regarding the feeding of their ruminant livestock. The large amounts of DM that shrub legumes such as Calliandra and Gliricidia can produce in the wet season may be better used if the leaves of these legumes were preserved by drying for use in the dry season. As demonstrated by results presented in Chapter 5, the added advantage of such a practice would be the improvement in nutritional value of the Calliandra leaves achieved through the drying process. With regards to Gliricidia leaves, at least no deterioration in its nutritional value would result from the sun-drying process and storage for six months (Panjaitan, 2001).

There is little doubt that, if available, the combination of fresh or dried Gliricidia and Calliandra leaves would offer a superior supplement to a basal low quality forage diet. This is evident from the limited examination undertaken on the legume mix in the current study (see Section 3.5). Further investigation should be carried out to determine the combination of the legumes that could be used to optimize the nutritional value of a diet. Similarly, with regards to Kenaf seeds, further improvements may be achieved if the oils from these seeds were extracted and the

resultant Kenaf seed meal used as a protein supplement. This would be an economic choice as the cost of oil extraction would need to be balanced against the nutritional advantage gained. If Kenaf oil already is a commercially viable commodity then this could improve the viability of Kenaf seed as a feed.

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