Chapter 1

Introduction

Ruminants have a fundamental advantage over monogastric animals in that they possess a forestomach with micro-organisms that assist the host animal to exploit nutrients from fibre-based diets that they cannot digest by their own enzymes. As a result, the extent to which ruminants can utilise energy and essential nutrients from fibrous feeds depends on the efficiency of the rumen micro-organism ecosystem. Consequently, feeding ruminants means feeding both rumen microbes and the host.

The importance of fibrous feedstuffs, in particular cereal straws, in ruminant feeding has been recognised for a long time. There are some major reasons for continuing to study ways of making better use of this highly available source of feed. Firstly, cereal crops provide food for the majority of people all over the world. Consequently, by-products (straws) are produced in huge amounts annually. Secondly, in many parts of the world, especially in Asia, available grazing land is becoming more and more limited and over-grazed. Thirdly, exploitation of cereal straws and other fibrous agro-by-products as feeds for ruminants will assist countries to meet the requirement for development of sustainable agriculture.

Unfortunately, straws have a low feeding value. They have a low concentration of crude protein, soluble carbohydrates and minerals, and high concentration of lignin and fibrous structures. These features bring about a low feed intake and low

digestibility by ruminants. Therefore, if animals are solely fed on straws, they may not obtain enough energy and essential nutrients to meet their requirements even for maintenance.

To overcome the nutritional disadvantages of straws, many techniques have been developed and evaluated in a large number of studies. The application of these techniques, however, is variable depending on the prevailing situation of feeding systems. Treatment or supplementation of straws or a combination of both have been studied and implemented for a long time. In most developed countries, where ruminants are kept under highly intensive conditions, treatment of cereal straws is based on high-technology infrastructure which needs relatively high inputs. On the other hand, in the developing countries, the most applicable methods require low inputs and will be taken up by a large number of farmers only if they fit into local farming practices and are appropriate for local conditions. Thus, how to make better use of straws, in particular rice straw in Asia, still remains one of the most challenging questions for the animal scientists.

Currently, there appears to be a consensus that ammoniation and supplementation of straw, especially rice straw, are the most feasible methods for feeding many of the large populations of ruminants in Asia. However, different supplements have different feeding values and have different effects on animal performance. Therefore, it is important to ask: "which supplement is better for production of ruminants, and is urea treatment as effective for promoting animal production as supplementation of straw based diets".

The experiments presented in this thesis were designed to evaluate the effects of different feeding strategies on performance of sheep (Exp.1) and cattle (Exp.2) fed on cereal straws in Australia and Vie Nam, respectively.

Chapter 2

Literature review

2.1 Rumen environment

2.1.1 Rumen environment

The rumen provides an environment that favours the growth of micro-organisms which in turn make an important contribution to the nutritional metabolism of the host. Rumen contents consist of a mixture of ingested foods, rumen-organisms, fermented products, saliva, etc. The rumen is a complex ecosystem in which there is constant interaction between feeds, micro-organisms, and the host animal. The rumen environment contains a number of unique features that make it ideal for anaerobic microbes and which cause it to differ from most other anaerobic systems, as follows:

- pH about 6.5
- temperature: 38-40°C (is near optimum for many enzyme systems)
- continuous flows of nutrients: end-products removed and new substrates come in
- well mixed by contraction, and
- long residence time of feeds.

The rumen medium is controlled by many factors such as the quality and quantity of feeds eaten, salivation and rumination, diffusion and secretion into the rumen, absorption of nutrients from the rumen, and removal of material

down the digestive tract. These factors maintain stable conditions for anaerobic micro-organisms.

When feeds are ingested, they are diluted with a copious amount of saliva which plays a role in maintaining the rumen contents in a fluid state and facilitates access of micro-organisms to the plant materials. The amount of saliva depends on species of animal, types and quantity of diets, and environmental conditions (Van Soest 1983). Normally, cattle secrete about 150 l per day, and sheep about 10 l. Together with the absorption of VFA, salivation continuously adjusts the pH of the ruminal fluid, thus ensuring continuous fermentation.

The flux of ions between the rumen and blood maintains a fairly stable osmotic pressure in the rumen which is close to that of blood (McDonald *et al* 1970). Osmotic pressure promotes the flow of liquid out of the rumen to the omasum, while VFA absorption helps osmotic pressure stay within the necessary limits.

The rumen environment is anaerobic. However some oxygen is introduced through feed and water, and some may diffuse across the rumen wall. This oxygen leads to unfavourable conditions for anaerobic metabolism including the synthesis of methane. It will thus poison obligate anaerobic organisms (Hungate 1966).

Gases, mainly methane and carbon dioxide and a small amount of hydrogen, are produced in the rumen as end products of fermentation. Rumen gases are mostly eliminated by eructation. Bloat occurs when stable foams are produced in the rumen. The foam causes closure of the oesophageal sphincter, preventing eructation. Overwhelming gas production beyond the limits of eruction

capacity, or accumulation of gases in the rumen for any reason, both lead to bloat.

End-product removal has important effects upon the ecological balance allowing the rumen to be a continuous culture system. Undegraded dietary materials, as well as the biomass of micro-organisms, are regularly passed down the digestive tract maintaining the rumen micro-organisms at a fairly constant level. The rate of turnover of rumen contents is determined by a number of factors such as the rumen volume, salivary flow rate, uptake of water across the rumen wall and the rates of rumen contraction, feed intake and fermentation.

2.1.2 Rumen micro-organisms

2.1.2.1 Rumen microbial species

There are four kinds of rumen micro-organisms: bacteria, protozoa, fungi and bacteriophages. The relative numbers and volumes of some main species are shown in Table 2.1. All of them are anaerobic and depend on energy derived from the fermentation of dietary materials. Each type of micro-organism occupies its over ecological niche and plays a different role in rumen digestion and makes different contributions of nutrients to the host.

2.1.2.1.1 Bacteria

Bacteria are present in the rumen in the highest numbers compared with the other organisms, i.e. around 10^{10} to 10^{11} per ml of rumen fluid. Fonty and Gouet (1988) reported that in both lambs and calves, the rumen is rapidly colonised by

an abundant and diverse population of bacteria in the first days after birth, probably transferred from mother to offspring in saliva during licking or by aerosol. The density of bacteria increases gradually during the first three weeks and then remains at a stable level. According to Preston and Leng (1987) there are four main groups of bacteria: bacteria free in rumen fluid (about 30% of total); bacteria attached to feed particles; bacteria adhering to the epithelial lining of the rumen; and bacteria attached to protozoa. Although free bacteria are a relatively small component of the total, they still play an important role in determining the rate of rumen fermentation. Because they are 'transmittable' they can be attached to newly ingested materials. Thus, the number of free bacteria in rumen fluid can indicate the rate of colonisation to new material.

Table 2.1 Relative volumes and numbers of microbial organisms in the rumen contents of sheep fed alfalfa and wheaten chaff. (Data from Warner (1962), quoted by Van Soest 1983).

Group	Numbers per ml ($\times 10^6$)	Mean cell volume (μ^3)	Net mass* (mg/100ml)
Small bacteria	16,000	1	1,600
Selenomonads	100	30	300
Oscillospera flagellates	1	250	25
Ciliate protozoa			
entodium	0.3	10,000	300
dasytricha, diplodinium	0.03	100,000	300
isotricha, epidinum	0.011	1,000,000	1,100

* Estimated cell weight per 100 ml rumen fluid assuming a density of 1.0

Hungate (1966) suggested tha.. bacteria can be classified into 10 groups as follows:

1) Cellulolytic bacteria. Impor ant species include *Ruminococcus flavefaciens, Ruminococcus albus, Bacterioc es succinogenes, Clostridium loch headii.*

2) Hemicellulose digesting b cteria, for instance *Butyrivibrio fibrisolvens, Lachnospira multiparus.*

3) Amylolitic bacteria, such as *Selemonas ruminatium, Bacteriodes amylophilus.*

4) Bacteria using sugars.

5) Bacteria using acids.

6) Proteolytic bacteria.

7) Ammonia producing bacteria, *Peptostreptococcus elsdenii* for example.

8) Bacteria producing methan e such as *Methanobacterium ruminatium, M. formicium.*

9) Lipolytic bacteria.

10) Vitamin-synthesising bacte ia.

Bacteria are the principal agents for fermenting plant cell wall material. The most important species are *Ruminococcus flavefaciens, Ruminococcus albus, Bacteriodes succinigenes, Butyrivibrio fibrisolvens* (Cheng *et al* 1984). These enzymes are capable of breaking down cellulose, hemicellulose, and peptic substances into cellobiose, glucose and/or VFA. In order to digest fibrous material, bacteria need to be attached to the material, partly through the damage brought about by fungal invasion (Bauchop 1988).

2.1.2.1.2 Fungi

The main species of rumen fungi include: *Sphaeromonas, Piromonas, Neocalliamstix,* but only one genus, *Neocallimastix* has been fully described (Orpin and Joblin 1988). Normally, the presence of anaerobic fungi in the rumen can be found from an early stage of life. In sheep, they become established within the second week after birth (Orpin 1988) and the mode of transmission of them could be direct oral contact, by aerosol or by faecal contamination. However, the development of rumen fungi depends on diet, even in mature animals (Orpin 1988, Fonty and Gouet 1988).

The rumen fungi play a role as pioneers that firstly attack fibrous material. The mechanism of attachment of fungi to feedstuffs is summarised by Preston and Leng (1987). First, sporangia of fungi reach newly eaten fibrous material and invade the tissue. Then they germinate and grow through the plant particles. In this way they reduce the tensile strength of feed particles and increase particle breakdown by rumination. Another important role of fungi in fibre digestion is that they create 'damage' on the surface of plant material (Bauchop 1988), creating an 'open door' through which bacteria enter and colonise the cell interior. Without fungi, bacteria can not so easily attach to the plant material.

Most fungi are cellulolytic. Moreover, their capacity to degrade the hemicellulose-lignin structure and solubilise lignin is recognised (Preston and Leng 1987). Even though they can not actually digest the lignin, they may allow fibre that is physically protected by lignin to be fermented by rumen bacteria.

2.1.2.1.3 Protozoa

According to Williams and Coleman (1988) there are 17 genera of protozoa present in the rumen. However, protozoa are generally classified into two groups: small entodiniomorphs (large Entodiniomorphid protozoa) and the

large holotrich protozoa (mainly *Isotricha* or *Dasytricha* spp) (Bauchop 1988, Orpin 1988, Preston and Leng 1987). The small protozoa appear to prefer starch-fibre diets, while the larger types mainly occur in animal fed on sugar-fibre diets (Preston and Leng 1987, Kruezer and Kirchgessner 1988).

Protozoa population densities in the rumen vary from 10^5 to 10^6 per ml of rumen fluid (Hungate 1966). The fluctuation in numbers of protozoa depends largely on diet. Preston and Leng (1987) indicated that protozoal population densities in the rumen of cattle and sheep fed fibrous diets are less than 10^5/ml, whereas they can reach up to $4x10^6$/ml on diets high in starch and sugar. The number of protozoa is much smaller than that of bacteria. However, because protozoa are the biggest among rumen micro-organisms, about thousand times bigger than bacteria, they may represent up to 40% of rumen microbial biomass (Mackie 1987).

According to Mackie (1987), the ciliate protozoa are largely associated with plant material and may be responsible for some 30-40% of microbial fibre digestion. Williams (1988) reported that rumen protozoa can degrade and metabolise the principal proteins, carbohydrates and lipids in ingested feeds. They have both cellulolytic and proteolytic activities (Mackie 1987). Therefore they play an important role in the degradation of plant material in the rumen. Methanogenic bacteria are also often found in close association with protozoa.

However, many researchers (see Nolan, Leng and Demeyer 1988) seem to agree that protozoa play a negative role in rumen digestion. Protozoa, first of all, are counted as predators in the rumen ecosystem. They graze ingested plant fragments as well as bacteria and fungal zoospores to form their own biomass (Nolan 1988, Bauchop 1988) That means that the larger the population of protozoa, the smaller the number of bacteria and fungi present in the rumen.

Unfortunately, rumen protozoa appear not to be readily passed down the digestive tract and so provide only a small fraction of their biomass to the host as a source of protein and other nutrients. Actually, they sequester in the rumen for a long time. Preston and Leng (1987) summarised several ways in which protozoa are selectively retained in the rumen. Firstly, they are mainly attached to large particles of plant material. Secondly, they are not separated from the bolus during and after rumination. Thirdly, they tend to preferentially sequester onto the rumen wall. This means that their full biomass is not readily available for digestion in the lower digestive tract. In summary, the effects of protozoa on bacterial and fungal populations indirectly influence the rate of fermentation in the rumen. They occupy niches in the rumen that might otherwise be occupied by bacteria which are more completely 'harvested' by the ruminant host This is a fundamental principle of the defaunation technique which has been shown to increase production in ruminants in a variety of circumstances (Bird and Leng 1984, Bird 1988).

2.1.2.2 Interactions between rumen micro-organisms

The rumen is a very complex ecosystem in which the micro- organisms depend on each other as well as compete with each other for nutrients. Energy for their growth is con... as ATP during the fermentation of plant materials to rumen fermentation products, such as VFA, CH_4, CO_2, NH_3. The interaction influences the microbial population and the relative proportions of different species present, which in turn affects the fermentation pattern in the rumen.

2.1.2.2.1 Bacteria - bacteria interactions

In the rumen the interaction between bacteria appears to be to the mutual benefit of both (Preston and Leng 1987). The important relationship between

bacteria is the dependence on each other for certain nutrients. Some species ferment the primary nutrients in feed, and the others ferment products of the primary groups. In turn the fermented products of the latter will be available nutrients for the former. One example of mutual interaction between bacteria is hydrogen producing and consuming species. Hydrogen produced by certain species, *Ruminococcus* for instance, is produced as follows:

$$NADH + H_2 \Leftrightarrow NAD^+ + 1/2\ H_2$$

In fact, produced hydrogen is normally kept at about 10^{-4} atmosphere (Van Soest 1983) due to the consumption of hydrogen by methanobacteria. Given this, the bacteria in the rumen live and 'work' together as an active consortium.

2.1.2.2.2 Bacteria - protozoa interactions

It is well known that protozoa compete strongly with bacteria for their food (Hungate 1966, Van Soest 1983, Coleman 1988, Nolan 1988). On the other hand, they engulf bacteria, kill and digest them. Coleman (1988) found the number of bacteria engulfed by *Ent. caudatum* is about 3,000 to 4,000 bacteria/protozoan/hour. Coleman (1988) reported that the presence of protozoa in the rumen reduces the number of bacteria by 40-90%. Protozoa also consume intermediate products, decreasing the availability of substrate needed for bacterial fermentation. Jouany (1988) found that an addition of starch favours the development of entodiniomorphid cilliates, whereas holotrichs respond more to an addition of soluble sugars. It is clear that protozoa can either directly or indirectly limit the bacterial population and thus decrease the bacterial growth in and outflow from the rumen.

2.1.2.2.3 Bacteria - fungi - protozoa interactions

The interactions between these groups are obviously complex (Preston and Leng 1987). Jouany (1988) reported that elimination of protozoa decreased digestibility. The reason may be that protozoa can degrade and metabolise the principal proteins, carbohydrates and lipids in digested feed (Williams 1988, Orpin 1985). They have cellulolytic (Mackie 1987), lipolytic (Latham *et al* 1972) and proteolytic (Nolan 1988) activities. They therefore make an important contribution to the degradation of plant material in the rumen. However, because elimination of protozoa in the rumen can increase the number of both bacteria and fungi, defaunation can bring about an increase in the apparent digestibility of ingested feed (Bird and Leng 1978, Bird and Leng 1984, Bird 1988). In terms of protein supply to the host, this interaction may be very important because it can determine the amount of microbial biomass which passes lower down the tract. Ultimately, this causes a change in the P:E ratio in the materials absorbed by ruminants.

2.2 Digestion and metabolism in the rumen

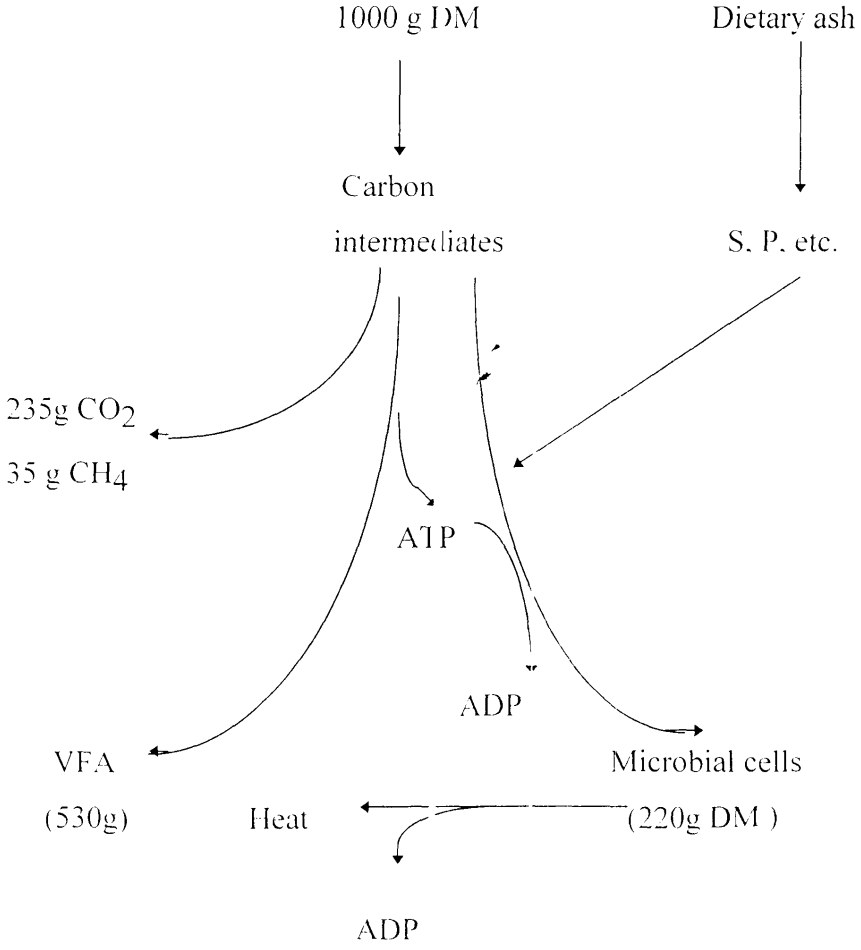
The overall scheme of digestion of organic matter in the rumen is presented in Fig 2.1

The rumen digestion and metabolism includes such processes as: degradation of complex materials, synthesis of macromolecules (microbial biomass), absorption of nutrients via rumen wall and passage of nutrients into the lower digestive tract. These processes involve both micro-organisms and the host. Micro organisms use energy and fermentation intermediates to maintain their vast population and so produce the absorbable nutrients for the ruminant.

2.2.1 Carbohydrates

In nature, carbohydrates can be found in the forms of cellulose, hemi-cellulose, lignin, pectin, starch and sugars. Quantitatively, carbohydrates, of which cellulose is the most abundant, are very important to the ruminant as they provide the source of energy primarily for the rumen micro-organisms and then for the host.

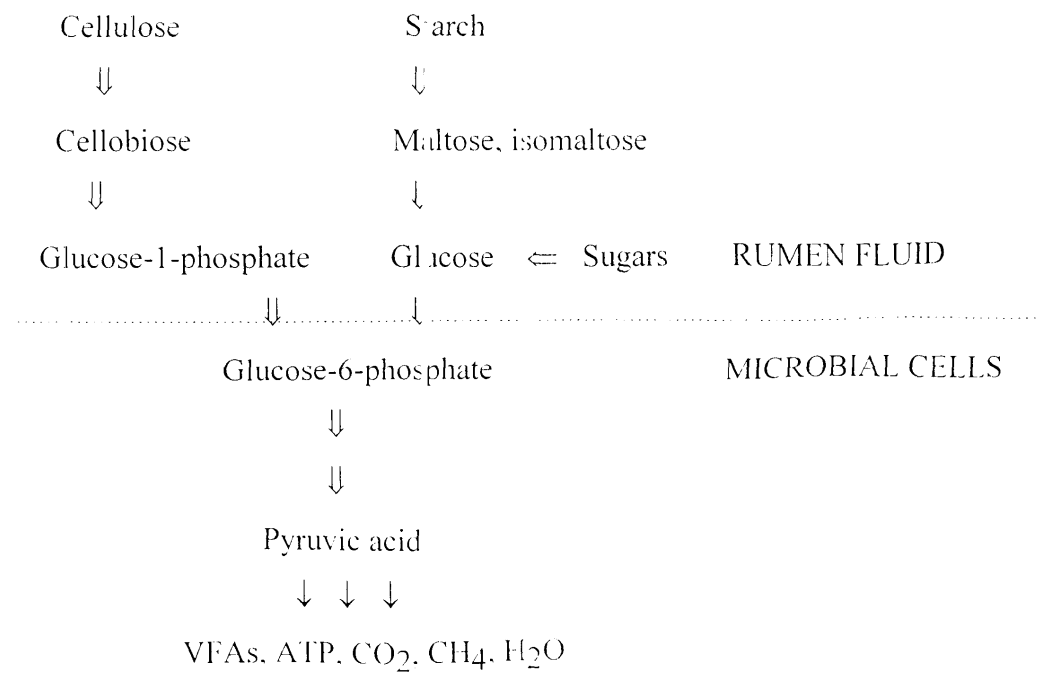
Fig. 2.1 The features of digestion of 1000g organic matter in the rumen. The values (g) are indicative of the amounts typically in an efficient rumen (Source: Nolan 1994)



The degradation of carbohydrates in the rumen involves two stages: the breaking down of complex carbohydrates to simple sugars and the intra-cellular metabolism of these sugars by micro-organisms.

Enzymes secreted by cellulolytic micro organisms break down cellulose into cellobiose which is then converted either to glucose or glucose-1-phosphate. Starch and dextrins are first digested by amylases to maltose and isomaltose, then by maltases to glucose or glucose-1- phosphate (McDonald *et al* 1992). Glucose and glucose-1-phosphate are rapidly taken up and metabolised intra-cellularly by rumen organisms In the microbial cell, glucose is used either as building monomers for synthesis of microbial protein, lipid and other macromolecules, or completely fermented to VFAs via several pathways of metabolism in which pyruvate is a key intermediate (Leng 1970) to produce ATP and end-products (Fig.2.2).

Fig. 2.2 Commonly accepted metabolic pathways of carbohydrates in the rumen.



Bacteria use released ATP from the fermentation process to maintain cell integrity and synthesise polymers. The rate of rumen fermentation depends upon the rate of supply of starch or sugar. The microbial cell production (yield of dry matter in grams per mol ATP or Y_{ATP}) is affected by the amount of ATP used for non-growth processes including cell maintenance. The fraction of the available ATP used for processes other than growth is a function of the mean residence time of microbes in the rumen and is thus lower for higher dilution rates. This means that a high turnover rate leads to reduced residence times for individual microbes in the rumen and therefore they must be able to grow rapidly to avoid being eliminated from the rumen. de Vries *et al* (1970) suggested a model to predict Y_{ATP} as follows:

$$1/Y_{ATP} = M_e/\mu + 1/Y_{ATP}\,(max)$$

where M_e = maintenance coefficient (mmol ATP/g cells per day)

μ = specific growth rate (dilution rate d^{-1})

ATP (max) = the maximum theoretical yield of cell per mol ATP (about 33g cell DM/day)

In practice, under good rumen conditions Y_{ATP} may be of the order of 10 - 14 (Leng 1993). Leng (1970) showed that, one-third of 4 kg of fermented carbohydrates provides the precursors for microbial cells and about 1,300 g are produced at a Y_{ATP} of about 14.5.

The most important end-products of fermentation of carbohydrates are volatile fatty acids (VFAs) including acetic, propionic, butyric and valeric, and carbon dioxide and methane. The proportions of VFAs produced are dependent upon the type of diet (Table 2.2). As can be seen in Table 2, acetic is the predominant acid, particularly in diets high in fibre. According to Beever *et al* (1988), total

VFA yield per mole of CHO is 1.90; 1.80 and 1.67 mol for high forage, high cereal and high molasses diet, respectively. The amount of total produced VFAs may reach 4 kg/head/day in cows (McDonald *et al* 1992). VFAs are mostly absorbed directly from the rumen, reticulum and omasum. The amount of VFAs which pass into the small intestine is probably small and hardly significant (McDonald *et al* 1992). Weston and Hogan (1968) estimated that 76% of ruminal VFA was absorbed from the rumen, 19% from omasum and abomasum, and 5% passed into the small intestine.

Table 2.2 Volatile fatty acids (VFA) (mmoles/l) in the rumen liquor of cattle or sheep fed on various diets (McDonald *et al* 1992).

Animal	Diets	Total VFA	Individual VFA proportions			
			acetic	propi	butyric	others
Sheep	Young ryegrass	107	0.60	0.24	0.12	0.04
Cattle	Mature ryegrass	137	0.64	0.22	0.11	0.03
Cattle	Grass silage	108	0.74	0.17	0.07	0.03
Sheep	Chopped lucerne hay	113	0.63	0.23	0.10	0.04
Cattle	40% long hay					
	+ 6% concentrates	96	0.61	0.18	0.13	0.08
Sheep	Hay : concentrate					
	1 : 0	97	0.66	0.22	0.09	0.03
	0.6 : 0.4	87	0.61	0.23	0.13	0.02
	0.2 : 0.8	70	0.4	0.4	0.15	0.05

A source of glucose is essential for ruminants to produce milk lactose, for potential growth to provide NADPH for lipid synthesis and as a source of energy for red blood cells and the nervous system. Because soluble sugars and starches in feeds are readily fermented, the amount of glucose which is passed down and absorbed in the small intestine is small. Thus ruminants have to synthesise glucose endogenously. Propionic acid and amino acids are the most common precursors for synthesis of glucose. Bergman *et al* (1966) and Leng (1970) indicated that 50-60% of the glucose is synthesised from propionate while Cridland (1984) suggested that propionic acid may contribute 80 - 90% of the glucose synthesised in sheep on roughage diets. In short, VFA production in the rumen becomes an important point of the digestion of carbohydrates in the rumen.

Gas produced from fermentation is a mixture of carbon dioxide (40%), methane (30-40%), hydrogen (5%) and other gases. The rate of gas production can be as high as 30 l/h in the cow (McDonald *et al* 1992), of which methane production ranges from 8.5 to 21.4 mol/d (Beever 1993).

Methane is formed through reaction that requires hydrogen as:

$$CO_2 + 8H \Rightarrow CH_4 + H_2O$$

Methanogenic bacteria, by this reaction, generate approximately 1 ATP and reduce hydrogen content in the rumen. Methane production varies depending on the pattern of VFA production which is determined by microbial species existing in the rumen (Chalupa 1980). Owing to methane formation, digestible energy is lost (from 3 to 10% of total energy intake, Beever 1993), so it is desirable to reduce the rate of methane production by microbial manipulation. The basic principle of increasing propionate : acetate ratio in order to decrease methane production is used. Chalupa (1980) reported that some feed additives such as ionophores (monensin, lasalocid) can promote propionate and reduce

methane production. Leng (1991, 1993) also emphasised that manipulation of methane production in the rumen is very necessary not only to increase the efficiency of energy in rumen digestion, but also to reduce emission of methane from ruminants which contributes to the "greenhouse" effect.

There are a number of factors that influence the extent to which cellulose is digested. Firstly, the rate of cellulose degradation depends on the degree of lignification of plant material, as lignin and related substances are resistant to attack by anaerobic micro organisms. Secondly, high availability of starch and simple sugars, can significantly increase the rate of cellulose fermentation as soluble carbohydrates provide rumen microbes with readily substrates to enable them to develop microbial biomass. Another factor that influences the rate of carbohydrate degradation is an imbalance of nutrients in diets. A shortage of nitrogen and minerals, particularly sulphur, can reduce the rate of microbial development thereby decreasing the rate of fermentation in the rumen. Last, but not least, rumen ecology which is, in effect, the combined interactions between all rumen microbial species can considerably affect the digestion of carbohydrates as discussed before.

2.2.2 Protein

In monogastric animals, a constant supply of amino acids, especially essential amino acids, from the diet is required to maintain production. In contrast, in ruminants total nitrogen content of the diet, or crude protein content, may be more important than true protein content since the rumen microbes can exploit non-protein nitrogen (NPN) and convert NPN into their true protein. Therefore, protein digestion in the rumen is synonymous with nitrogen metabolism. On the other hand, dietary protein which passes unfermented out of the rumen, so-called bypass protein, still plays important role in providing amino acids to the

host animal, in particular when nitrogen requirement of rumen microbes has been satisfied by NPN. There are two main sources of nitrogen entering the rumen, i.e. dietary and endogenous. Natural feedstuffs vary widely in the amount of nitrogen (Bo Goh 1975) which is present in different chemical compounds such as protein, amino acids, peptides, nucleic acids, amides and other non-protein nitrogenous compounds. The digestion and metabolism of nitrogen in the rumen, thus depends much on the types of compounds in which it is contained. The digestion and metabolism of nitrogenous materials in the rumen takes place via metabolic pathways in which catabolism and synthesis of protein are simultaneously occurring. The basic principles of nitrogen metabolism in the rumen are outlined in Fig.2.3, in which the maximal microbial biomass and bypass protein are the most important targets in protein nutrition for ruminants.

Ingested proteins are broken down by proteolytic activity, mainly provided by bacterial proteases which are secreted by the rumen micro organisms, into simpler compounds and finally to NH_3, the end product of protein catabolism, as follows:

protein → polypeptides → peptides → amino acids → NH_3

The rate of degradation of protein is affected by a number of factors such as its chemical attributes (solubility secondary and tertiary structure, cross-linking, etc.), dilution rate, rumen microbial profile, treatment of protein (Preston and Leng 1987, Beever 1993, Nolan 1993, 1994).

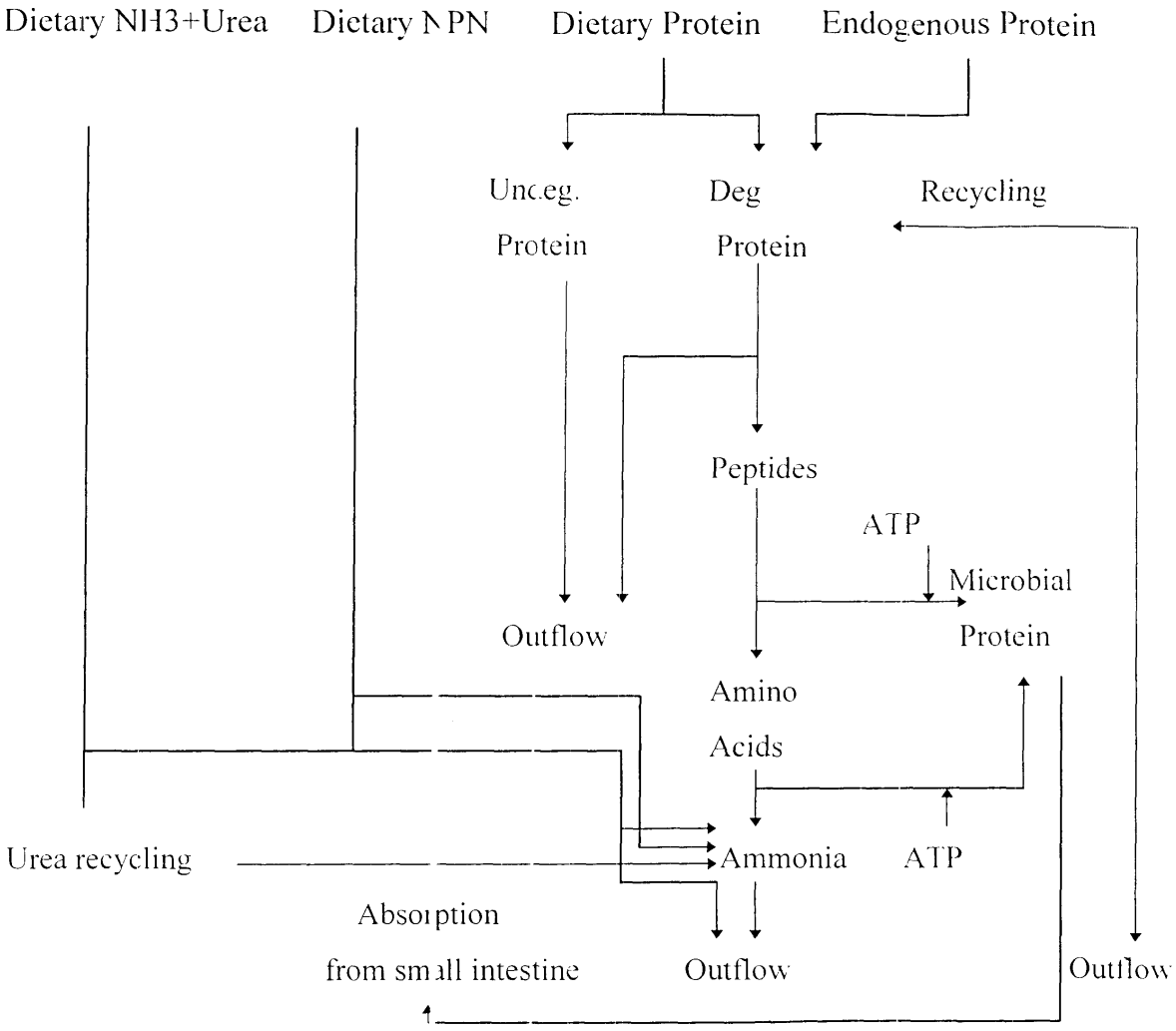
In forages, proteins are the main nitrogenous materials which represent 70 % or more of the total nitrogen present, although the non-protein nitrogen (NPN) fraction may at times be as high as 30% (Nolan 1993). Fresh forage diets, high in protein and soluble carbohydrates, support the development of proteolytic

micro organisms leading to specific activities in the rumen of more than nine times those found in animals given low-protein, hay based diets (Nolan 1993). Furthermore, Beever (1993) noticed that on fresh forages, particularly on fresh legumes, the rate of dissimilation of dietary crude protein is rapid and often exceeds the microbial requirements for ammonia. Application of nitrogen fertiliser to pasture increases both crude protein content of the pasture and the rate of degradation of nitrogen materials in the rumen (Nolan 1993, Beever 1993). By contrast, cereals contain only modest levels of crude protein (mainly present as true protein) with a reasonable rate of degradation (Beever 1993). Animal protein is broken down more rapidly than plant protein, but the former is seldomly completely degradable while, given enough time, the latter are completely degradable, but are lysed at a slower rate (Nolan 1993).

Chemical and physical treatments of protein supplements, e.g. heat, formaldehyde, tannins and alkali, can reduce their degradability in the rumen and therefore increase the proportion that escapes to the small intestine (Preston and Leng 1987, Nolan 1993).

As mentioned earlier, the majority of dietary protein in the rumen is degraded to amino acids and ammonia. Micro organisms use these products to synthesise their own protein and microbial protein is the major source of protein in duodenal digesta (Hungate 1966, Church 1975, Preston and Leng 1987, Nolan 1993, Beever 1993; Rooke *et al* 1987). Hungate (1966) suggested that about an amount of 100 - 150 g bacterial cells was produced from 1 kg of carbohydrate fermented. However, Church (1975) was critical of Hungate's estimate, and suggested that the maximum possible yield of microbial protein may reach 200g/kg of digested organic matter/day. Recently, Nolan (1993) estimated that the maximum output of microbial protein is about 150g (~ 300g cell DM) per kg fermentable feed polysaccharide.

Fig.2.3 Schematic representation of nitrogen metabolism in the rumen (Source, McDonald *et al* 1992).



Amino acids may be incorporated into microbial protein or deaminated intracellularly to various fatty acids and ammonia (Nolan, 1993). Moreover, according to Nolan (1993) micro-organisms are capable of synthesising ten essential amino acids for the host from simple carbon compounds and ammonia which is either available in the diet or derived from degradation of protein or endogenous sources. Many bacteria, however, use peptides rather than amino acids as precursors for protein synthesis. The reason for it may be that peptides

are part of the way to protein therefore bacteria need less ATP to synthesise their protein.

Rumen ammonia is derived from two main sources, ingested materials and secretion of urea and other endogenous sources of nitrogen. It is also released by rumen protozoa (Nolan 1993) Ammonia can be incorporated into microbial matter, absorbed through the rumen wall or passed down the lower digestive tract. Under grazing condition, Beever (1993) determined that only 30% of total ammonia nitrogen produced in the rumen was incorporated into microbial matter whereas 63% was absorbed across the rumen wall. However, these figures may be variable because incorporation of ammonia nitrogen into microbes must be dependent on factors such as nitrogen intake and energy (ATP) availability in the rumen. Absorbed ammonia may be used to synthesise amino acids in the rumen wall cells (Nolan 1993), or pass to the blood stream. Because ammonia is continuously absorbed from the rumen and urea produced from it is secreted into the rumen in the form of urea, it can be said to be recycled. Kameoka (1962) (cited by Church 1975) found that 14% of ^{15}N labelled nitrogen was recycled into the rumen. In work with sheep fed continuously on alfalfa, Nolan and Leng (1972) reported that 1.2g of rumen ammonia were derived from plasma urea. If the diet is very low in crude protein, the quantity of recycled nitrogen may exceed that absorbed from the rumen (McDonald *et al* 1992).

The ammonia in the rumen liquid is the key intermediate in microbial degradation and synthesis of protein, especially for some bacterial species (McDonald *et al* 1992, Nolan 1993, Leng 1993). Thus, if dietary protein is low or the protein resists degradation, the concentration of rumen ammonia will be low and the growth of organisms will be slow if supplementary nitrogen is not provided. As a consequence, carbohydrate breakdown will be retarded.

Estimates of the optimum concentration of ammonia in the rumen liquor vary widely, from 85 to over 300mg/l (McDonald *et al* 1992). Perdok and Leng (1988) suggested that, 50mg/l rumen liquid is optimum for highest feed digestibility in the rumen, whereas a level of 200mg/l is required for the highest feed intake. It is likely that there is no one 'optimum' concentration of rumen ammonia, but that the concentration will depend on diet and conditions in the rumen.

2.2.3 Lipid

Normally, feeds for ruminants are relatively low in fat content. Forage plants and many seeds usually contain about 4 - 6% of fat (Hilditch 1956). On the other hand, in many oil bearing seeds which are used as supplements in ruminant feeding, the lipid concentration may reach up to 36%, e.g. in linseed (Bo Gohl 1975). Plant lipid is mainly made up of glycerides, waxes, sterols and phospholipids. Lipid can be found in plant materials in the form of fatty acids, lipo-proteins or lipid-bound sugars. Dietary lipids of grazing ruminants comprise mainly galactolipids, sulpholipids and phospholipids. In contrast, animals receiving cereal or seed oil-concentrates will have triglyceride as the major dietary lipid (Hilditch 1956).

In monogastric animals, the digestion and absorption of feed lipid occurs in the small intestine with an important contribution of bile salts. By contrast, in ruminants the situation is different, owing to the activities of rumen micro organisms. The major evidence of lipid metabolism in the rumen is microbial hydrolysis and hydrogenation of feed lipids and *de novo* synthesis of cellular lipids by the rumen micro organisms (Harfoot and Hazlewood 1988).

The first step in fat metabolism in the rumen is the hydrolysis of ester linkages by microbial enzymes, and this step is a prerequisite for the biohydrogenation of

unsaturated fatty acids (Harfoot and Hazlewood 1988). Lipolysis in the rumen is done mainly by lipolytic bacteria and protozoa which are capable of secreting esterase and lipase (Lough 1970, Harfoot and Hazlewood 1988). According to Harfoot and Hazlewood (1988) the bacterial enzymes are entirely extracellular. Phospholipase activity is largely associated with rumen bacteria (Latham *et al* 1972, Dawson and Kemp 1969, Harfoot and Hazlewood 1988). Protozoa, play an important role in lipolytic activity. Latham *et al* (1972) found that about 30% of total lipolytic activity could be associated with protozoa.

It is well known that the tissue lipids of ruminants are more saturated than those of non-ruminants. The reason, which is now widely accepted, is that hydrogenation takes place in the rumen (Harfoot and Hazlewood 1988). Before hydrogenation, the hydrolysis of fatty acids from their esterified forms occurs. To investigate the role of different micro-organisms in biohydrogenation, Kemp (1969) eliminated protozoa from the rumen of sheep and found that the rate of biohydrogenation of linoleic and oleic acids in the rumen was not necessarily dependent on the presence of protozoa. The reason may be that bacteria are largely responsible for biohydrogenation in the rumen, while protozoa are of only very minor importance (Harfoot and Hazlewood 1988). In contrast, Chalupa *et al* (1967) showed that reductions in the numbers of ciliate protozoa have been associated with reduced hydrogenation. The role of particles in biohydrogenation is not clear. However some 80% of the biohydrogenation of linoleic acid occurred in association with fine food-particles (Harfoot and Hazlewood 1988).

The net effects of hydrolysis and biohydrogenation are the long-chain free fatty acids which constitute the major lipid class in digesta as they pass from the rumen to the lower digestive tract. Of the total free and bound long-chain fatty acids in digesta, stearic acid is the major free fatty acid which accounts for

about 40% of the fatty acids in digesta from ruminants on high forage diets (Katz and Keeney 1966) and as much as 80% in digesta from animals ingesting high grain diets (Keeney 1970).

One interesting aspect of lipid metabolism in the rumen is that high levels of dietary lipid may cause some negative effects on rumen microbes, and therefore affect lipolysis and biohydrogenation. It is commonly agreed that a high level of dietary lipid can be toxic to rumen protozoa (Hungate 1966, Dawson and Kemp 1969, Ikwuegbu and Sutton 1982, Murphy *et al* 1990, Armentano *et al* 1993). Fatty acids, especially if unsaturated, inhibit microbial growth and their toxicity has been explained by their greater surface activity and consequent effect in changing the permeability of cell membranes (Dawson and Kemp 1969). Ikwuegbu and Sutton (1982) reported that with 40 ml of linseed oil given per day, the rumen of sheep was almost defaunated. Latham *et al* (1972) found that low-roughage diets increased the number of viable bacteria in cows by 0.6 to 1.6 log units compared with those fed high roughage diets, but decreased the number of lipolytic bacteria by up to 3 log units. At the same time, low-roughage diets reduced the numbers of ciliate protozoa.

The level of dietary fat also influenced other aspects of rumen digestion. Murphy *et al* (1987) and Armentano *et al* (1993) have found that high dietary fat content decreases not only the proportion of DM digested in the rumen but also total DM digestibility. Furthermore, excessive amounts of fat may shift volatile fatty acids towards more propionic acid and less acetic acid, thus reducing milk yield and milk fat of milch animals (Chalupa 1984).

To make better use of fat supplements in feeding ruminants, especially high yielding dairy cows, it is necessary to protect fat from rumen fermentation. Saturated fats such as tallow, or rumen protected forms like Ca-soaps of long

chain fatty acids are particularly useful because of their minimal effect on rumen microbial activity (Palmquist and Jenkins 1980, Klusmeyer *et al* 1991). Furthermore, because bypass fat can be digested and absorbed in the small intestine with little conversion for fat synthesis, the energetic efficiency of the conversion of ME into energy in body fat is very high (close to 90%) while efficiency decreases when fats are first broken down to simpler fatty acids in the rumen (Van Soest 1983).

In conclusion, digestion in the rumen and metabolism of compounds in the host are major aspects of ruminant nutrition. Owing to symbiotic micro organisms in the rumen, the process of conversion of the complex feed materials into absorbable matter is achieved. This is advantageous for ruminants in comparison with non-ruminant animals, especially when the feed available is high in complex carbohydrates. On the other hand, rumen fermentation is wasteful of ingested energy. Thus, based on the understanding of rumen digestion, it is essential to optimise the digestive efficiency in both the rumen and in the host tissues in order to maximise utilisation of the absorbed nutrients by the host.

2.3 Improving feeding value of straws by treatments

The idea of treating cereal straws to improve their nutritive value has attracted people for more than a hundred years (Sundstol 1988). There are many methods of treating straws which can be classified into physical, chemical, physico-chemical and biological. These methods aim at improving the feeding value of straw by increasing its digestibility, feed intake or a combination of both. A number of methods described in the literature are technologically feasible; however their use has been limited in many circumstances for economic

reasons (Doyle *et al* 1986). The application of any technique, therefore must be carefully matched with the prevailing farming practices.

2.3.1 Physical treatments

Physical treatments of cereal straws include soaking and wetting, chopping, grinding and pelleting, steaming, and gamma irradiation. In general, physical treatments do not significantly affect the chemical composition of straws.

The main beneficial effect of soaking and wetting is an increased feed intake which is brought about by the reduced dustiness of the feed (Doyle *et al* 1986). In contrast, some workers have found negative effects of the soaking method. Firstly, dry matter was lost (8-14%, Dumlao and Perez 1976) indicating some soluble cell contents were removed by the soaking process. Secondly, soaking decreased *in vitro* digestibility of dry matter (Ibrahim and Pearce, 1983). Thirdly, wetting can reduce both feed intake and digestibility of organic matter (Devendra 1983).

Chopping feeds does not improve the performance of ruminants (Mathison 1976, Drennan 1980, Devendra 1983), but this method can facilitate feeding (Doyle *et al* 1986). On the other hand, grinding can improve feed intake (Stone *et al* 1969) as well as digestibility (Moore *et al* 1972) due to an increase in surface area of feed particles which provides more opportunity for rumen microbes to attach to plant materials (Stone *et al* 1969, Millett *et al* 1970).

Effects of steaming straws under high pressure (1 - 42 kg/cm^2) have been tested by a number of workers (Hart *et al* 1981; Rangnekar *et al* 1982; Garrett *et al* 1981). Doyle *et al* (1986) suggested that 'steaming exerts physical effects through the separation of cell wall structures and chemical effects including the

cleavage of bonds between cell wall constituents, the degradation of hemicellulose, and hydrolytic action of acids resulting from these forces'. This method, however, is not practically feasible due to its disadvantages such as the loss of organic matter, the lower digestibility, and more importantly, the high cost of the treatment. As with steam treatment of straws, gamma irradiation (dose levels from 0, 5, 10, 25, 100 to 200 Mrad) does not improve either feed intake or dry matter digestibility (Arthur 1971, McManus *et al* 1972) and is also costly. Also the treatment has to be done in 'high technology' centres and therefore straw has to be transported to a central location which can be costly.

2.3.2 Chemical treatments

Chemical treatments of feeds have been widely studied for a long time. Chemicals used include acids, alkalis or oxidising reagents. Methods developed include wetting, soaking or spraying with these types of chemicals.

Although sodium hydroxide has long been known to be an effective reagent which can improve (about 4% *in vitro*) digestibility of straws (Chandra and Jackson 1971, Shin *et al* 1981, Ibrahim and Pearce 1983), its use has been very limited because of its high cost, low availability and harmfulness to handlers as well as to the environment. On the other hand, calcium hydroxide, or its relatively inexpensive precursor, lime with its reasonable effects on feeding value of straws may to be more feasible in Asian farming systems. Depending on levels of lime used, DMD is increased by about 20% *in vitro* (Dumlao and Perez 1976) or 15% by evaluation using nylon bag method (Haque *et al* (1981). Unfortunately, soaking feeds in calcium hydroxide solution appears necessary as spraying appears to be ineffective. This means that a large vessel and a large amount of water are required for its application. Poor farmers in many developing countries can not afford these facilities, and as a result, they can not

adopt this technique. Acids (sulphuric for example), oxidising reagents (e.g. sulphur dioxide, chlorinated compounds) have also been tested as a means of improving feeding quality of straws (Yu *et al* 1975, Goering *et al* 1973, Ben-Ghedalia and Miron 1981, Teck *et al* 1982, Han 1977, Crosthwaite *et al*). Similar to alkalis, they are hazardous to people and the environment. The cost involved in these chemical treatments is also high, making their use inappropriate.

2.3.3 Ammoniation or urea-treatment of straws

In contrast to the above-mentioned chemicals, ammonia and urea have been intensively investigated and widely used as a means of improving the feeding value of low digestibility feeds. Interest in the use of anhydrous and aqueous ammonia for treatment of straws has increased considerably in Europe and North America (Owen and Urio 1984) while, in Asia, scientists have concentrated on the use of urea due to difficulties of transportation and handling of gaseous and liquid ammonia (Doyle *et al* 1986). Urea has successfully been used to treat straw in many Asian countries such as Sri Lanka, Bangladesh, India, Thailand, Philippines, Indonesia and recently China (Doyle et al 1986, Wanapat 1990, Dolberg and Finlayson 1995).

Many workers (Waiss *et al* 1972, Wanapat *et al* 1984, Wanapat 1990, Ibrahim *et al* 1984) have tested the effects of anhydrous and aqueous ammonia on digestibility of rice straw and found that DMD and OMD were increased by 12-15% and 5-20%, respectively. When aqueous and anhydrous ammonia were used to treat straw, Sundstol *et al* (1979) reported that ammonia levels of 3 to 4% of dry matter significantly improved digestibility and little benefit was obtained when the levels were increased further. They also suggested that because ammonia is a slow reacting chemical, treatment time must be longer

when temperature is lower. Moisture content is another important factor affecting the quality of treated straws. According to Waiss *et al* (1972), a moisture level of 30% is optimum, whereas Sundstol *et al* (1979) suggested that the optimal level might be 50%. A very common method of urea treatment of cereal straws is ensiling. Urea is applied to the straw at concentrations from 2 to 6 % of straw DM. The duration of the treatment varies from 1 week (high urea level) to 3 weeks. Urea can be used alone or combined with other compounds (lime, concentrates, for instance).

Most of the studies on urea ensiling of straw have shown a significant increase in feed intake, digestibility, and yield of animal (Jackson 1978, Preston and Leng 1987, Dolberg and Finlayson 1995, Oosting *et al* 1993, Fondevila *et al* 1993). Doyle *et al* (1986) have summarised the effects of urea-ammonia treatment of rice straw on dry matter intake, digestibility and live weight gain from experiments with growing or mature buffaloes and cattle (Table 2.3). Saadullah *et al* (1983) and Wanapat *et al* (1984) reported that urea treatment increased digestibility of rice straw DM by 5 to 10%.

Recently, Dolberg and Finlayson (1995) demonstrated that urea-treatment of straw has been successfully taken up by a large number of farmers in China. The quantities of straw treated since 1985 in China are shown in Table 2.4. These authors also reported the clear effects of urea treatment on the nutritional value of straw as well as the performance of animals. For example, treatment with urea at 5% tended to lead to higher feed intake by animals than treatment at 3% urea. Generally, treated straw had a higher rate of DM degradation *in vitro* (by approx. 10%), which significantly improved the performance of animals.

Table 2.3 Dry matter intake (DMI), digestibility (DMD) and live weight (LW) change of non-lactating cattle and buffaloes fed untreated (None), urea-supplemented (US) or urea-treated (UT) rice straw-based diets (adapted from Doyle *et al*, 1986).

Treatment	Storage time (days)	Total DMI (kg/100 kg LW)	Straw DMI (kg/100 kg LW)	DMD (%)	LW change (g/day)	References
A. Buffaloes						
None			2.1	50	-182	Wanapat et al, 1982
5% UT	21	-	2.3	52	79	"
5% UT	21	-	2.0	51	-	Sriwattanasombat &
5% UT	21	2.1	-	58	-	Wanapat, 1985
None			2.0	43	-130	Wongsrikeao &
3% UT	21		2.2	53	-50	Wanapat, 1985
6% UT	21		2.5	55	210	"
B. Cattle						
US		2.6	-	49	-	Hossain et al, 1982
5% UT	10	3.3	3.1	51	110	Saadullah et al, 1982
5% UT	21-28	2.9	2.1	48	75	Wanapat et al, 1982
None		2.3	1.3	-	73	Perdok et al, 1982
5% UT	7	3.1	2.7	-	310	Khan & Davis, 1982
US		2.9	2.7	46	75	"
4% UT	28	2.6	1.6	-	364	Perdok et al, 1982

One important aspect of ammoniation of straws is its positive impact on the action of rumen micro-organisms. Fondevila *et al* (1993) studied the effect of ammonia treatment of barley straw on the dynamics of its degradation in the rumen and concluded that "although treatment increased the soluble fraction of

untreated and treated straw by 52 and 65g/kg respectively, from 0 to 72 h of incubation, increasing the differences between straws from 45 to 58g/kg in that period, actually microbial action on the treated roughage was the more important factor accounting for a difference between untreated and treated straw of 131g/kg after 72 h incubation". When supplementing straw with barley or unmolassed sugar-beet pulp, Chen *et al* (1992) found that microbial yield per unit DOMI for a diet based on ammonia treated straw could not be improved substantially. They suggested "The practical implication of these findings is that ammonia treated straw does not differ from barley or unmolassed sugar-beet pulp as an energy source for microbial protein production in terms of the yield of microbial protein per unit DOMI".

Table 2.4 Quantities of straw treated in China from 1985 to 1992 (Dolberg and Finlayson 1995).

Year	Million tons
1985	0.003
1986	0.042
1987	0.148
1988	1.480
1989	1.830
1990	2.570
1991	3.870
1992	6.000 (estimated)

2.4 Supplementation of straws with nitrogen compounds

2.4.1 Supplementation with non-protein-nitrogen

On low quality straw-based diets, the primary limitation to the growth of rumen micro-organisms is often the concentration of ammonia in the rumen fluid (Preston and Leng, 1987). The optimal concentration of rumen ammonia for microbial growth will vary depending on diet. Preston and Leng (1987) reviewed that with forages containing considerable amounts of crude protein it is highly likely that organisms adhering to the fibre depend on the nitrogen within the plant cell wall. The efficiency of growth of these organisms may be less affected by the level of ammonia in the rumen fluid. However, 50 mg NH_3-N/litre of rumen fluid is recommended as most suitable for growth of microbes, whereas the level of ammonia that maximises feed intake was reported to be 250 mg NH_3-N/l in case of straw-based diets (by Perdok and Leng, 1988). It is necessary that ammonia concentration is continuously high in the rumen fluid to support the growth of micro-organisms as well as to encourage maximal feed intake by animals. Preston and Leng (1987) recommended that when rumen ammonia levels are less than 150 mg/litre, the effects of adding urea should be monitored under the prevailing farm conditions to determine the optimum level of supplementation to support the growth of rumen microbes.

A common source of fermentable nitrogen, which has been widely used, is urea. The reason for this is that urea is hydrolysed rapidly in the rumen to carbon dioxide and ammonia, which is then synthesised into microbial amino acids and proteins by rumen bacteria. The advantages of using urea are that it is: (1) highly available; (2) relatively cheap; (3) safe and easily handling and (4) a rich source of ammonia. The application of urea supplementation may be by:

- treatment of straws,
- addition to the diets,
- molasses-urea block.

Urea treatment and molasses-urea block appear most advantageous and economical (Preston and Leng 1987). An interesting example of urea supplementation for dairy cows in India was reported by Leng (1991). By using strategic supplementation of dairy cattle, mainly using the molasses-urea block, the milk production in certain parts of India was increased by nearly 30% in comparison with the previous two years.

The effects of NPN supplementation on sheep were almost the same as that for other ruminants. Williams (1982), Leng (1992) and Smith (1984), reported that both NPN and protein-nitrogen supplements increased feed intake, digestibility and the performance of sheep The increase in roughage intake was marked, especially when NPN was concurrently used with protein supplements. For instance, a supplementation of oats plus urea increased by 27% the intake of roughage by sheep (Smith, 1984). Experiments conducted by Soetanto (1986) on sheep fed roughage based-diets showed that a diet without urea caused a loss in live weight (35 g/day), whereas diets with 3 and 6 % urea resulted in a daily live weight gains of sheep of 19 and 24 g, respectively. However, NPN supplementation on its own may not significantly affect wool growth. The reason for this is that wool growth is responsive to the proportion of sulphur-containing amino acids which are more readily supplied by protein-rich feed supplements (Reis 1979; Kempton 1979, Williams 1982).

2.4.2 Supplementation with bypass proteins

There are two main production systems in which bypass proteins are emphasised with different objectives:

1) High milking animals and feedlot-beef cattle. The animals in these systems are normally of high genetic merit for productivity and therefore escape proteins (and other nutrients) are needed to maximise animal genetic potential.

2) Ruminants in low-input systems. In these systems, ruminant feed is mostly based on low quality roughage or by-products. Supplementation with bypass proteins aims to optimise the utilisation of local fibrous-feed resources, and therefore improve net income for the producers.

For the former, feeding standards have been compiled and are widely used in many intensive husbandry systems (NRC, 1966; 1985). The following discussion focuses on the latter systems.

Leng (1987) claimed that 'the balanced nutrient approach to feeding livestock in drought potentially saves 30% or more of feed for productive purposes which would otherwise be wasted as heat'. In livestock consuming fibrous diets, even when microbial growth in the rumen is supported by non-protein nitrogen and other soluble nutrients, the absorbed nutrients may be still imbalanced so that their utilisation by the animal is not as efficient as in the case of a balanced nutrient supply. Lindsay *et al* (1982) for example, reported the effects of feeding urea/sulphur and bypass protein with hay, live weight changes of growing cattle were -0.32kg/d in a urea/sulphur supplemented group, and +0.22 kg/d in group given urea/sulphur plus 500g bypass protein. Hennessy (1984) showed that supplementation of young cattle with protein concentrates in the dry season significantly increased their mature body weight as cows (Table 2.5). Stobbs *et al* (1987) supplemented cows on a subtropical pasture with 1 kg/day of formaldehyde-treated casein and found they produced 14.7 kg milk/day compared with 12.3 kg/day in non-supplemented cows.

Table 2.5 The effects of protein supplementation of young cattle on their mature body weight (kg) as adults. (Hennessy, 1984)

Feeding system	1978	1981	1982
Native pasture (no supplementation)	197	329	320
Native pasture plus bypass protein)			
Group1	259	387	382
Group2	264	397	397

Smith and Kenney (1987) also reported that cattle given 900 g lupin or cottonseed meal/day increased pasture intake 11 and 41% respectively compared with non-supplemented cattle. Their live weight gains were 119 and 321 g/day respectively, compared with a loss in weight of 214 g/day of control animals.

Supplementation with bypass protein not only supports live weight gain and milk production of cows, but also improves their reproductive performance. Hennessy (1986) reported that conception rates of grazing cows were markedly improved by supplementation with 1.5 kg/day of cotton seed meal for 2 months during a period when only carpet grass was available. Whilst a well-balanced energy supplement increased live weight gains, conception rates were remarkedly improved (Table 2.6). Du Huyvetter *et al* (1993) compared the effects of supplying different quantities of ruminally undegradable protein (UDP) to cows before breeding and found that those receiving 25% UDP (119 g of UDP/490 g of CP) returned to oestrus later than cows fed 50 % UDP (245/490).

As with cattle, the effects of supplementing sheep with bypass protein sources have also been positive. Smith and Kenney (1987) showed that lupin supplementation not only increased live weight gain but also supported better wool growth and reproduction of sheep (Table 2.7). Stephenson and Bird (1987) studied the effects on grazing ewes of N supplementation and demonstrated that both non-protein nitrogen (urea plus ammonium sulphate) and meat meal increased live weight gain of ewes and birth weight of lambs; however meat meal gave a greater improvement with 131 g/d live weight gain and 4.0 kg of birth weight compared with 38g/day and 3.5 kg of non-supplemented animals.

Table 2.6 Information on live weight and conception rate of lactating beef cows (with first calf at foot) grazing dry native pasture obtained by appropriate supplementation. (Source: Hennessy, 1986)

Supplement	Live weight (kg)	Pregnancy (%)
Nil	302	10
Energy	332	20
Bypass protein	343	60

According to Fattet *et al* (1984), there was a marked increase in the feed utilisation efficiency of sheep when a protein supplement was given. In their experiment, sheep fed fish meal at 120 g/day although losing total body energy, were able to deposit tissues, to increase protein growth and increase live weight (Table 2.8).

Table 2.7 The effects of lupin grain supplement on production of lambs fed on poor quality hay. (Smith and Kenney, 1987)

Observations	Nil	Lupin	Wheat
Birth weight (kg)	3.7a	4.0b	3.9ab
Lamb growth from birth to 6 weeks(g/day)	155a	196b	175b
Lamb survival	81	93	82
Milk production (ml/h)	39a	62d	49b
Fleece weight (kg)	2.8a	3.0b	2.9ab
Tender fleece (%)	40a	10b	30a
Barren ewes next lambing (%)	29	9	23

[Note: Values followed by the same letter are not significantly different ($P<0.05$)]

In hot environments, animals suffer through an inability to dissipate their heat load. Depression of feed intake is a way by which ruminants can limit heat production because heat is continuously generated from rumen fermentation and during metabolism of absorbed substrates in tissues (McDowell 1972). Many researchers have reported that high temperatures decrease feed intake, in particular roughage intake, and performance (milk, live weight gain) of ruminants (NRC 1981, Moreau 1983, Mc Dowell 1972). Supplementing ruminants with concentrates, especially escape nutrients enables them to minimise the decline in intake with rising temperature and help them to perform better (Preston and Leng 1987). Bunting *et al* (1992) found that, lambs supplemented with escape protein retained more N when exposed to high temperature than unsupplemented lambs (2.8 vs 3.6 g N/day). Thus, in hot environments, where poor feed supply is usually coupled with high

temperatures, the addition of bypass protein to fibrous diets for ruminant could improve the performance of animals.

Table 2.8 Effects of bypass protein supplement on partitioning of body tissue reserves of sheep fed straw-based diets (Source: Fattet *et al.* 1984).

Parameters	No supplement	120g fish meal/day
Initial weight (kg)	45	43
Final weight (kg)	45	49
Daily weight gain (g/d)	0	68
Intake (g/d):		
Straw	860	760
Molasses	43	43
Fish meal	0	120
Changes in carcass composition:		
Protein (g)	-140	+890
Energy (MJ)	-59	-16
Fat (kg)	-1.4	-0.9

The effect of protein supplements on performance of ruminants has been widely demonstrated. However, nutritional benefit brought about by protein supplements is variable depending on: 1) the amount of ruminally fermentable dietary nitrogen, which is most affordable by urea treatment or urea addition and which Preston and Leng (1984) suggested, for poor quality diets, should be raised to a minimum of 30g/kg DOM, and 2) degradability of supplemented proteins in the rumen.

Microbial protein production may be limited by a shortage of ruminally degradable nitrogen when ruminants are given protein sources that are resistant to rumen degradation. When studying ruminal characteristics and internal amino acid flows of steers fed corn-based diets and supplemented with a combination of corn gluten meal/blood meal, Ludden and Cecava (1995) found that the supplements increased non-microbial protein flow at the duodenum but tended to decrease microbial nitrogen flow. It is possible that microbial growth in their study was limited by the availability of ruminally available nitrogen.

2.5 Effects of protein supplementation on feed intake and digestibility

The effects of protein supplementation on feed intake and digestibility of ruminants may be either additive or substitutive. In animals on low quality fibrous diets, the bypass proteins acted as true supplements, not substitute feeds. In contrast, on high quality diets, they acted as a substitute feed rather than as true supplements. Doyle *et al* (1986) fed increasing amounts of a 2:1 mixture of oat grain and sunflower meal and found that the lowest level (175g/d) was mostly additive with the intake of the control, but higher feeding levels were completely substitutive.

Supplementing ruminants with high levels of protein or concentrates may reduce both feed intake and digestibility by the animals. Preston and Leng (1987) suggested that, in practice, protein supplements should not be given in amounts higher than 20% of total dietary dry matter. They wrote "the 20% limit is to prevent intake of supplement from reducing intake of digestible energy of the basal diet and lesser amounts may be more economical".

Last but not least, there is an important consideration that supplementation with bypass protein should always be a second priority, implemented only after an optimum provision of nitrogen and/or soluble proteins for rumen micro organisms. This approach must be more comprehensive when and where ruminant feedings are based on low quality roughages. Therefore, the overall strategic scheme of nitrogenous compound supplementation for ruminants is:

Step 1: Provide non-protein nitrogen to maximise the microbial growth

Step 2: Provide bypass protein to maximise and balance the absorbed amino acids and protein : energy ratio.

2.6 Feeding values of cotton seed, sunflower seed, copra and palm kernel cake

2.6. 1 Cotton seed

Cottonseed is an important commercial source of plant protein concentrates. The cotton seed consists of two parts, the hull from which the staple cotton lint and cotton linters arise and the kernel from which the oil and meal are obtained. One ton of cotton seed yields about 200kg of oil, 500kg of cotton seed meal and 300kg of hulls (Bo Gohl, 1981 .

The chemical composition of products derived from cottonseed has been reported by Bo Gohl (1981) (Table 2.9). The protein of cottonseed meal is of good quality, but has the disadvantages common to other oil seeds of having a low content of cystine, methionine and lysine (McDonald *et al* 1978). One noticeable problem of using cottonseed as animal feed is the pigment *gossypol* (0.03-0.2%, McDonald *et al* 1978; 0.4-1.4%, Bo Gohl 1981) which has an inhibiting effect on digestive enzymes (Bo Gohl, 1981). Luckily, ruminants

show no ill effects even when fed on large quantities of cottonseed meal (McDonald *et al* 1973). However, calves are susceptible to the harmful effects of gossypol because of incomplete rumen development (Bo Gohl 1981), and therefore, it has been recommended that a concentrate for calves under five months of age should not contain more than 10 to 15% cotton seed meal (Van Loenen, 1985). In addition, the biological effect of gossypol can be prevented by adding of iron, in the form of ferrous sulphate (Van Loenen, 1985).

2.6.2 Sunflower meal

Native to Central America, sunflower is now grown all over the world, mostly for its oilseed. The sunflower meal is produced when the oil is removed from the seed by hydraulic pressure or solvent extraction. Solvent extraction meals contain about 200g crude fibre and 390g crude protein/kg and have metabolisable energy content of about 10.4 MJ/kg for cattle and 11.2 MJ/kg for sheep (Mc Donald 1992). The composition of sunflower meal varies according to the quality of the original seed and the method of processing (Van Loenen, 1985).

The meals are useful sources of protein which is low in lysine but has about twice as much methionine as does soya protein (Mc Donald *et al* 1992). Amongst oilseeds, sunflower seed appears to have the highest sulphur amino-acid content (Bo Gohl 1981). Proteins in sunflower meals have a higher degradability (ranges from 0.77 to 0.84) compared to those in coconut meal (0.43) or fish meal (0.16 - 0.71) (Mc Donald *et al* 1992).

2.6.3 Coconut meal and copra

Chemical composition of copra is shown in Table 2.9. The edible kernel of the ripe fruit of the coconut palm is called *copra* when it is dried to a moisture content below 6%. The residue from copra after pressing or extraction to remove oil is called copra meal or cake (Bo Gohl 1981). On the average, 1000 nuts will produce about 180 kg copra and processing of this copra yields about 110 kg oil and 55 kg meal (Van Loenen, 1985). Copra and coconut meal are good energy concentrates for simple stomached animals (McDonald *et al* 1992). They have the disadvantage, however, of a tendency to develop rancidity in storage (Bo Gohl 1981, McDonald *et al* 1992). It is also claimed that it will increase the fat content of the milk, therefore the maximum safe amount for dairy cows seems to be 1.5 to 2 kg daily (Van Loenen, 1985). Although the protein of copra is low in lysine and histidine, neither protein quality nor fibre content is limiting where ruminant animals are concerned, and for them coconut meal provides an acceptable and very useful protein supplement (McDonald *et al* 1992).

2.6.4 Palm kernel cake

The fruit of the palm tree yields to kinds of oil. Palm oil is obtained from the freshy covering and palm kernel oil from the kernel of fruit. The residues after extraction of the kernels is called palm kernel cake (Van Loenen, 1985). This feed has a relatively low content of protein compared with cotton seed meal (Table 2.9). However, the protein is of high quality (Bo Gohl 1981, Devendra 1982). The first limiting amino acid for non-ruminants which is usually methionine. One important advantage of this feed is that the ratio of calcium to phosphorus is more favourable than many other oil seed residues (McDonald *et al* 1992). Normally, palm kernel cake is dry and gritty and is not readily acceptable by all types of animals (Bo Gohl, 1981). For monogastric animals, its high fibre content (about 15%) appears to reduce its apparent digestibility

(McDonald *et al* 1992). On the other hand, palm kernel cake is largely used for feeding cattle, especially for da ry cattle (Bo Gohl 1981, McDonald *et al* 1992).

Table 2.9 The chemical composition of cottonseed, sunflower seed, coconut meal and palm kernel (after Bo Gohl 1981)

Feeds	DM (%)	As % of dry matter						
		CP	CF	Ash	EE	NFE	Ca	P
Cotton seed								
whole seed, India	94.3	20.6	21.5	4.9	20.1	32.9		
whole seed Egypt	91.2	21.5	23.2	5.5	26.2	23.6		
Sunflower seed								
seed with hulls, Chile	93.7	2.3	27.9	3.1	42.0	14.7	0.21	0.59
oilcake without hulls, Uganda	91.0	34.1	13.2	6.6	14.3	31.8	0.30	1.30
Coconut meal								
copra, Malaysia	50.0	7.4	3.0	2.0	68.0	19.6	0.03	0.26
copra, Philippines	51.3	9.7	4.3	2.9	64.4	18.7		
Palm kernel								
kernel, Malaysia	92.0	7.9	3.9	1.7	54.0	32.5	0.09	0.31
kernel oil cake, Ghana	88.2	15.8	29.7	3.7	23.0	27.8	0.21	0.47

2.7 Summary and conclusions

The rumen is a complex ecosystem in which micro-organisms live, reproduce and metabolise the materials ingested by the animal. The actions of and the interactions between the rumen microbes take place in a symbiotic relationship with the host ruminant. Feeds ingested by the animal determine the rate of

development and the pattern o ' metabolism of the rumen micro-organisms. The rate at which micro-organisms ferme nt feeds to volatile fatty acids, and grow and leave the rumen in turn af ects strongly the overall nutrition of the animal. Optimising the rumen digestio n in order to maximise nutritional efficiency for the host animal is therefore ess ntial in ruminant feeding.

Many intensive studies have been done to discover the nature of the rumen ecosystem and their both posi ive and negative impacts on ruminant nutrition (Hungate 1966, McDonald *et al* 1970, Van Soest 1983, Cheng *et al* 1984, Preston and Leng 1987, Bauchop 1988, Orpin 1988, Williams and Coleman 1988, Bird 1988, Mackie 1987, Nolan 1988). There has been a high recognition of the role of rumen bacteria fungi and protozoa in the digestive processes occurring in the rumen. Conversion of complex plant materials into simple, absorbable nutrients for the host is the most important role of rumen bacteria and other micro-organisms. In addition, rumen microbes are able to convert non-protein-nitrogen into their own protein which contributes the most important source of amino acids absorbed in the gut, in particular in ruminants given poor quality diets (Preston and Leng 1987, Beever 1993, Nolan 1993). Therefore, the rate of microbi l cell production affects both the rate of rumen digestion and the quantity and quality of proteins reaching the sites of absorption in the gut.

On the other hand, rumen microbes appear to have some negative effects on feed utilisation efficiency. Firstly, most reviewers have agreed that rumen fermentation of dietary protei ns and other nutrients that are digestible in the small intestine, is wasteful in erms of protein and energy. As a result, feeding ruminant with bypass nutrien s, especially proteins, has been a controversial topic (Kempton 1979, Hennessy 1984, Fattet *et al* 1984, Leng 1987, Smith and Kenney 1987, Preston and Leng 1987, Doyle *et al* 1988, Dhuyvetter 1993).

Secondly, protozoa often play a negative role in ruminant nutrition by competing with bacteria for their food or by engulfing bacteria leading a decreased number of the rumen bacteria (Hungate 1966, Van Soest 1983, Preston and Leng 1987, Bird 988, Coleman 1985, 1988, Leng 1988, Nolan 1988).

Despite these negative aspects of rumen microbial digestion, the benefits of the rumen ecosystem are overwhelming Because of the activities of rumen microbes ruminants can survive and produce on high fibre feedstuffs that are highly available throughout the world. Cereal straws are an important potential feed for ruminants because they are an abundant and under-utilised by-product of grain-food production in most parts of the world. Unfortunately, cereal straws are too poor when fed alone to meet the nutritional requirement of ruminants for their production, or even for their maintenance (Bo Gohl, 1973, 1981, Sundstol 1988). Thus, there has been a need for finding techniques to improve the feeding value of cereal straws.

Many techniques have been investigated, developed and tested. Unfortunately, various constraints appeared to prevent their application in many circumstances, in particular in developing countries (see Doyle *et al* 1986). Furthermore, techniques must be economically acceptable. Thus, development of ways of making better use of cereal straws remains an interesting domain for research.

Treatment and supplementation appear at present to be the most feasible methods of making efficient use of cereal straws as feed for ruminants. Many studies dealing with treatment of straws have been reported. Physical treatment has proved to be unacceptable in most developing countries because of the high requirement for facilities and high cost involved (Doyle *et al* 1986). Moreover, physical treatment basically does not improve feeding value of the feed (Arthur

1971, McManus 1972), and at times even decreases feed intake and digestibility (Ibrahim and Pearce 1982, Devendra 1983). In contrast, various chemical treatments appear to increase feed intake, digestibility and performance of ruminants (Chandra and Jackson 1971, Shin *et al* 1981, Dumlao and Perez 1976, Yu *et al* 1975, Goering 1973, Sundstol 1978, Doyle *et al* 1986). However, in practical terms, ammonia and urea appear to be the most suitable chemicals for treating straws to improve their nutritional value. Reasons for this are that it is: relatively inexpensive, simple techniques are required, these chemicals are less harmful to people and animals and good results can be expected (Sundstol 1978, Doyle *et al* 1986, Leng 1991, Preston and Leng 1987, Dolberg and Finlayson 1993).

Supplementation of straws with non-protein-nitrogen and protein concentrates, on the other hand, is generally effective. Urea can be added into diets to ensure an optimum level of rumen ammonia in order to maximise rumen microbial growth (Kempton 1982, Williams 1982, Smith 1984, Perdok and Leng 1988, Preston and Leng 1987).

The next step of supplementation is using bypass protein. A large number of papers indicate that bypass protein supplementation could markedly increase intake, digestibility and performance of ruminants fed on fibrous or straw-based diets (Hennessy 1986, Smith and Kenney 1987, Leng 1987, Stephenson and Bird 1987, Preston and Leng 1987, Fattet *et al* 1984, Bunting *et al* 1992, Sibanda 1993). However, different sources of protein supplements gave different effects on ruminant nutrition depending on factors such as the nature of the protein, the amounts used in feeding, etc (Leng 1987, Moore *et al* 1988, Preston and Leng 1987). Furthermore, the consequences of protein supplementation on feed intake may be either additive or substitutive, depending

on the nature of the basal diets and their level of availability to the animal (Kempton 1982, Scott and Hibberd 1990, Beck *et al* 1992).

Overall, when considering how to make better use of cereal straws as ruminant feeds, the conclusion from research and practical application is that ammoniation of straw is one of the most practical and least expensive ways of meeting the requirements of animals for maintenance or a low level of production. Bypass protein can be used if the objective is to reach higher levels of production in animals of higher genetic merit, or to maximise reproductive efficiency. However, whether or not to use protein concentrates must be decided on the basis of cost and sustainability.

The work done and presented in this thesis was designed to:

1) Evaluate different protein-rich supplements when used as bypass protein sources for Australian sheep with high genetic potential for wool growth when given a diet of oaten chaff with urea and minerals (designed to optimising rumen microbial growth). The protein sources tested were: cotton seed meal, sunflower meal, copra and palm kernel cake.

2) Evaluate different feeding strategies suitable for use with cattle fed on rice straw in Viet Nam. The strategies compared were: supplementation with whole cotton seed or copra , and treatment with urea.