THE NUTRITIVE VALUE OF RICE STRAW VARIETIES FOR RUMINANTS

by

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<th>Definition</th>
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<tbody>
<tr>
<td>ADF</td>
<td>= acid detergent fibre</td>
</tr>
<tr>
<td>CP</td>
<td>= crude protein</td>
</tr>
<tr>
<td>DE</td>
<td>= digestible energy</td>
</tr>
<tr>
<td>DM</td>
<td>= dry matter</td>
</tr>
<tr>
<td>DMI</td>
<td>= dry matter intake</td>
</tr>
<tr>
<td>DML</td>
<td>= dry matter loss</td>
</tr>
<tr>
<td>GE</td>
<td>= gross energy</td>
</tr>
<tr>
<td>HC</td>
<td>= hemicellulose</td>
</tr>
<tr>
<td>IVOMD</td>
<td>= <em>in vitro</em> organic matter digestibility</td>
</tr>
<tr>
<td>ME</td>
<td>= metabolisable energy</td>
</tr>
<tr>
<td>MJ</td>
<td>= mega joule</td>
</tr>
<tr>
<td>N</td>
<td>= nitrogen</td>
</tr>
<tr>
<td>NDF</td>
<td>= neutral detergent fibre</td>
</tr>
<tr>
<td>OM</td>
<td>= organic matter</td>
</tr>
<tr>
<td>OMD</td>
<td>= organic matter digestibility</td>
</tr>
<tr>
<td>PD</td>
<td>= potential degradability</td>
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Abstract

Rice straw is abundantly available in Indonesia and is an important source of ruminant feed, especially in the dry season. However, rice straw has poor nutritive value as a ruminant-feed, a limitation which must be overcome. Many methods have been attempted to improve the nutritive value of rice straw, including treatment (chemical and physical), supplementation and selection of varieties of straw with high nutritive value. The objectives of this study were: (i) to evaluate the difference in the nutritive value of rice straw varieties using chemical composition and digestibility measurements, (ii) to assess a range of methods for improving the nutritive value of rice straw and (iii) to study the effect of urea treatment and rice straw quality on the colonisation of ruminal fungi and the characteristics of stem tissue structure.

The first experiment (Chapter 3) was conducted to evaluate the nutritive value of various rice straws and the effect of urea treatment, using measurements of chemical composition (N, NDF, ADF, HC, lignin and silica) and IVOMD (in vitro organic matter digestibility). Yanco tailings and straws from eight varieties of rice were used. Chopped straw from each part of each variety was treated with urea, and untreated and urea treated straws were analysed. The experiment used an 8 x 2 x 2 factorial design.

The chemical composition and IVOMD varied within and between varieties. In all varieties the N content was higher in the upper part than the lower part. The IVOMD of the lower part was higher than that of the upper part except for llb, Pld and Yrl varieties. Urea treatment consistently increased the N content and IVOMD of both parts in all varieties. There was no consistent effect of urea treatment on the other chemical components.

Linear regression used to assess the relationship between IVOMD and chemical composition showed very poor relationships. This indicated that no chemical component could be used as a single parameter to predict rice straw digestibility.
Multiple regression showed that 60% of the variation in IVOMD was accounted for by N, HC, lignin and silica.

A second experiment (Chapter 4) was conducted to evaluate the digestibility of rice straws and the effect of urea treatment using a rumen degradability (in sacco) study. Four varieties from Experiment 1 were chosen to represent medium (IVOMD; >40%, <50%) and low (IVOMD < 40%) quality. Ground samples were put in nylon bags before suspension in the rumen for various incubation times. The results showed that the dry matter loss of the rice straws was higher for the lower parts than for the upper parts for all varieties except IIb. Urea treatment increased the rumen degradability of rice straw as indicated by the higher rate of the degradation constant and the potential degradability in urea treated straw than untreated straw.

A third experiment (Chapter 5) was conducted using straw from the variety Pld to formulate isoenergetic (7MJ/Kg DM of ME) and isonitrogenous ruminant rations. All diets also contained lucerne hay. The diets could best be described as being based on the following components. (rice straw, RS; oaten hay, OH; lucerne hay, LH; Urea treated rice straw, URS; rice straw with added urea, RS+U)

Diet 1 49% RS, 32% LH
Diet 2 55% URS, 25% OH, 11% LH
Diet 3 49% RS+U, 20% OH, 18% LH

Nitrogen retention and the apparent digestibility of DM, OM and ADF were not significantly different between the three diets. Diet 2 had higher DMI/metabolic weight (DMI/W0.75) and DE intake than Diet 3. The DMI/W0.75, DE intake and apparent digestibility of NDF were not significantly different between Diets 1 and 2, suggesting that legume forage supplementation offers an alternative means by which to increase the feeding value of rice straw. Based on these results, it appears that the best way to prepare rations is to use urea ensiled rice straw and untreated rice straw supplemented with legume forage.
A fourth experiment (Chapter 6) was conducted to investigate variation in the digestibility of rice straw between varieties and the effect of urea treatment. This experiment involved measurement of ruminal fungi colonisation and observation of plant structure and the susceptibility of the latter to rumen microbial attack. Stem internodes from three rice varieties representing low and medium quality were used in this study. Ten fragments of rice straw stem were put in nylon bags and incubated in the rumen for 24, 48 or 72 h. Observation under scanning electron microscopy (SEM) revealed that sporangia of ruminal fungi had colonised the Dong and Yrl varieties after both 48 and 72 hours of incubation. The substrate of Ilb was colonised after 24 and 72 hours incubation. All populations tended to be lower after 72 hours incubation than the shorter incubations. Urea treatment decreased the time required for ruminal fungi colonisation. All urea treated samples were colonised after 24 hours of incubation. The structure of unincubated stem tissues was similar for all three varieties. Parenchyma tissue was most susceptible to rumen microbial attack. Urea treatment increased the extent of degradation of inner vascular bundles after 24 hours of incubation.

This study concluded that IVOMD of rice straw differed between the varieties studied but this variation could not be explained by chemical composition, ruminal fungi colonisation or stem tissue structure. Urea treatment increased the digestibility of all rice straw varieties studied, and thus the feeding value of rice straw may be improved by ensiling it with urea prior to feeding or by supplementing it with forage legumes.
DECLARATION

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

All experiments involving animals were approved by the University of Adelaide Animal Ethics Committee with approval number W/51/94.

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

[Signature]

Dwi Yulistiani
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CHAPTER 1

INTRODUCTION

Fibrous agricultural residues such as rice straw are abundantly available throughout tropical agricultural systems. In a rice-based farm system, rice straw is a major component of the crop residues that are classified as poor quality (Schiere and Ibrahim, 1989). Rice straw is commonly used as a ruminant feed in tropical countries. This residue is available on most farms in Indonesia and is an important source of fodder, especially in the dry season. It is estimated that there are 9 million ha of rice paddy grown annually in Indonesia, and these can produce around 72.5 million tons of rice straw (Nari, 1986).

Doyle et al., (1986) compiled data on the chemical composition and in vitro organic matter digestibility (IVOMD) of rice straw from many countries and reported that these parameters varied widely. In addition, a number of researchers (Cheva-Isarakul and Cheva-Isarakul, 1985; Sannasgala et al., 1985; Roxas et al., 1985) found substantial variation in the chemical composition, IVOMD and organic matter degradability of rice straw varieties. This variation may be due to genetic and environmental factors, and differences in the proportion of plant botanical fractions, such as leaf to stem ratio, in the straw (Pearce, 1985; Walli et al., 1988). From the literature, it can be concluded that the chemical composition and nutritive value of rice straw varieties are inconsistent.

Rice straws are characterised by low protein, mineral and energy levels and consequently, have a poor nutritive value for ruminants. According to Jackson (1977b), the protein content of rice straw is only 3-5%. In addition, rice straw has low phosphorus and available calcium contents. Low actual digestibility of rice straw (35 - 55%) (Doyle et al., 1986) results in a low voluntary feed intake (less than 2% of body weight) and hence low energy intake (Jackson, 1977b).
The nutritive value of rice straw can be improved by pre treatment, supplementation with high quality feed (Doyle et al., 1986; Castrillo et al., 1991; Smith, 1993) and genetic selection (Doyle et al., 1986; Khush et al., 1988).

Digestion of rice straw by rumen microorganisms can be detected visually using electron microscopy (Akin, 1979). Microscopic techniques give information on factors that affect forage degradation as well as the rate and extent of degradation (Akin, 1979) and the variation in digestibility of forage (McManus, 1981). This information can not be fully explained from the chemical analysis (McManus, 1981).

The main objectives of the current study were to:

(1) evaluate the differences in the nutritive value of rice straw varieties using chemical composition and digestibility (in vitro, in sacco, and in vivo) measurements;

(2) assess a range of methods for improving the nutritive value of rice straw as feed for ruminants, and;

(3) study the effect of urea treatment and rice straw quality on ruminal fungi colonisation and stem tissue structure before and after degradation in the rumen.
CHAPTER 2
REVIEW OF LITERATURE

2.1 Characteristics of Rice Straw

Rice straw is one of the by-products of rice cultivation. Others include rice bran, rice husks, and broken rice. Rice straw is different from other cereal straws because its characteristics are affected by the cultural practices of rice growing (sloughy soil and multiple cropping). Rice straw also has a high silica content (12-16%, Barber et al., 1981) and low lignin content (6-7%, Jackson 1977a) compared with other cereal straws. Silica limits the digestibility, and hence the utilisation, of rice straw when fed to ruminants (Van Soest, 1982). The relationship between silica and digestibility is also affected by a compensatory association with lignin. The sum of silica and lignin is more closely related to digestibility than either one is individually (Van Soest, 1982). Silica is absorbed in large quantities and this element is present in all parts of the paddy plant (Grist, 1986) because silica is crucial to maintain the erectness of the leaves. This function is more important when the plant is fertilised with high quantities of nitrogen (Grist, 1986). Because of the higher silica content in the leaf blades and sheath, rice straw internodes have a higher digestibility than the leaves (Walli et al., 1988). In other cereal straws (wheat, barley, etc.) internodes are very poorly digested (Ørskov, 1988) due to a high lignin content (Jackson 1977a). A comparison of the fibre composition and silica content of rice straw with other cereal straws is shown in Table 2.1.

The thickness of rice straw is affected by tillering and sowing (Grist, 1986). Thick sowing and high tillering will tend to give thinner straw per unit area (Staniforth, 1979). The structure of the internodes of rice straw is similar to other cereal straws in appearance, however, the internodes of irrigated rice varieties have air cavities and its vascular bundles are located between the air cavities (Staniforth, 1979). When rice is grown under irrigation, the plant may not mature and senesce evenly. Occasionally, new vegetative tillers may appear late in the development of the plant,
and, therefore, the plant may be green when the grain is mature. As a result, the bottom part of the plant is more digestible than the top (Doyle et al., 1986). *In vitro* organic matter digestibility of stubble increases when the stubble is left ungrazed (Hart and Wanapat, 1985), probably due to ratooning (regrowth of the paddy crop from stubble) and continuing growth of unproductive tillers (Khush et al., 1988).

Table 2.1 Fibre composition and silica content of barley, oat, wheat and rice straw (% dry matter).

<table>
<thead>
<tr>
<th>Straw</th>
<th>CC</th>
<th>Cell wall</th>
<th>HC</th>
<th>Cellulose</th>
<th>Lignin</th>
<th>Silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>19</td>
<td>81</td>
<td>27</td>
<td>44</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Oats</td>
<td>27</td>
<td>73</td>
<td>16</td>
<td>41</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Wheat</td>
<td>20</td>
<td>80</td>
<td>36</td>
<td>39</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Rice</td>
<td>21</td>
<td>79</td>
<td>26</td>
<td>33</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

CC, cell content; HC, hemicellulose

(After Jackson, 1977a)

2.2 Defining the Nutritive Value of Roughage

Nutritive value describes the proportion of nutrients in a feedstuff available for metabolic use by animals. The nutritive value of a feed is determined by the concentration of nutrients in the feed, the amount eaten, the proportion of the nutrients digested and the efficiency with which the absorbed nutrients are used (Pearce et al., 1988). For cereal straws, the chemical composition and digestibility are commonly used to assess nutritive value (Pearce et al., 1988). Akin (1979) suggested that microscopic evaluation can aid the assessment of nutritive value by providing an insight into the potential degradation by rumen microorganisms.

2.2.1 Chemical Composition

Chemical analysis is the starting point for the definition of nutritive value (Schneider and Flatt, 1975). The primary purpose of laboratory analysis is to characterise forages and feedstuffs so that nutritive value and performance of
livestock can be related to chemical composition (Van Soest, 1988). Chemical analysis methods may be used to determine the content of the specific nutrients contained in the feedstuffs (Church, 1991) e.g. acid detergent fibre determination by the procedure of Goering and Van Soest (1970). The main components of foods are water and dry matter. Dry matter (DM) consists of in-organic and organic matter. Organic matter (OM) contains protein, carbohydrate, lipids, nucleic acids, organic acids and vitamins (McDonald et al., 1991).

**Water and ash:** In a proximate analysis, water content (moisture) is determined by the weight loss after the samples are dried at 100°C to constant weight. This method is satisfactory for all foods, but not for silage, due to the loss of some volatile material during drying. The ash content is determined by the ignition of samples at 500°C until all carbon has been removed. The residue is the ash and represents the inorganic constituents. Organic matter constitutes the weight loss by ignition (McDonald et al., 1991).

**Protein:** Proteins are the major nitrogen-containing compounds in the plant. Crude protein content is usually calculated from the nitrogen content of the food, which is determined by a modification of the Kjeldahl technique. With this method, food is digested with sulphuric acid, which converts all nitrogen into ammonia, except the nitrogen in the form of nitrate and nitrite. This ammonia is liberated by adding sodium hydroxide to the digest, distilling off and collecting in standard acid. The quantity of ammonia collected is determined by titration. It is assumed that the nitrogen is derived from protein containing 16% nitrogen, therefore, the approximate protein content is calculated by multiplying the nitrogen content by 6.25 (McDonald et al., 1991). Because nitrogen is not only derived from protein but also from other origins, the protein content determined in this way is expressed as crude protein (McDonald et al., 1991). The actual protein value depends on the amount/proportion of amino acids absorbed in the small intestine (Vérité, 1980). Crude protein analysis of agricultural by-products is important, however as this
analysis can measure increases in crude protein content resulting from chemical or physical treatments (Donefer, 1982).

Fibre: The carbohydrates in the food are contained in two fractions, crude fibre and the nitrogen free extract. The crude fibre is determined from the organic residue of the ether extract. However, crude fibre results are imprecise and far from adequate as a measure of nutritive value (McDonald et al., 1991; Van Soest, 1982). Therefore, an alternative procedure using a detergent analysis system was proposed by Van Soest (McDonald et al., 1991). The detergent analysis system is designed to replace the proximate analysis method for crude fibre (the Weende system; Reed and Van Soest, 1985), and was developed to solve analytical problems with forages and residues from lignocellulosic materials, particularly for ruminant diets (Van Soest and Robertson, 1980). Detergent analysis separates cell contents, which have a true digestibility greater than 90%, and cell wall components (Reed and Van Soest, 1985). This separation is carried out using neutral detergent fibre analysis (Van Soest and Robertson, 1980).

Neutral detergent fibre (NDF) is the residue of the sample after extraction with boiling neutral detergent solutions of sodium lauryl sulphate and ethylene diamine tetra acetic acid (EDTA). This residue mainly consists of lignin, cellulose and hemicellulose, and can be regarded as plant cell wall material (Van Soest and Robertson, 1980). The cell wall components are less readily available to animals (Morrison, 1976) and are only partly digested by rumen microorganisms (Van Soest, 1982).

Acid detergent fibre (ADF) is the residue after refluxing the sample with 0.5 M sulphuric acid and cetyltrimethyl ammonium bromide (CTAB). It represents the crude lignin and cellulose fractions, and also silica. This residue can be used for sequential estimation of lignin, cutin, cellulose, indigestible nitrogen and silica (Van Soest and Robertson, 1980). The ADF and lignin content can be considered as an
estimate of digestibility, while NDF is used as an estimator of the potential intake of the forage either within or between species of forage materials (Marten, 1981).

**Gross Energy:** The quantity of energy present in a food is measured by converting chemical energy in the food into heat energy. This conversion is carried out by oxidising the food by burning it. The quantity of the heat resulting from the complete oxidation of a unit weight of food is known as the gross energy (GE). The GE value of a food is an inaccurate estimate of the energy actually available to the animal because gross energy does not take into account the energy losses during digestion and metabolism. The first loss of energy is the faeces. Thus, the apparent digestible energy (DE) is measured from the subtraction of gross energy in the faeces from gross energy in the particular food (McDonald *et al.*, 1991). The metabolic energy (ME) of a food is the digestible energy less the energy lost in urine and combustible gases. The combustible gases lost from the rumen consist almost entirely of methane. Methane production is measured using a respiration chamber. When no respiration chamber is available, methane production can be estimated as 8% of gross energy intake (Van Es, 1980). MAFF (1984) suggested that ME can be estimated from GE and IVOMD using $\text{ME} = 0.81 \times (\text{GE} \times \text{OMD})$, where OMD is *in vitro* organic matter digestibility. Net energy $= \text{ME} - \text{H}$.

### 2.2.2 Digestibility

Chemical analysis determines the potential value of a food for supplying a particular nutrient. Yet it can not give clear differences between the nutritive value of straws for ruminants. The next step in determining the nutritive value of feedstuffs after chemical analysis is digestibility analysis (Schneider and Flatt, 1975).

#### 2.2.2.1 Techniques for measuring digestibility

*In vivo* digestibility experiments involve the food being investigated being given to target animals in known amounts, and the output of faeces being measured (McDonald *et al.*, 1991). *In vivo* digestion of a nutrient is the percentage consumed
in the ration which does not appear in the faeces, or the percentage of the nutrients that have disappeared during the passage of the feed through the digestive tract (Schneider and Flatt, 1975). Digestibility trials provide a more accurate estimate of the nutritive value of a food by accounting for losses that occur during digestion, and absorption (McDonald et al., 1991; Pearce et al., 1988; Schneider and Flatt, 1975). The feeding trial study is used to determine the digestibility of feed. These data are needed to formulate feed ration of farm animals (Schneider and Flatt, 1975). The disadvantage of in vivo methods in ruminants is that methane arising from the fermentation of carbohydrates is lost by eructation and not absorbed. This loss leads to overestimation of the digestible carbohydrate and digestible energy content of ruminant feed. In addition, the feed residue in the faecal materials is not only from feed but also from endogenous material (e.g. rumen bacteria, hair, abraded epithelial cells) (Van Es, 1980; McDonald et al., 1991). Therefore the values obtained in digestibility trials are termed apparent digestibility coefficients (McDonald et al., 1991).

Digestibility experiments are laborious to perform and expensive (Minson, 1981). Numerous attempts have been made to determine the digestibility of foods using in vitro or in sacco methods which primarily study the microbial phase of ruminant digestion in the rumen (Schneider and Flatt, 1975).

An in vitro method using rumen liquor and pepsin in a two-stage digestion was devised by Tilley and Terry (1963). This involves incubation of ground samples in buffered rumen liquor under anaerobic conditions in the first stage, in order to make conditions as similar as possible to those in the rumen. The samples are then acidified with hydrochloric acid (HCl) to pH 2 to kill bacteria followed by incubation with pepsin. There are many disadvantages to this method, including the time required to produce the results, which Morrison (1976) puts at no less than six working days. In addition fresh rumen liquor must be available, which can be a problem if there is no access to experimental animals (Morrison, 1976; Nefzaoui
fresh rumen liquor must be available, which can be a problem. (Morrison, 1976; Nefzaoui and Vanbelle, 1985). The most difficult factor to control in this method is the variation in the activity of the rumen fluid, even when animals are fed on the same forage before collection (Minson, 1981; Nefzaoui and Vanbelle, 1985). Differences in rumen fluid content lead to variation within and between replicates (Nefzaoui and Vanbelle, 1985). However, compared to chemical analysis methods such as detergent fibre (Morrison, 1976), this method may be a helpful method for plant breeders for screening purposes (Schneider and Flatt, 1975; Marten, 1981; Nefzaoui and Vanbelle, 1985) and can be used to predict the forage qualities of vast numbers of, or greatly varying, forage samples grown in limited quantity (Marten and Barnes, 1980).

An alternative method to two-stage in vitro rumen liquid digestibility, is in vitro enzyme solubility (Nefzaoui and Vanbelle, 1985). This method is based on cellulolytic activity, using a cellulase enzyme which is extracted from the fungi Trichoderma reesei (Nefzaoui and Vanbelle, 1985). The first stage of in vitro enzyme solubility is to incubate ground samples with acid pepsin to remove the cell contents. This is followed by incubation with cellulase. This method has disadvantages, such as a slightly lower precision than the two-stage in vitro rumen fluid (due to the commercial brands of enzyme not being pure and varying considerably in their digestion activity) (Nefzaoui and Vanbelle, 1985). Nevertheless, it has several advantages, including good reproducibility, repeatability. Furthermore it does not require the use of animals (Nefzaoui and Vanbelle, 1985; Minson, 1981). In vitro techniques do not give good estimates of digestibility of high starch feeds, such as cereal grains without the inclusion of an amylase digestion phase in the technique. In sacco methods involve the placement of samples in bags made from indigestible fabrics (such as nylon, dacron or silk), directly suspended in the rumen for various incubation periods. In sacco methods are used to rapidly assess the digestibility in the rumen of small samples of feeds (Marten, 1981). This method provides a similar environment to that experienced during in vivo rumen fermentation, and gives information on the possible extent of degradation of feed
material occurring in the rumen (Ørskov, 1985). The rate and extent of digestion can be measured by loss of dry matter or specific nutrients after incubation in the rumen (Marten, 1981). The rate of degradation of food in the rumen is a useful measurement for prediction of voluntary feed intake (Forbes, 1995; Ørskov, 1985). Despite this, the nylon bag (rumen degradability) method has disadvantages, including difficulties in standardisation and considerable variability between animals used (Marten and Barnes, 1980).

**Microscopic evaluation of forage digestion in the rumen**: Electron microscopy can be used to show the rate of forage degradation by rumen microorganisms (Akin, 1979). There are three types of microscopy, each of which is complementary (Akin, 1979). Assessment using light microscopy (LM) and scanning electron microscopy (SEM) provides information on the forage micro anatomy and the extent and rate of degradation of various forages by rumen microorganisms in the rumen. Transmission electron microscopy (TEM) gives information on the mode of action of microorganisms that associate with forage degradation (Akin, 1979).

The reliability of scanning electron microscopy studies in assessing degradation of forages by rumen micro-organisms has been reported by Akin et al. (1986). Chemical analysis of components such as lignin fails to reflect the variation in digestibility of the forage and the effects of alkali treatment (McManus, 1981). McManus et al. (1977) and McManus et al. (1979) used SEM to study the correlation between chemical and physical properties of forage materials, and reported that inorganic structures closely resemble the original plant fraction structure. Akin et al. (1984) reported that during digestion of feed, there are physical interrelationships between micro-organisms and the feed. Akin and Amos (1975) used young fescue leaf and reported that diverse bacterial types degraded mesophyll and, in some cases, phloem tissues without prior attachment. However, degradation of bundle sheath and epidermal cell walls appeared to be preceded by attachment of large cocci, in which the physical association varies with tissue types. Silva and
Ørskov (1988) used SEM to examine the degradation of ammonia-treated and untreated barley straw and reported that bacterial colonisation of untreated barley straw differed quantitatively and qualitatively among animals given different diets. On the other hand, Horn et al. (1989) reported that fungi colonisation was not affected by the type of animal diet, but by the type of straw incubated in the nylon bag.

Microscopic techniques are very useful in forage breeding programs because they give information on factors that affect forage degradation, as well as the rate and extent of degradation. According to (Akin 1979) the limitations of this method are as follows:

(i) tissues such as mesophyll are often collapsed in the high vacuum of the electron microscope, although plant cell walls are rigid;
(ii) the results can not be statistically compared because of the difficulty in obtaining numerical data;
(iii) viewing zones must be representative, and;
(iv) electron microscopy equipment is expensive.

2.2.2.2 Plant factors that affect digestion

Chemical factors

The low digestibility of straw is largely due to the lignification of cell walls. Lignification starts after the growth of the plant ceases (Crampton and Harris, 1969). Lignin becomes part of the cell wall during formation and thickening of secondary walls (Jung, 1989 and Harris, 1990). Increasing maturity of a pasture, or of individual leaves and stems, usually leads to higher cell wall content and lower dry matter digestibility because of an increasing stem proportion, cell wall thickening or a lower content of protein and other cell solubles (Wilson 1994).

Plant cell walls principally consists of pectin, hemicellulose, cellulose and lignin (Hatfield 1990). Lignin is a polymeric of phenylpropanoid units (Amstrong and
Gilbert, 1991) which is most commonly associated with the reduced digestibility of lignocellulosic feed material (Van Soest, 1982). The association of lignin with cellulose and hemicellulose inhibits the availability of carbohydrates (Amstrong and Gilbert, 1991; Chesson, 1988; Neilson and Richards, 1978). Because secondary thickened lignin protects cell walls, as a whole, from microorganism attack, cell walls that are extensively lignified are poorly degraded (Chesson, 1988; Crampton and Harris, 1969). As a consequence the digestibility of plant cell walls depends upon the lignin content. However, total lignin content has been an unreliable predictor of dry matter digestibility, especially for comparison across species (Wilson, 1994). This is due to the fact that lignin is not a homogenous polymer, but varies in its predominant monomers and in chemical linkages (Akin, 1988). Phenolic components in the lignin protect cell walls from bacterial attack (Crampton and Harris, 1969; McAllister et al., 1994) because phenolic compounds interfere with the attachment of cellulolytic bacteria to cellulose (McAllister et al., 1994). p-coumaric acid, which is a phenolic monomer of precursor lignin, is detrimental to digestibility and also toxic to rumen micro-organisms at low concentrations (Akin, 1988; Jung, 1989; Varet and Jung 1986). Therefore, the higher p-coumaric acid content, the lower the digestibility of the cell wall (Jung, 1989).

Besides phenolic components, the other cell wall component that limits fibre digestibility is silica (Borneman and Akin, 1990). Higher levels of silica are found in rice straw compared to other cereal straws. Silica, together with lignin, strengthens and rigidifies plant cell walls making them poorly assessable to rumen micro-organisms (Van Soest, 1982).

**Plant tissue structure**

Cereal straw contains a high proportion of cell types with lignified secondary walls (Harris 1990). Secondary thickened lignin is the primary factor determining the susceptibility of structural polysaccharides to microbial attack, and protects the cell wall as a whole (Chesson, 1988). Observations using scanning electron microscopy
reveal that each cell type has a different extent of lignification, and therefore, has different susceptibility to rumen microbial attack (Wilson, 1994). The extent of the lignification of the cell walls of plant tissue can also be detected using histochemical reactions. Acid phloroglucinol reacts with the tissue most resistant to biodegradation, while chlorine sulphite stains the lignocellulosic fibre that is less resistant to enzymatic degradation (e.g. leaf sclerenchyma and stem parenchyma) (Akin, 1986). Tissues that have positive reactions to acid phloroglucinol have the highest concentration of phenolics (Akin et al., 1990a) and include the xylem, the mestome sheath and the sclerenchyma in leaf blades and the epidermal sclerenchyma ring, the vascular tissue and the mature parenchyma in stems (Borneman and Akin 1990) Anatomical studies defining lignified cells within any plant part are important in relation to digestibility (Wilson, 1990).

2.2.2.3 Rumen microbial factors that affect fibre digestibility

Digestion in the rumen involves fermentation performed by rumen microorganisms, which consist of bacteria, fungi and protozoa (Ørskov, 1992). The presence of cellulolytic bacteria in the rumen gives ruminants the ability to survive on poor-quality fibrous forage (Ørskov, 1992). Cellulolytic bacteria *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Bacteroides succinogenes* are the bacteria that commonly attach to the cell wall (Chesson and Forsberg, 1988). These bacteria hydrolise cellulose and hemicellulose (Church, 1983), and are found on cut the edges of cell walls and damaged areas (Chesson and Forsberg, 1988) However, the most active bacterium in degrading highly ordered cellulose, such as cotton, is *Bacteroides succinogenes* (Chesson and Forsberg, 1988; Steward and Bryant, 1988).

The other rumen microorganism that plays an important role in fibre digestion in the rumen are an-aerobic ruminal fungi which have been discovered in the rumen by Orphin (1975). These fungi play an important role in the digestion of poor quality forages such as straw (Gordon and Phillips, 1995; Fonty and Joblin, 1991; Horn,
The population of rumen fungi is more abundant when the diet is rich in fibrous material (Fonty and Joblin, 1991; Grenet et al., 1989). Ruminal fungi have a higher capability to degrade plant cell walls than fibre-degrading bacteria (Borneman and Akin, 1990), because fungi selectively colonise the plant tissues with thick or lignified cell walls (Grenet et al., 1989; Grenet and Barry, 1988). Electron microscopy observations revealed that rumen fungi have the ability to degrade the mestome sheath and xylem tissues of leaf blades (Borneman and Akin, 1990). In the process of degradation of schlerenchyma walls, rumen fungi predominantly colonise the schlerenchyma and vascular tissues of leaf blades by invasion of the fungal rhizoid or rhizomycelia (Fonty and Joblin, 1991). Certain strains of ruminal fungi are higher degraders of plant fibre than the other strains (Gordon and Phillips, 1995).

Considering the important contribution of cellulolytic bacteria and ruminal fungi to the digestion of low quality forages by ruminant, utilisation of low quality forages could be improved through manipulation of the rumen microbial population (Leng and Devendra, 1995; Gordon and Phillips, 1995). One alternatives to increase rumen microbes is the control of protozoa in the rumen, using feed additives. This could enhance microbial growth and the efficiency of the flow of energy and protein from the rumen (Leng and Devendra, 1995). The population of protozoa needs to be controlled because protozoa ingest and digest bacteria as their main protein source (Ushida et al., 1991). This proteolytic activity reduces microbial yield in the rumen. Thus, protozoa directly influence the proportion of protein and energy in the absorbed nutrients (Williams and Coleman, 1988). In addition, control of protozoa could increase the population of ruminal fungi, however, the mechanism of this effect is still unknown (Fonty and Joblin, 1991). The inoculation of superior strains of anaerobic fungi into the rumen is another alternative to manipulating the population of microorganisms in the rumen. (Gordon and Phillips, 1995).
2.3 Variation in the nutritive value of rice straw

2.3.1 Chemical composition

Data from many countries compiled by Doyle et al., (1986) showed that the chemical composition and IVOMD of rice straw varies widely (Table 2). The cell wall constituents, which are expressed as NDF, are high (up to 86% of the dry matter). This indicates that cell contents are often present in small amounts (Doyle et al., 1986). Similar results have been reported by Cheva-Isarakul and Cheva-Isarakul (1985) based on an experiment using seven varieties of rice straws in Northern Thailand. Protein content of the straws ranged from 2.2 - 5.8%, ADF ranged from 68-79%, and NDF ranged from 49-57%. The crude protein content of rice straw usually ranges from 2.2 to 9.5% (Doyle et al., 1986; Jackson, 1977b).

The phosphorus content of rice straw is only 0.02 to 0.16 %, which is less than the level of 0.4 % needed for growth and normal fertility in ruminants when it is fed as the only source of nutrients (Jackson, 1977b). Although rice straws contain calcium levels from 0.25-0.55%, the presence of oxalate renders much of this calcium unavailable to ruminants. Calcium supplementation is required when feeding rice straw as a basal diet (Jackson, 1977b). According to Roxas et al. (1985), the chemical composition of straw is thought to be affected by genetic factors (variety) and environmental factors (soil fertility, fertiliser application, botanical fraction, season, etc.). Differences in genetic and environmental factors may be responsible for the large range in rice straw chemical composition.
Table 2.2 Variation in the chemical composition and *in vitro* digestibility of rice straw (% dry matter)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>4.2</td>
<td>2.2 - 9.5</td>
</tr>
<tr>
<td>NDF</td>
<td>75</td>
<td>61 - 86</td>
</tr>
<tr>
<td>ADF</td>
<td>52</td>
<td>41 - 63</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>23</td>
<td>6 - 29</td>
</tr>
<tr>
<td>Cellulose</td>
<td>38</td>
<td>30 - 50</td>
</tr>
<tr>
<td>Lignin</td>
<td>6</td>
<td>4 - 10</td>
</tr>
<tr>
<td>Silica</td>
<td>13</td>
<td>7 - 18</td>
</tr>
<tr>
<td>IVOMD</td>
<td>42</td>
<td>37-55</td>
</tr>
</tbody>
</table>

(After Doyle *et al.*, 1986)

2.3.2 Digestibility

Most of the reported digestibility studies on rice straw varieties have been completed using *in vitro* and *in sacco* methods. Few studies have been completed using *in vivo* digestibility experiments on cereal straws as a single feed (Pearce, 1985). A study on the *in vitro* organic matter digestibility conducted by Cheva-Isarakul and Cheva-Isarakul (1985) showed that the IVOMD of seven varieties of rice straw in Thailand varied from 37-54%. The *in vivo* digestibility varied from 37-55% for buffalo and cattle and from 35-55% for sheep and goats (Doyle *et al.*, 1986). Rumen degradability studies on various rice straw varieties were carried out by Ibrahim *et al.* (1989), who showed that the undegradable organic matter content of rice straw ranged from 27-50%. Due to the low digestibility and subsequent low intake (2% of body weight) (Jackson, 1977b) animals lost weight when fed with rice straws only (Doyle *et al.*, 1986).
2.4 Factors influencing the nutritive value of rice straw

2.4.1 Variety

It is commonly assumed that wide differences occur between different straw varieties (Doyle et al., 1986). The height of the rice plant is influenced by the variety (Grist, 1986) and the height affects the proportion of the botanical fractions (leaf and stem). The variation in nutritive value between varieties is mainly due to differences in the leaf to stem ratio (Ørskov, 1988). Despite this, no differences or small differences have been found between straw varieties grown within a particular region (Pearce, 1986). For example, Sannasgala et al. (1985) in Sri Lanka, and Cheva-Isarakul and Cheva-Isarakul (1985) in Thailand, did not find significant differences in the chemical composition and in vitro digestibilities between varieties. In addition, Walli et al. (1988) conducted a degradability study of two varieties of rice straw and reported no differences in the organic matter degradability between varieties. In contrast, Roxas et al. (1985) found that variety has an effect on the organic matter, crude protein and neutral detergent fibre content of rice straw.

2.4.2 Environment

During the pattern of growth of a plant (germination, growth and reproductive development, maturity, senescence and death) and in combination with other factors such as availability of soil nutrients, water supply, the ambient temperature, intensity of light and incidence of disease, there are some opportunities to change the chemical composition and digestibility of the plant (Pearce, 1986). The main nutritional changes are due to changes in the proportions of the main morphological fractions of the plants (i.e. leaf blades, leaf sheaths, stem internodes) and the chemical composition of each fraction (Pearce, 1985). Sannasgala et al. (1985) and Roxas et al. (1985) pointed out that levels of nitrogen fertilisation may affect crude fibre and silica content. Increased levels of nitrogen fertilisation tended to increase crude protein content of the straws. Furthermore, there is a seasonal effect on OM and NDF content, as well as in vitro digestibility (Roxas et al., 1985). In vitro digestibilities are higher in the wet season, however, OM and NDF content are lower
at this time. By contrast, Subarinoto et al. (1991) found in vivo dry matter and organic matter digestibilities were higher in the dry season.

2.4.3 Botanical Fraction

Cereal straws consist of the true stem, that consists of a variable number of internodes, separated by the nodes. The node is the point at which the leaf arises. The leaf has two parts, a leaf base or sheath which encloses the internodes of the stem for a certain distance, and the expanded leaf blade which is carried at an angle to the stem (Staniforth, 1979). The proportions of these fractions in rice straw will depend mostly on the height of the stubble left in the field (Staniforth, 1979). Differences in botanical composition, particularly leaf:stem ratio of each variety, will affect the nutritive value of rice straw varieties. This is because leaf is less degradable than stem. However, the largest source of variation appears to be differences in nutritive value of leaf and stem from different varieties (Orskov, 1991). Winugroho (1981) found that the proportion of dry matter (DM) in leaf blades ranged from 15 to 27%, in leaf sheaths from 23 to 30%, and, in stem internodes from 16 to 37%. The mean IVOMD of these three fractions was 52, 45 and 54% respectively. Walli et al. (1988) found that the ash and silica content in the leaves was higher than in the internodes. Due to the lower silica content in the stem, rice straw stem is more digestible than rice straw leaf (Jackson, 1977b; Pearce, 1985; Walli et al., 1988). The variability in the nutritive value and proportion of the internodes, leaf sheath and leaf blades of rice straw is presented in Table 2.3.
Table 2.3 Chemical composition and IVOMD (*in vitro* organic matter digestibility) of internodes, leaf sheaths and leaf blades of rice straw (% dry matter).

<table>
<thead>
<tr>
<th></th>
<th>Internodes</th>
<th>Sheaths</th>
<th>Blades</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>CP</td>
<td>2.7</td>
<td>1.7-6.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Total ash</td>
<td>15</td>
<td>11-20</td>
<td>20</td>
</tr>
<tr>
<td>NDF</td>
<td>81</td>
<td>77-85</td>
<td>82</td>
</tr>
<tr>
<td>ADF</td>
<td>60</td>
<td>55-64</td>
<td>57</td>
</tr>
<tr>
<td>HC</td>
<td>21</td>
<td>13-28</td>
<td>25</td>
</tr>
<tr>
<td>Cellulose</td>
<td>47</td>
<td>38-51</td>
<td>39</td>
</tr>
<tr>
<td>Lignin</td>
<td>5</td>
<td>4-6</td>
<td>4</td>
</tr>
<tr>
<td>IVOMD</td>
<td>42</td>
<td>34-54</td>
<td>45</td>
</tr>
</tbody>
</table>

CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; HC, hemicellulose.

(After Doyle et al., 1986)

2.5 Potential Methods to Improve The Nutritive Value of Rice Straw

Even in high quality rice straw, crude protein content is only 6%, and digestible organic matter is only 50%. Hence the nutritive value of this straw is insufficient to support ruminants when fed alone (Schiere and Ibrahim, 1985). Schiere and Ibrahim (1985) proposed many methods to overcome the limitation of this straw, including:

1. pre-treatment;
2. supplementation, by feeding a concentrate or additional grass (to provide additional nutrients and to provide essential elements needed by rumen microorganisms to stimulate rumen function);
3. genetic selection.

2.5.1 Pre-treatment

Many processing methods or pre-treatments to improve the nutritive value of fibrous feed have been investigated. These methods can be classified as physical,
chemical or biological. Usually, pre-treatment improves the nutritive value of straw by increasing its digestible energy content, by increasing feed intake, or by a combination of the two. The maximum effect of pre-treatment in increasing straw digestibility can only be achieved when an adequate level of energy and other essential nutrients, such as nitrogen and minerals, are available. Pre-treatments can range from simple procedures, such as chopping or soaking, to elaborate processes such as controlled fermentation. Economic constraints, however, often rule out the use of pretreatments under practical conditions (Doyle et al., 1986) .

2.5.1.1 Physical treatment

Some physical processing methods such as chopping and grinding do not affect the chemical composition of straw (Minson, 1963). The purpose of chopping is to reduce wastage and to facilitate feeding. Grinding increases voluntary intake and rate of passage, but at the same time it decreases in vivo digestibility (Minson, 1963). Steaming results in the separation of cell wall structures, and has chemical effects through the cleavage of bonds between cell wall constituents. This treatment increases in vitro digestibility of the straw. In vivo studies, however, indicate that steaming does not increase digestibility of the straw because diets containing treated straw have lower digestibilities of organic matter and cellulose (Ibrahim, 1983).

2.5.1.2 Chemical treatment

The objective of chemical treatment of fibrous feed is to increase digestibility and intake by the target animal (Ibrahim, 1983). The effect of the treatment is to disrupt the cell wall structure by breaking or weakening complexes of lignin-carbohydrate bonds, and solubilising lignin and releasing carbohydrate (Smith 1993; Ibrahim, 1983). The increase in digestibility leads to an increase in clearance rate in the rumen, thereby increasing intake (Doyle, 1983; Smith, 1993). Moreover, pre-treatment alters the fractional digestion rate of fibre in the rumen by influencing the growth and activity of the microbial population. There are three groups of chemicals
used for treating fibrous feed: alkalis, acids and oxidative reagents (Doyle et al., 1986).

Alkali treatment is the most commonly investigated treatment to improve the nutritive value of fibrous residues (Smith, 1993). Alkali pre-treatment breaks the ester bond within hemicellulose and lignin (Kristensen et al., 1981; Chesson, 1988). Some of the hemicellulose become water soluble (Klopfenstein, 1978; Jackson, 1977a) and the tight structure is opened for invasion by cellulolytic enzymes (Kristensen et al., 1981). Sodium hydroxide is a most effective treatment (Smith, 1993), however, its practicality is ruled out because of its cost and availability, the need to take care when handling concentrated solutions, and the risk of soil pollution (Doyle et al., 1986; Smith, 1993). Garret et al. (1979) studied the effect of sodium hydroxide-treated rice straw on the performance of lambs, and reported that alkali treatment increased the digestibility of organic matter and cellulose with a consequential increase in live weight gain when the diet was comprised of a large proportion of the treated straw.

Experiments in the use of ammonia for treating straws have been carried out under a range of conditions. Ammonia levels of 3 to 4% of dry matter significantly improved digestibility with little benefit obtained from further increasing the levels (Sundstøl et al., 1978). Appropriate methods of treatment as determined by the form of ammonia and type of material to be treated have been described and discussed by Sundstøl et al. (1978). Besides increasing the digestibility, ammonia treatment has another advantage in that it increases the nitrogen content. Therefore, ammonia treatment has positive effects on intake (Doyle et al., 1986). However, for the smallholder, ammonia treatment may not be feasible because it is difficult to obtain, transport and handle.

Asian scientists have concentrated on the use of urea instead of ammonia to pre-treat rice straw (Doyle et al., 1986). Urea is the best source of ammonia because it is
widely available in rice growing areas and farmers are already familiar with its use. However, urea is very toxic to ruminants when fed even in moderate amounts and has not been gradually introduced over a period of weeks. Urea treatment is a method in which straw is treated by ammonia, which is released from urea. Ammonia, in the presence of water produces the alkali, ammonium hydroxide (Schiere and Nell, 1993; Schiere and Ibrahim, 1989). The effect of urea treatment is to increase the nitrogen content of the straw, and it is the best alternative for farmers when protein supplementation is very expensive (Kiangi et al., 1981). Indonesia, in particular, is one of the tropical countries where urea is affordable for the small farmer, therefore, urea treatment is more promising than other treatments (Schiere and Nell, 1993).

The main factors affecting the process of urea treatment are concentration of urea, duration of treatment, initial quality of the straws (Schiere and Ibrahim 1989), moisture content (Schiere and Ibrahim, 1989; Castrillo et al., 1991) and environmental temperatures (Castrillo et al., 1991). Various concentrations of urea have been used to treat rice straw up to 7.5% (Cloete and Kritzinger, 1984a; Williams et al., 1984a). Schiere and Ibrahim (1989) suggested that treatment with 4% urea is a sufficient level to improve the digestibility of rice straw. However, Wanapat (1987) found urea concentration at 5% was better than 3%. Animals fed on 5% urea-treated rice straw had a significantly higher intake and dry matter digestibility than those fed on 3% urea-treated rice straw.

Duration of treatment is related to environmental temperatures, moisture content and the addition of urease (Castrillo et al., 1991). High temperatures accelerate ureolysis, and optimum digestibility can be obtained when the temperature ranges between 15 and 25°C (Jayasuriya and Pearce 1983; Cloete and Kritzinger 1984a; Williams et al., 1984a). Williams et al. (1984a) found no effect on digestibility when the straw was treated at a temperature 5.5°C. Cloete and Kritzinger (1984a) and Dias da Silva et al. (1988) considered that temperatures of 22-24°C for 4 to 6 weeks were enough to obtain an improvement in the nutritional value of rice straw.
Moisture content of the straw significantly affects the degree of urea hydrolysis and degree of improvement in degradability when the straw is incubated in the rumen for 48 hours (Williams et al., 1984a). The increase in moisture content increases hydrolysis and, as a result, straw degradability is increased. Williams et al. (1984a) investigated the effect of urea and moisture content on straw degradability. DM of treated straw below 60% increased hydrolysis as well as degradability. Williams et al. (1984a) found DM degradability of straw with a moisture content of 40%, treated with urea at 35.3 and 105.9 g/kg straw DM respectively was similar (55.8 and 55.9% respectively). The degradability of treated straw at the same level of urea (70.5 g urea per kg straw DM) with different moisture contents (25, 40 and 55%) resulted in different straw degradabilities (48.1; 55.8; and 55.3%, respectively). It seems that treatment with 35.3 g/kg urea and 40% moisture content is sufficient to improve DM degradability of straw.

Urea treatment increases nitrogen availability for microbes which promotes straw digestibility (Shiere and Ibrahim, 1989). Cloete et al. (1983) found that the voluntary feed intake of sheep fed on urea treated wheat straw was 46.7% higher than untreated wheat straw. In addition, Kaaschiester et al. (1983) found that urea treatment of rice straw increased in vitro organic matter digestibility (IVOMD) of straw from 39 to 54%. Crude protein content increased from 6.1 to 7.0% and dry matter intake reached 3.3% of body weight. It was concluded that the increase in intake was due to the higher content of nitrogen in the treated straw. In contrast, Doyle et al. (1986) concluded from the experimental evidence that urea treatment is only considered to improve the nutritive value of rice straw for maintenance requirements.

2.5.1.3 Biological treatment

Biological treatment methods include composting, ensiling and fungal growth. In the process of composting, organic materials are decomposed through biochemical
processes involving microorganisms. During the composting process, organic matter losses, especially neutral detergent solubles, result in a consequential increase in the ash and lignin content (Doyle et al., 1986; Smith, 1993). Therefore composting is unlikely to increase the feeding value of rice straw (Doyle et al., 1986).

Ensiling is performed to preserve green fodder in a silo or pit with an anaerobic fermentation process. Ensilage seems to be a useful means of conserving straw with a high moisture content at harvest (Doyle et al., 1986; McDonald et al., 1991).

The effect of fungal growth on the chemical composition and the nutritive value of fibrous residues is dependent on the type of fungus, the nature of the residue and the condition of growth (Doyle et al., 1986). Rice straw is usually used as a medium for the growth of mushrooms. Straw that has already been used for growing mushrooms (Volvariella displasia) for period of 30 days has a reduced OM and NDF content and an increase in ash, CP and lignin content. When this spent straw was fed to sheep, it was consumed by sheep at the same level as untreated straw when the diet was only supplemented with minerals (Doyle et al., 1986). Burrow et al. (1979) found that when the fungus Coprinus cinereus was grown in barley straw for ten days, the digestibility of the straw was increased from 45 to about 55%, but there was a 12% dry matter loss from the straw, and the fungus attacked both cellulose and hemicellulose but did not attack lignin. Similar results were obtained by Sundstøl et al. (1993) during the treatment of barley straw grown with the fungi Stropharia rugosoamulata, Pleurotus sajorcajo and Pleurotus eryngi. The DM losses ranged from 27.7 to 40.4%. However, the in vitro dry matter digestibility of treated straw which was grown with these fungi was higher than untreated straw (44.7, 46.2, 40.6 and 35.7% respectively). Fungus does not increase the nutritive value of straw because of the decrease in hemicellulose and cellulose contents after treatment.
2.5.2 Supplementation

Supplementation of rice straw is essential to satisfy the sheep requirements for maintenance. Rice straw has a low digestibility, as indicated by very low voluntary intake by ruminants, which in turn causes insufficient energy intake (Doyle et al., 1986). Smith (1993) reported that most crop residues do not contain adequate soluble nitrogen, fermentable carbohydrate or essential minerals. This imbalance in nutrients result in poor animal performance.

Treatment methods usually only increase intake and digestibility of the treated materials but still do not overcome the imbalance of nutrients that exist in crop residues (Smith, 1993). Urea treated rice straw is similar in nutritive value to medium quality grass. Therefore, supplementation of treated straw with grass, tree leaves, and concentrate is required in production rations (Schiere and Ibrahim, 1989). Zorrila-Rios et al. (1989) stated that ammoniated straw needs to be supplemented with a suitable source of energy to enhance utilisation of the added nitrogen. In their experiment, it was found that digestibility of nitrogen in ammoniated straw increased as supplementation of whole shelled corn increased. This indicates that supplementation of carbohydrate to diets decreased rumen ammonia concentration because of the more efficient utilisation of nitrogen by rumen microbes. In addition, nitrogen balance was improved by ammoniation and by feeding a suitable energy source. Cronje (1990) stated that supplementation with a by-pass starch (maize for example) provides a source of glucose precursors to animals. However, maize also provides considerable amounts of additional fermentation starch in the rumen. This presence of excessive, readily-fermentable starch in the rumen may cause a depression in intake. An experiment conducted by Zorrila-Rios et al. (1989) showed that supplementation of ammoniated straw with 10 g/0.75 kg BW whole shelled corn did not reduce straw intake and resulted in an increase in the nitrogen digestibility of ammoniated straw. However, intake was reduced when the level of supplementation with whole shelled corn increased. Doyle et al. (1986) suggested that supplementation with available energy at up to 10
- 15% of total dietary dry matter will increase the intake of low quality roughage only when nitrogen and minerals are not limiting factors.

The protein requirements of ruminants are derived from the digested remains of rumen microorganisms and dietary by-pass protein (Leng, 1986). According to Church (1983), protein produced by rumen microorganisms provides an adequate supply of protein to the tissues in most animals which have moderate production levels. Rapidly growing animals and high producing cows require greater supplementation of amino acids than those supplied by the microorganisms. This protein must be digested and absorbed in the gut/small intestine. To accomplish this, animals must be fed on protein that escapes degradation by rumen microorganisms, but which can be digested in the intestines (by-pass protein). Jackson (1977a) stated that a protein content of at least 8-10% of the diet is required for production. Males (1987) stated that supplementary protein with a low quality roughage resulted in a linear increase in dry matter intake, cellulose, hemicellulose and energy digestibility. Optimum digestibility occurred when dietary crude protein supplementation was 8.5%.

Kaaschieter et al. (1984) found that supplementation of treated rice straw with coconut cake resulted in a significant increase in milk production compared to gliricidia and leucaena supplementations. This result indicated that both energy and protein limited the milk production of buffaloes when they were fed on treated rice straw, or on treated rice straw supplemented with legumes only. Experiments conducted by Cronje and Weites (1990) using cottonseed meal (as a protein source) and maize (as an energy source) demonstrated that supplementation with the former increased average daily gain and wool growth rate, while the latter decreased roughage intake. However, the feed conversion ratio indicates that responses to protein supplementation will depend on the level of energy absorbed.
2.5.3 Genetic selection

No deliberate attempts have been made to include straw quality in cereal breeding selection programs. The main objective of cereal breeding is to increase the grain yield (Doyle et al., 1986). Improvement of straw quality through genetic selection may be cheaper for farmers, but would involve extensive research. Feed value could be improved without reducing grain yield and quality (Khush et al., 1988). Bainton et al. (1987) studied 46 rice straw varieties and found breeding early-maturing and short-stemmed varieties for high grain yield did not lower the nutritive value of the straw. The longer time from planting to flowering or grain maturity resulted in an increase in leaf blade content. The leaf blade has a higher content of silica and lower digestibility (Walli et al., 1988). Therefore, selection for early maturity is likely to lead to an improvement in straw digestibility (Kush et al., 1988). Bainton et al. (1987) found shorter plant varieties have higher contents of stem and leaf sheath and lower contents of leaf blade. They reported stems as having the highest in vitro enzyme solubility, compared to leaf sheaths (43.8% vs 25.5%). They also found shorter varieties have a higher harvest index and mature earlier than taller varieties. There are significant correlations between botanical fraction, height, days of maturing and harvest index. Taller varieties have more leaves, therefore straw from shorter rice varieties should have a higher feeding value. Capper (1988) suggested that high-yielding rice varieties are shorter and mature earlier compared to traditional varieties. Furthermore, Khush and Khumara (1987) suggested that the development of early-maturing rice varieties allows farmers to grow rice two or three times a year. This condition provides benefit to farmers in continuously supplying fresh straw, which has better palatability than straw which has been stored for long periods.

2.6 Conclusions

From this review of the literature the following can be concluded:

1. The nutritive value of rice straw varies widely in many areas and also between varieties.
2. Rice straw is a low quality roughage that has a low nutritive value. Many methods have already been investigated to improve the nutritive value of rice straw, however, to be applied practically, the methods must be economically viable. Improvements through genetic selection are the cheapest methods for the farmer.

3. Despite previous studies on the nutritive value of rice straw varieties and many methods of treatment to improve the nutritive value of rice straw based on chemical composition and digestibility, this information is still not sufficient to explain the reasons for variability in the nutritive value of rice straw varieties.

4. Microscopic evaluation may help to explain the factors that affect the digestibility of forages. This information will be very useful for the breeding selection of forages.

Based on this review, the aims of the current study were to

1. evaluate the differences in the nutritive value of rice straw varieties using chemical composition and digestibility (in vitro, in sacco and in vivo) measurements;

2. assess a range of methods for improving the nutritive value of rice straw as a feed for ruminants, and

3. study the effect of urea treatment and rice straw quality on ruminal fungi colonisation and stem tissue structure before and after degradation in the rumen.
CHAPTER 3

CHEMICAL COMPOSITION AND IN VITRO ORGANIC MATTER DIGESTIBILITY OF UNTREATED AND UREA TREATED RICE STRAW VARIETIES FOR RUMINANTS.

3.1 Introduction

Rice straw is a crop residue that is widely available in tropical countries (Ibrahim et al., 1988) and is used in an attempt to meet the energy requirements of growing and lactating ruminants (Colucci et al., 1992; Doyle et al., 1986). However, its nitrogen content and digestibility are too low to meet the nutrient requirements of ruminants. These limitations must be overcome if it is to be used as a food source. The classical approach has been to treat crop residues physically or chemically (Colucci et al., 1992). Sodium hydroxide (Jackson, 1977) and ammonia (Sundtol and Coxworth, 1978) have been the most widely used chemicals to improve straw quality. Urea has also been used to treat straw, and this treatment involves the conversion of urea to ammonia by the action of bacterial urease (Williams et al., 1984a).

The economic feasibility of adopting chemical treatment to improve the feeding value of rice straw in developing countries has increasingly been questioned (Schiere and Nell, 1993; Capper, 1988; Colluci et al., 1992) due to the cost of chemicals and labour. An alternative method of improving the feeding value of straw would be to examine the prospects for increasing the nutritive value of crop residues through plant breeding and selection of varieties with straw of high nutritive value which also retain a high grain yield (Capper, 1988). Givens et al. (1988) found that considerable variability exists in the quality of untreated straw and suggested that identification of the highest quality straw for ruminant production may prove cost effective compared to chemical treatment.

The objectives of the study reported in this chapter were (i) to evaluate the chemical composition and IVOMD of several varieties of rice straw and the relationship
between these two measures, and (ii) to assess the effect of urea treatment on the nutritional value of the rice straws.

3.2 Materials and Methods

3.2.1 Sample preparation

Eight, semi-dwarf varieties of rice (tailings and straw) were obtained from the Yanco Agricultural Institute, Yanco, Leeton, N.S.W. These were Doongara (Dong), Amaro (Amr), Illabong (Ilb), Pelde (Pld), Millin (Mil), Langi (Lan), YRL-39 (Yrl) and YRM-43 (Yrm). Tailings and straws (see Table 3.1 for a description of the botanical fractions) were dried in a forced draught oven at 60°C for 48 hours. Dried materials were then chopped into three cm lengths. Sub-samples of the chopped straws was then ground in a laboratory hammer mill with a 1mm screen. Ground samples were stored in air-tight containers prior to chemical analysis and an in vitro digestibility study.

3.2.2 Botanical fraction separation

To compare the relative proportion of the botanical fractions, the upper part of each variety was dissected into four components and the lower part into three components. The components were rachis (for the upper part only), leaf blade, leaf sheath and stem. Each component was weighed and then oven dried at 60°C to constant weight.

3.2.3 Urea treatment

For urea treatment, 200 g samples of chopped straw from each part of each variety were prepared by spraying with urea solution and mixing thoroughly to provide urea and moisture levels of 40 g/kg and 400 g/kg of dry matter respectively. Treated straws were then kept in air-tight plastic bags at 22°C for six weeks. The bags were then opened and the contents dried at 50-60°C for 48 hours (Ibrahim et al., 1988). The treated straws were then ground as in section 3.2.1.
3.2.4 Chemical analysis
Dry matter (DM), organic matter (OM) and nitrogen (N) content of the samples were determined using the methods of the AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF), permanganate lignin and silica (insoluble ash) were determined using the methods of Goering and Van Soest (1970). Hemicellulose (HC) was calculated by subtracting ADF from NDF values (Goering and Van Soest, 1970).

3.2.5 In vitro digestibility
In vitro organic matter digestibility (IVOMD) was determined using the two-stage technique of in vitro digestibility described by Tilley and Terry (1963). Rumen fluid was collected with a stomach tube from three 50 kg Merino sheep which were being fed a maintenance ration of 1200g DM/day, consisting of 50% lucerne and 50% oaten chaff. The ration was fed in equal meals each day at 0900 and 1700.

3.2.6 Statistical analysis
The experiment used an 8x2x2 factorial design involving 8 straw varieties, upper and lower parts and either untreated or urea treated straw. Analysis of variance using Genstat 5 (Lawes Agricultural Trust, 1994) was carried out for the values for N, NDF, ADF, hemicellulose, lignin, silica and IVOMD. Significant differences were tested using a 95% confidence interval.

3.3 Results
For comparative purposes, a large proportion of the results have been presented as figures in this chapter. All raw data used to generate the figures are presented in Appendix 1.

3.3.1 Botanical fraction
The lower parts of the straw contained more stem than the upper parts (Table 3.1). The data were not replicated and therefore statistical analysis was not done. The samples were already dried when obtained, and some of the leaf had already
Table 3.1 Distribution of the botanical fractions on the upper and lower parts of eight varieties rice straw (% dry matter)

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Dong</th>
<th>Amr</th>
<th>Ilb</th>
<th>Pld</th>
<th>Mil</th>
<th>Lan</th>
<th>Yrl</th>
<th>Yrm</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Rachis</td>
<td>19.6</td>
<td>24.1</td>
<td>33.4</td>
<td>24.1</td>
<td>8.7</td>
<td>17.7</td>
<td>21.4</td>
<td>27.3</td>
<td>22.0</td>
</tr>
<tr>
<td>Leaf blade</td>
<td>59.8</td>
<td>41.1</td>
<td>34.2</td>
<td>47.4</td>
<td>22.6</td>
<td>39.3</td>
<td>46.4</td>
<td>25.9</td>
<td>39.6</td>
</tr>
<tr>
<td>Leaf sheath</td>
<td>12.2</td>
<td>20.6</td>
<td>19.8</td>
<td>18.0</td>
<td>41.7</td>
<td>27.8</td>
<td>21.9</td>
<td>29.4</td>
<td>23.9</td>
</tr>
<tr>
<td>Stem</td>
<td>8.3</td>
<td>14.3</td>
<td>12.6</td>
<td>10.5</td>
<td>27.0</td>
<td>15.2</td>
<td>10.3</td>
<td>17.4</td>
<td>14.5</td>
</tr>
<tr>
<td>Lower Leaf blade</td>
<td>14.0</td>
<td>6.1</td>
<td>10.6</td>
<td>19.4</td>
<td>1.9</td>
<td>11.1</td>
<td>22.5</td>
<td>5.4</td>
<td>11.4</td>
</tr>
<tr>
<td>Leaf sheath</td>
<td>44.4</td>
<td>47.6</td>
<td>43.8</td>
<td>37.1</td>
<td>53.6</td>
<td>54.0</td>
<td>37.3</td>
<td>53.1</td>
<td>46.4</td>
</tr>
<tr>
<td>Stem</td>
<td>41.6</td>
<td>46.3</td>
<td>45.6</td>
<td>43.5</td>
<td>44.4</td>
<td>34.8</td>
<td>40.2</td>
<td>41.6</td>
<td>42.3</td>
</tr>
</tbody>
</table>

Dong, Dongara; Amr, Amarro; Ilb, Ilabong; Pld, Pelde; Mil, Milin; Lan, Langi; Yrl, YRL-39; Yrm, YRM-42.
separated from the stem. Considering the difficulties experienced in obtaining complete straw samples, the values for leaf sheath, blade and rachis are approximate only.

3.3.2 The effect of part, variety and urea treatment on chemical composition

There was a highly significant (P<0.01) three-way interaction between part, variety and urea treatment for N content (Table 3.2). The nitrogen content of the upper part of untreated straw of all varieties was higher than the lower part. The range in N content of the upper part was 8.1-11.1 g/kg, while in the lower part it was 5.8-8.3 g/kg. Urea treatment significantly increased the nitrogen content of both parts in all varieties (Fig. 3.1). Urea treatment reduced the difference in N content between parts and varieties. N content in the upper part, after treatment with urea ranged from 16 - 18.6 g/kg, while in the lower part it ranged from 15.5 - 20 g/kg. The highest increase in N content, after treatment with urea, was in the lower part of variety Ilb (from 5.8 to 20 g/kg). The N content of the treated lower part of the Ilb variety was higher than its treated upper part. The lowest increase in N content after urea treatment was in the upper part of Yrm (from 11.1 to 18.2 g/kg).

The interaction between urea, part and variety on NDF content was significant (P<0.05) (Table 3.2). The NDF content of the upper and lower parts varied between varieties and between treatments (Fig. 3.2). Before treatment with urea, the NDF content of upper and lower parts was significantly different (P<0.05) between the varieties Dong, Ilb, Yrl, Yrm. After urea treatment, the NDF content of upper and lower parts was not significantly different except in varieties Amr and Lan. Urea treatment significantly increased the NDF content in the lower part of Yrl (from 704 to 743 g/kg), and significantly decreased the NDF content in the upper part of varieties Dong (from 739 to 705) and Lan (from 723 to 682 g/kg).

Urea treatment significantly (P<0.05) increased the ADF content of rice straw (Table 3.3). There was a highly significant (P<0.01) interaction between part and
variety for ADF content (Table 3.2). The lower parts of Amr, Ilb and Lan varieties contained significantly higher ADF levels than their upper parts. The lowest ADF content was in the lower part of Dong (515 g/kg), while the highest content was in the lower part of Ilb (621 g/kg). The variation in ADF content was higher in the lower part than in the upper part (Fig. 3.3).

Urea treatment significantly (P<0.05) reduced the HC content of rice straw (Table 3.3). There was a highly significant (P<0.01) effect of variety on HC content, where Ilb containing the lowest HC (Table 3.3). The lower part had significantly lower HC than the upper part (Table 3.3). Interaction between the various factors had no significant effect on HC content.

There was a significant (P<0.05) effect of lignin content of the interaction between part and urea treatment (Table 3.2). The lignin content of the upper part of untreated straw was significantly higher than that of the lower part. After treatment with urea, however, the lignin content was not significantly different between upper and lower parts (Fig. 3.4). There was a highly significant (P<0.01) effect of variety on lignin content (Table 3.2). Ilb had the highest lignin content (78 g/kg) compared with other varieties (Table 3.3).

The silica content was not significantly different between upper and lower parts in any varieties except Amr, where the lower part contained a higher silica level (P<0.05) (Fig. 3.5). There was no significant effect of urea treatment on silica content.

3.3.3 The effect of part, variety and urea treatment on IVOMD

There was a highly significant (P<0.01) interaction between part, variety and urea treatment on IVOMD (Table 3.2).
Table 3.2: Significance level from analysis of variance of chemical composition and IVOMD of rice straws

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degree of freedom</th>
<th>N</th>
<th>s.e.m.</th>
<th>NDF</th>
<th>s.e.m.</th>
<th>ADF</th>
<th>s.e.m.</th>
<th>HC</th>
<th>s.e.m.</th>
<th>Lignin</th>
<th>s.e.m.</th>
<th>Silica</th>
<th>s.e.m.</th>
<th>IVOMD</th>
<th>s.e.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main factor:</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part (P)</td>
<td>1</td>
<td>**</td>
<td>0.097</td>
<td>N.S.</td>
<td>2.329</td>
<td>*</td>
<td>3.395</td>
<td>*</td>
<td>2.995</td>
<td>*</td>
<td>1.378</td>
<td>N.S.</td>
<td>1.634</td>
<td>**</td>
<td>3.694</td>
</tr>
<tr>
<td>Variety (V)</td>
<td>7</td>
<td>**</td>
<td>0.195</td>
<td>**</td>
<td>4.656</td>
<td>**</td>
<td>6.790</td>
<td>**</td>
<td>5.990</td>
<td>**</td>
<td>2.756</td>
<td>**.</td>
<td>3.267</td>
<td>**</td>
<td>7.389</td>
</tr>
<tr>
<td>Urea (U)</td>
<td>1</td>
<td>**</td>
<td>0.097</td>
<td>**</td>
<td>2.329</td>
<td>*</td>
<td>3.395</td>
<td>*</td>
<td>2.995</td>
<td>*</td>
<td>1.378</td>
<td>N.S.</td>
<td>1.634</td>
<td>**</td>
<td>3.694</td>
</tr>
<tr>
<td>Interaction:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PxV</td>
<td>7</td>
<td>**</td>
<td>0.276</td>
<td>**</td>
<td>6.584</td>
<td>**</td>
<td>9.602</td>
<td>N.S.</td>
<td>8.471</td>
<td>N.S.</td>
<td>3.898</td>
<td>N.S.</td>
<td>4.621</td>
<td>**</td>
<td>10.45</td>
</tr>
<tr>
<td>UxV</td>
<td>7</td>
<td>**</td>
<td>0.276</td>
<td>N.S.</td>
<td>6.584</td>
<td>N.S.</td>
<td>9.602</td>
<td>N.S.</td>
<td>8.471</td>
<td>N.S.</td>
<td>3.898</td>
<td>N.S.</td>
<td>4.621</td>
<td>**</td>
<td>10.45</td>
</tr>
<tr>
<td>UxP</td>
<td>1</td>
<td>**</td>
<td>0.138</td>
<td>N.S.</td>
<td>3.292</td>
<td>N.S.</td>
<td>4.801</td>
<td>N.S.</td>
<td>4.236</td>
<td>*</td>
<td>1.949</td>
<td>N.S.</td>
<td>2.310</td>
<td>**</td>
<td>5.224</td>
</tr>
<tr>
<td>UxPxV</td>
<td>7</td>
<td>**</td>
<td>0.390</td>
<td>*</td>
<td>9.311</td>
<td>N.S.</td>
<td>13.38</td>
<td>N.S.</td>
<td>11.98</td>
<td>N.S.</td>
<td>5.512</td>
<td>N.S.</td>
<td>6.534</td>
<td>**</td>
<td>14.78</td>
</tr>
</tbody>
</table>

s.e.m. = standard error mean  
** P<0.01  
* P<0.05  
N.S. = non significant  
N  = nitrogen  
NDF  = neutral detergent fibre  
ADF  = acid detergent fibre  
HC   = hemicellulose  
IVOMD= in vitro organic matter digestibility
Fig. 3.1 The effect of part, variety and urea treatment on the nitrogen content of rice straw
(Bar is the value of the confidence interval 95% = ± 0.65; Dong, Dogara; Amr, Ammaro; Ilb, Ilabong; PId, Pelde; Mil, Milin; Lan, langi; Yrl, YRL-39; Yrm, YRM-42)
Fig. 3.2 The effect of part, variety and urea treatment on the NDF content of rice straw

(Bar is the value of the confidence interval 95% = ±15.56; Dong, Dongara; Amr, Ammaro; Ilb, Ilabong;
Pld, Pelde; Mil, Milin; Lan, Langi; Yrl, YRL-39; Yrm, Yrm-42)
Fig. 3.3 The effect of part and variety on the ADF content of rice straw (Bar is the value of the confidence interval 95% = ±16; Dong, Dongara; Amr, Ammaro; Ilb, Ilabong; Pld, Pelde; Mil, Milin; Lan, Langi; Yrl, YRL-39; Yrm, YRM-42)
Fig. 3.4 The effect of part and urea treatment on the lignin content of rice straw
(Bar is the value of the confidence interval 95% = + 3.3)

Fig. 3.5 The effect of part and variety on the silica content of rice straw
(Bar is the value of the confidence interval 95% = + 7.7; Dong, Dongara; Amr, Amaroo; Ilb, Ilabong; Plid, Plide; Mil, Milin; Lan, Langi; Yrl, YRL-39; Yrm, YRM-42)
Table 3.3 Mean values of mean factor effects of variety, part and urea treatment on NDF, ADF, HC and lignin content.

<table>
<thead>
<tr>
<th>Chemical composition (g/kg)</th>
<th>NDF</th>
<th>ADF</th>
<th>HC</th>
<th>Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Means of varieties:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dong</td>
<td>694c</td>
<td>528c</td>
<td>166a</td>
<td>60.2b</td>
</tr>
<tr>
<td>Amr</td>
<td>722ab</td>
<td>570ab</td>
<td>152a</td>
<td>59.5b</td>
</tr>
<tr>
<td>Ilb</td>
<td>712b</td>
<td>592a</td>
<td>120b</td>
<td>77.6a</td>
</tr>
<tr>
<td>Plb</td>
<td>737a</td>
<td>583a</td>
<td>154a</td>
<td>58.5b</td>
</tr>
<tr>
<td>Mil</td>
<td>716ab</td>
<td>568ab</td>
<td>148a</td>
<td>60.4b</td>
</tr>
<tr>
<td>Lan</td>
<td>714ab</td>
<td>558b</td>
<td>156a</td>
<td>66.4b</td>
</tr>
<tr>
<td>Yrl</td>
<td>729ab</td>
<td>575ab</td>
<td>153a</td>
<td>68.7ab</td>
</tr>
<tr>
<td>Yrm</td>
<td>717ab</td>
<td>579ab</td>
<td>140ab</td>
<td>68.1b</td>
</tr>
<tr>
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</tr>
<tr>
<td><strong>Significance</strong></td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td><strong>Means of parts:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>720</td>
<td>564</td>
<td>155</td>
<td>67.9</td>
</tr>
<tr>
<td>Lower</td>
<td>716</td>
<td>574</td>
<td>142</td>
<td>61.9</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>2.32</td>
<td>3.39</td>
<td>3.0</td>
<td>1.38</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td>N.S.</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td><strong>Means of treatment:</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>untreated</td>
<td>721</td>
<td>559</td>
<td>162</td>
<td>62.2</td>
</tr>
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<td>urea treated</td>
<td>714</td>
<td>579</td>
<td>135</td>
<td>67.6</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>2.32</td>
<td>3.39</td>
<td>3.0</td>
<td>1.38</td>
</tr>
<tr>
<td><strong>significance</strong></td>
<td>**</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

s.e.m. = standard error of treatment mean.

**, P<0.01 (highly significant)

*, P<0.05 (significant)

N.S. = non significant
different letters in the same column indicate significant difference (P<0.05)

NDF, neutral detergent fibre; ADF, acid detergent fibre; HC, hemicellulose
Fig. 3.6 The effect of part, variety and urea treatment on IVOMD of rice straw
(Bar is the value of the confidence interval 95% = + 24.7; Dong, Dongara; Amr, Ammaro;
Ilb, Ilabong; Pld, Pelde; Mil, Milin; Lan, Langi; Yrl, YRL-39; Yrm-YRM-42)
The IVOMD of the lower part of all varieties was higher than that of the upper part except in Ilb and Yrl (Fig. 3.6). The IVOMD of the lower part of untreated straw ranged from 325 - 498 g/kg whereas in the upper part it ranged from 325 - 439 g/kg. The IVOMD of the lower part of the Dong variety was significantly (P<0.5) higher than other varieties except for Amr and Yrm. Urea treatment increased the IVOMD of both parts in all varieties. The IVOMD in the upper part after treatment with urea ranged from 438 - 522 g/kg, whereas in the lower part it ranged from 458 - 562 g/kg. After urea treatment there was no significant difference in IVOMD between upper and lower parts in any variety. The increase of IVOMD in response to urea treatment was higher in the upper part than the lower part (Fig. 3.6).

3.3.4 Relationship between chemical composition and IVOMD

The relationship between the IVOMD and chemical composition of rice straw was investigated; the regressions are depicted in Figs. 3.7 - 3.12. The linear regressions to assess the relationship of cell wall components (NDF, ADF, HC, lignin) and silica with IVOMD showed that the relationship between these parameters was poor. Therefore, multiple regression using backward elimination was carried out. The highest coefficient of determination (R²= 0.60) was obtained, following the dropping of NDF and ADF content from the equation. The equation (P<0.001) was

\[ \text{IVOMD} = 997 + 7.34 \text{N} - 1.46 \text{HC} - 2.7 \text{Lignin} - 1.95 \text{Silica}. \]

3.4 Discussion

The chemical composition and the IVOMD varied between varieties and urea treatment consistently increased the N content and IVOMD of both parts in all varieties. The results suggest that the nutritive value of rice straw can be improved by treatment with urea.
Fig. 3.7 The relationship between nitrogen content and IVOMD of rice straw

Fig. 3.8 The relationship between NDF content and IVOMD of rice straw

Fig. 3.9 The relationship between ADF content and IVOMD of rice straw
Fig. 3.10 The relationship between hemicellulose content and IVOMD of rice straw

Fig. 3.11 The relationship between lignin content and IVOMD of rice straw

Fig. 3.12 The relationship between silica content and IVOMD of rice straw
The effect of part and variety on chemical composition

The N content of the upper part of all varieties was higher than the lower part. A similar difference was observed by Winugroho and Sutardi (1986). This difference may largely be due to the higher leaf content of the upper part (63.5%) (Table 3.1). This is supported by Sannasgala and Jayasuriya (1986), who reported the N content in leaves to be higher than in stems. Despite its higher N content, however, the upper part of rice straw does not necessarily have the highest nutritive value. Information on the digestibility of the respective parts (e.g. IVOMD, in sacco, in vivo) is required to make this assessment.

In addition to a difference between the N content of the upper and lower parts, there was a difference between the N content of the different varieties. The upper part of the Yrm variety had the highest total N content (11.1 g/kg), while the lower part of IIb and Yrl had the lowest total N content (5.8 g/kg). Roxas et al. (1985) and Ibrahim et al. (1988) also observed a difference in N contents between 4 and 6 varieties respectively. Roxas et al. (1985) found crude protein content varied with the growing season (wet and dry) and level of nitrogen fertiliser, while Ibrahim et al. (1988) found the crude protein content was not affected by the level of nitrogen fertiliser. In contrast, Cheva-Isarakul and Cheva-Isarakul (1985) observed no significant differences in CP content between seven varieties because of the large variation between samples obtained from a wide range of growing conditions (uncontrolled experiment). In the current experiment the samples were obtained from the same plot with the same treatment, and, therefore, the differences in N content between varieties are likely to be due to varietal, rather than environmental, differences.

There was a variation in NDF content between and within varieties (significant interaction between part and varieties); some varieties had the higher NDF content in the upper part and some in the lower part. In contrast, Sannasgala et al. (1985),
Cheva-Isarakul and Cheva-Isarakul (1985), and Chowdhury et al. (1995) reported no significant differences in the NDF content between varieties. In the current study the variation in the NDF content was higher in the upper part than in the lower part. The lower part of the Dong variety had the lowest NDF content (653 g/kg) while the upper part of variety Pla was the highest (750 g/kg). When the mean value of the parts is considered, the NDF content of the upper part was higher than the lower part. The higher NDF content in the upper part might indicate lower nutritive value because NDF usually is slowly and poorly digested (Pearce et al., 1988). However, the digestibility of the NDF is dependent upon the linkage between lignin and structural carbohydrate (Chesson, 1988) and the phenolic component of the lignin (Jung, 1989).

Lignin and silica content are associated with the lower digestibility of rice straw (Van Soest, 1982). In the current study, lignin content varied between varieties, with variety Ilb having the highest lignin content (78 g/kg). The mean value of parts showed that the upper part had a higher lignin content than the lower part. This result suggests that the lower part will have a higher nutritive value, however, digestibility assessment are required to confirm this.

Silica content in paddy plants is important to maintain the erectness of the leaf (Grist, 1986), therefore, it can be expected that the leaf will have a higher silica content than the stem. In the current study, silica content was not significantly different between parts except for the Amr variety in which the silica content of the lower part was higher than the upper part, even though the upper part contains more leaf. This suggests that the differences in the proportion of leaf had no effect on silica content. In contrast, Doyle and Chanpongsang (1990) and Walli et al. (1988) reported that leaf blade and leaf sheath contained more ash, and in particular, silica.

Analysis of chemical composition alone does not provide clear indications about the relative nutritive value of different rice straw parts and varieties. However, if the
mean value of parts is considered, there were differences between the upper and lower parts in chemical composition which might indicate that the lower part has a higher nutritive value than the upper part. In particular, it has lower NDF and lignin content. Digestibility assessment is required to provide an indication of the relative nutritional value.

The effect of urea treatment on chemical composition

Urea treatment consistently increased the N content of both parts in all varieties. A similar increase of N content of wheat and barley straw after urea treatment was observed by Cottyn and De Boever (1988). This increase could be due to urea, in addition to allowing treatment also providing N to treated straw. The increase in N content after urea treatment was higher in varieties with lower N content (Fig. 3.1). This suggests that rice straw with low N content could benefit more from urea treatment to improve nutritive value. However, the utilization of the N in the urea treated straw will depend upon its digestibility as some of the N bound to cell wall in urea treated straw was not fully utilised in the rumen (Hassen and Chenost, 1992).

The effect of urea treatment on the NDF (Fig. 3.2) was not consistent between part and variety. However if mean values of urea treatment are considered, it appears that urea treatment significantly decreases the NDF content of the rice straw (Table 3.3). Urea treatment increased ADF and lignin content and decreased hemicellulose content (Table 3.3). A similar result was reported by Seawalt et al. (1996) who observed the ADF content of corn stover increased with ammonia treatment. This is due to urea treatment resulting in partial solubilization of hemicellulose (Kiangi et al., 1981; Givens et al., 1988; Masson et al., 1988). The hemicellulose is probably rendered soluble in the neutral detergent solution (Van Soest et al., 1983/84 and Mason et al., 1988). The decreased hemicellulose content results in increased cellulose and lignin levels (Givens et al., 1988 and Mason et al., 1988).
Van Soest (1988) suggested that the analysis of lignin is the most obvious means by which to evaluate the efficiency of delignification. A chemical method to evaluate alkali treated straw must distinguish cleaved lignin from uncleaved lignin. Unfortunately, lignin analysis using 72% acid or through oxidation using permanganate solution did not distinguish cleaved from uncleaved lignin. In the current study, the lignin content was determined using permanganate lignin, therefore the change to lignin content after urea treatment was not consistent and only the lignin content of the lower part significantly increased due to urea treatment (Fig 3.4).

The effect of part and variety on IVOMD

The IVOMD of the lower part was higher than the upper part. Similar results have been reported by Winugroho and Sutardi (1986). Sannasgala and Jayasurya (1986) observed the IVOMD of the stem node and internode was higher than the leaf fraction. Moreover, Sannasgala and Jayasurya (1987) reported that the narrower the leaf:total stem ratio, the higher the IVOMD. Furthermore, Roxas et al. (1985) reported that variation in the semi-dwarf (improved) varieties had higher IVOMD than traditional varieties because semi dwarf varieties contained more stem. In contrast, Doyle and Chanponsang (1990) found the leaf blade of rice straw from 4 varieties had a higher IVOMD than leaf sheath and stem.

In addition to a difference between the IVOMD of the upper and lower parts within a variety, there was a difference between the IVOMD of parts between varieties. Bainton et al. (1991) found that although there were differences between varieties in in vitro digestibility, there was no consistent difference between modern and traditional varieties overall. In the current study all the varieties were semi-dwarf. The lower part of the Dong variety had the highest IVOMD (498 g/kg), while the lower part of variety Yrl and the upper part of variety Lan had the lowest IVOMD (325 g/kg each). Most of the upper parts in all varieties were significantly different to the lower parts, except for the IIb variety. The upper parts were generally low
quality (IVOMD<40%), except for variety IIb which was medium quality (IVOMD=43.9%), while the lower parts were of medium quality (40%<IVOMD<50%), except for varieties Pld and Yrl which were low quality (IVOMD 38 and 32.5%, respectively).

In contrast to the current study, which showed IVOMD of the lower part to be higher than the upper part, Winugroho and Sutardi (1986) reported the in vivo digestibility of the upper part was higher. Doyle and Chanponsang (1990) observed there was no particular part preference in the intake of rice straw when the straw was offered as whole straw, because the intake of fibrous material was governed by palatability and physical features rather than digestibility. The results from previous studies and the current study suggest that rice straw should not be separated into fractions when it is used as a ruminant feed. However, with a particular variety, such as Dong, where the lower part has a much higher digestibility than the upper part, offering the lower part only could result in better animal performance.

*The effect of urea treatment on IVOMD*

Urea treatment consistently increased the IVOMD of both parts in all varieties. A similar increase in IVOMD after urea treatment was observed by Cottyn and De Boever (1988) who reported the main effect of urea treatment on wheat and barley straw was to increase the nitrogen content and IVOMD. This increase in IVOMD is due to the ammonium hydroxide that was released during urea treatment causing the cleavage of the alkali-labile linkage between lignin and structural carbohydrates, increasing fibre digestibility (Hartley and Jones, 1978; Chesson, 1988).

There was no significant difference between the IVOMD of the upper and lower part after urea treatment. The greater increase in IVOMD in the upper part is due to a higher lignin and hemicellulose content in the upper part than in the lower part.
Alkali treatment disrupts the bond between lignin and structural carbohydrates and, therefore, structural carbohydrate, such as hemicellulose, becomes more assessible to microbial degradation (Chesson, 1988). In addition, a higher increase in IVOMD after urea treatment was obtained from low quality rice straws (IVOMD<40%), the increase ranging from 20 - 53%. Similar results have also been observed by Ramazin et al. (1986), Kernan et al. (1979) and, Capper (1988). In the current study the maximum response to urea treatment was obtained from the lower part of the YrI variety which had the lowest IVOMD before treatment with urea. Its IVOMD increased 53% after treatment with urea (from 325 to 499 g/kg; Fig. 3. 6). However, Ibrahim et al. (1989) reported that the maximum benefit of urea treatment was obtained with medium quality rice straw. In the current study, the increase in the IVOMD of medium quality straw (40%<IVOMD<50%) with urea treatment only ranged from 1 - 16%. The results from the current study indicate that the response to urea treatment is higher when the original quality is low.

**Relationship between chemical composition and IVOMD:**

The linear relationship between IVOMD and N, NDF, ADF, HC, lignin and silica was poor. Poor linear relationships between IVOMD and chemical composition have also been reported by Sannasgala and Jayasurya (1986) with rice straw, and by Mason et al. (1988) with wheat, barley and oats. In contrast, Van Soest et al. (1983/84) observed that the relationship between IVOMD and lignin content was significant if the data of treated and untreated straws were regressed separately, but was not significant for the combined data. The difference between untreated and treated populations did not allow crude lignin to become a meaningful measurement. Van Soest et al. (1983/84) suggested that, generally, urea treatment did not greatly change the lignin content. Treatments mainly resulted in a shift of the regression line. In the current study there was no consistent effect of urea treatment of parts and varieties on lignin content. When the untreated and treated data were separated to assess the relationship between lignin content and IVOMD, an R² of 0.057 was obtained for untreated straw, and of 0.081 for treated straw. This indicates that the
relationship between lignin content and IVOMD was poor with either separated or combined data.

Linear regressions between each chemical component and IVOMD both in the present study and in the literature, resulted in poor correlation. This indicates that each chemical component (N, NDF, ADF, HC, lignin and silica) can not be used as a reliable single factor to explain the variability in the IVOMD of rice straw varieties and their parts before and after treatment with urea. The results suggested that the chemical composition is a poor method of assessing the nutritive value of rice straws and subsequent improvement after urea treatment. IVOMD is a better method of assessing the nutritive value of rice straw varieties and improvement after urea treatment. This observation is supported by Ørskov et al. (1988) who reported that biological measurement is the most appropriate method to differentiate the nutritive value between varieties, botanical fractions and treatments of cereal straws.

Because of the poor linear relationship between chemical composition and IVOMD, the relationship between these two parameters was assessed using multiple regression analysis combining data on untreated and urea treated rice straws. The result showed that the IVOMD is a function of N, hemicellulose, lignin and silica with a determinant coefficient, of 0.60. This indicates that 60% of the IVOMD is reflected by the N, hemicellulose, lignin and silica content. The equation also indicates that HC, lignin and silica content have negative effects on the IVOMD. Alternatively, Bainton et al. (1991) reported that the in vitro digestibility is a function of ash content and days to maturity, with a coefficient determinant of 0.74. This result provides further evidence that straw digestibility is affected by a range of chemical parameters and not by any single chemical factor, and hence, IVOMD is a better means than chemical analysis alone of evaluating the nutritive value.
3.5 Conclusion

From the research presented in this chapter, the following conclusions can be drawn.

- There were significant differences in the nutritive value between the upper and lower parts and varieties.
- There was a significant difference in the IVOMD between varieties with the lower part of the Dong variety having the highest IVOMD.
- Chemical composition is a poor indicator of the nutritive value of rice straw as indicated by the poor relationship between IVOMD and chemical composition.
- Urea treatment was effective in improving the nutritive value of all varieties, the improvement being more effective in those varieties with a lower nutritive value.
- Having shown chemical composition cannot be used to explain the differences in in vitro digestibility, further research for evaluating nutritive value is required using in vitro and in sacco methods to assess the nutritive value of rice straw.
CHAPTER 4

RUMEN DEGRADABILITY OF THE UPPER AND LOWER PARTS OF UNTREATED OR TREATED RICE STRAW VARIETIES

4.1. Introduction

The chemical composition and IVOMD of urea treated and untreated straw from eight varieties of rice used in this study differed between varieties and between treatments (Chapter 3). This variation may result in differences in straw intake and animal growth rates (Ørskov et al. 1988).

The nutritive value of feeds is dependent upon nutrient concentration (chemical composition), digestibility and the level of voluntary intake (Ibrahim et al., 1989). Estimates of in vitro digestibility are often used as an index of feeding value because of the difficulties associated with conducting feeding trials. In vitro digestibility is only suitable to compare feeds, but does not provide information on intake. The voluntary intake of low quality feeds, such as straw, is governed by the amount of material in the reticulo rumen (both degradable and undegradable), the rate of digestion and the rate of passage of digesta out of the reticulo-rumen. Data obtained from rumen degradation studies provide information on both the degradable and undegradable fractions of feeds and also on the rate of degradation (Ibrahim et al. 1989).

Little work has been reported on the aspects of rumen degradability of rice straws (especially those bred in southern Australia) and its upper and lower parts or the effect of urea treatment. Therefore, the objectives of this study were:

1. to determine if there were differences in the degradation characteristics of the upper and lower parts of four varieties of rice straw; and

2. to observe the effects of urea treatment on degradability.
4.2 Materials and Methods

4.2.1 Animals and diets

Four, South Australian Merino sheep of 50 kg live weight fitted with rumen fistula were used for the experiment. The sheep were fed a maintenance ration (1.2 kg DM/day) which consisted of 50% oaten chaff and 50% lucerne chaff. This was fed in equal meals each day at 0900 and 1700.

4.2.2 Test feeds.

A range of low (IVOMD<40%) and medium (40%>IVOMD < 50%) quality rice straw varieties were selected, based on their IVOMD (Chapter 3; table 4.1). All varieties were also treated with urea as described in Chapter 3. Unground samples of untreated and urea treated straw were ground through a 2.5 mm screen and subsequently used for nylon bag studies.

Table 4.1 The quality of the upper and lower parts of selected rice straw varieties based on their IVOMD

<table>
<thead>
<tr>
<th>Part</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>upper</td>
<td>quality</td>
</tr>
<tr>
<td>lower</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dong</th>
<th>low</th>
<th>medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilb</td>
<td>medium</td>
<td>medium</td>
</tr>
<tr>
<td>Yrl</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td>Yrm</td>
<td>low</td>
<td>medium</td>
</tr>
</tbody>
</table>

Dong, Dongara; Ilb, Ilabong; Yrl, YRL-39; Yrm, YRM-42

4.2.3. Determination of degradation characteristics

The dry matter losses (DML) of straw samples when incubated in nylon bags, in vivo, were determined as described by Mehrez and Ørskov (1977). About 2 g of air dry samples were incubated in nylon bags suspended in the rumen for 8, 16, 24, 48
or 72 hours. The bags were incubated using the serial addition method which allowed removal of all bags at the same time. Each of the parts from each variety was incubated separately in the rumen of each sheep for each incubation period. Their degradation characteristics were described by the exponential equation of Ørskov and McDonald (1979);

\[ p = a + b(1 - e^{-ct}) \]

where:

- \( p \) = degradation at time \( t \), and \( a \), \( b \), \( c \) are constants defining the degradation characteristics of the sample.
- Constant:
  - \( a \) = the intercept, and equals the immediately soluble material.
  - \( b \) = the insoluble but degradable material
  - \( c \) = the rate of degradation of \( b \)

\( (a+b) \) = the potential degradability of the straw (PD)

Washing loss (solubility) without incubation in the rumen was determined by measuring dry matter loss caused by soaking the samples contained in the nylon bags in water at 30°C for 30 minutes and then washing them under running tap water until the water was colourless. The bags withdrawn from the rumen were also washed in the same manner, before being dried at 60°C to constant weight.

4.2.4 Statistical analysis

The experiment used a 4 x 2 x 2 factorial design involving 4 straw varieties, upper and lower parts, and either untreated or urea treated straw. Analysis of variance using Genstat 5 (Lawes Agricultural Trust, 1994) was carried out on the values of washing loss, DML (dry matter loss) and degradation characteristics.
4.3 RESULTS

For comparative purposes, a large proportion of the results have been presented as figures in this chapter. All raw data used to generate the figures are presented in Appendix 2.

4.3.1 Washing loss and dry matter loss

There was a significant (P<0.05) interaction between part, variety and urea treatment in the washing loss (Table 4.2). For untreated straw, washing loss was higher (P<0.05) in the lower part than in the upper part. Urea treatment increased the washing loss of both parts of Dong and Yrl (Fig. 4.1). There was no significant effect of urea treatment on the washing loss of the lower part of Ilb or Yrm (Fig. 4.1).

There was a significant interaction between part and variety on DML at incubation times up to 48 h (Table 4.2). The DML of the lower part was higher than the upper part, except for the Ilb variety where DML of the upper part was higher than the lower part (Fig. 4.2). The lower part of the Dong variety had the highest DML of the other varieties (Fig. 4.2).

The interaction between urea treatment and part was significant (P<0.05) on DML at incubation times of 8, 16 and 48 hours and was highly significant at 24 and 72 hours (Table 4.2). The untreated lower parts had a higher DML than the upper parts. Urea treatment increased the DML of the upper and lower parts (Fig. 4.3). After urea treatment, the DML of both parts was similar (Fig. 4.3). The increase in DML after urea treatment was higher in the upper part than in the lower part.

4.3.2 Degradation characteristics.

There were significant (P<0.01) differences between the degradation characteristics of the upper and lower parts in the immediately soluble material, the insoluble but degradable material and potential degradability (Table 4.3). The lower part had
significantly (P<0.05) higher immediately soluble material than the upper part. On the other hand, the upper part had significantly (P<0.05) higher insoluble but degradable material and potential degradability (Table 4.3). There was no significant effect of variety on degradation characteristics (Table 4.3).

Urea treatment increased (P<0.05) rate of degradation and increased (P<0.01) potential degradability. There was no significant effect of urea treatment on the immediately soluble material and on the insoluble but degradable material (Table 4.3). There was no significant interaction between part, variety and urea treatment on degradation characteristics.

4.4 Discussion
Urea increased both “c”, the rate of degradation and PD, the potential degradability of the rice straw.

The effect of part and variety on DML and degradation characteristics
The higher dry matter degradation of the lower part compared to the upper part (Fig. 4.2) could be due to the lower part containing more stem than the upper part (42.3% vs 14.5%). This result is supported by Nakashima and Ørskov (1990) and Walli et al. (1988) who observed that the stem internode was more degradable than the leaf. In the current study the lower part of the Dong variety had a higher DML than the other varieties. The higher DML of the upper part compared to the lower part of the Ilb variety is the likely reason for the significant interaction between part and variety.

The analysis of variance of the mean factor effects shows that the lower part had higher immediately soluble material, insoluble but degradable material and potential degradability values (Table 4.2). This could be due to the lower part having a
Table 4.2 Significance level from analysis of variance of washing loss and dry matter loss at various incubation times

<table>
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<th>Degrees of freedom</th>
<th>Incubation time (h)</th>
<th>Washing loss</th>
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<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Main factors</td>
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<tr>
<td>Part (P)</td>
<td>1</td>
<td>** (10)</td>
</tr>
<tr>
<td>Variety (V)</td>
<td>3</td>
<td>** (29)</td>
</tr>
<tr>
<td>Urea (U)</td>
<td>1</td>
<td>** (29)</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P x V</td>
<td>3</td>
<td>** (5.8)</td>
</tr>
<tr>
<td>V x U</td>
<td>3</td>
<td>* (5.8)</td>
</tr>
<tr>
<td>P x U</td>
<td>1</td>
<td>** (4.1)</td>
</tr>
<tr>
<td>P x V x U</td>
<td>3</td>
<td>* (8.2)</td>
</tr>
</tbody>
</table>

** (P<0.01) highly significant
* (P<0.05) significant
N.S. = non significant
values in the bracket are standard error of difference
Fig. 4.1 The effect of part, variety and urea treatment on washing loss of rice straw (Bar is the confidence interval 95%; Dong, Dongara; Ilb, Ilabong; Yrl-YRL-39; Yrm, YRM-42)
Fig. 4.2 The effect of part and variety on dry matter loss of rice straw (Bar is the standard error mean)
Fig. 4.3 The effect of part and urea treatment on dry matter loss of rice straw (Bar is the standard error mean)
Table 4.3 Mean values of main factor effects of variety, part and urea treatment on washing loss and degradability characteristics *in sacco* of rice straw according to the equation $p = a+b(1-e^{ct})$.

<table>
<thead>
<tr>
<th>Degradation characteristics</th>
<th>a (g/kg DM)</th>
<th>b (g/kg DM)</th>
<th>c (fraction/h)</th>
<th>PD (a+b) (g/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variety</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dong</td>
<td>249</td>
<td>372</td>
<td>0.039</td>
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<tr>
<td>Ilb</td>
<td>225</td>
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<td>573</td>
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<td>Yrl</td>
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<td>s.e.</td>
<td>8.12</td>
<td>14.47</td>
<td>0.0037</td>
<td>7.03</td>
</tr>
<tr>
<td>significance</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>Part</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>upper</td>
<td>210</td>
<td>404</td>
<td>0.032</td>
<td>614</td>
</tr>
<tr>
<td>lower</td>
<td>241</td>
<td>346</td>
<td>0.044</td>
<td>586</td>
</tr>
<tr>
<td>s.e.</td>
<td>5.74</td>
<td>10.21</td>
<td>0.0026</td>
<td>4.97</td>
</tr>
<tr>
<td>significance</td>
<td>*</td>
<td>*</td>
<td>N.S.</td>
<td>*</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>untreated</td>
<td>213</td>
<td>355</td>
<td>0.031</td>
<td>568</td>
</tr>
<tr>
<td>urea treated</td>
<td>238</td>
<td>395</td>
<td>0.045</td>
<td>632</td>
</tr>
<tr>
<td>s.e.</td>
<td>5.74</td>
<td>10.21</td>
<td>0.0026</td>
<td>4.97</td>
</tr>
<tr>
<td>significance</td>
<td>N.S.</td>
<td>N.S.</td>
<td>*</td>
<td>**</td>
</tr>
</tbody>
</table>

a = immediately-soluble material  
b = insoluble but degradable material  
c = the rate of degradation of b  
PD = (a+b) potential degradability  
s.e., standard error; **, P<0.01; *, P<0.05; N.S., non significant
higher stem content. This result is supported by Nakashima and Ørskov (1990) who found that the potential degradability and rate of degradation value in the internodes were higher than in the leaf. However, this differs from Walli et al. (1988) who observed no differences in potential degradability within botanical fractions.

In the present study, there were no significant differences between varieties in the degradation characteristics of the immediately soluble material, insoluble but degradable material, rate of degradation and potential degradability, even though the DML at all incubation times was highly significant. This might be due to variability in the measurement of factors used to calculate degradation characteristics. Walli et al. (1988) observed a variation of degradation characteristics between long (traditional variety) and short varieties of rice straw in the immediately soluble material, insoluble but degradable material, and rate of degradation between varieties. Moreover, Nakashima and Ørskov (1990) observed short and early varieties of rice straw had higher values of washing loss, immediately soluble material, and insoluble but degradable material than tall and late varieties. Despite this, the differences in the degradation between varieties could not be explained from the differences between the botanical fractions as there were no significant differences between leaf and stem proportions. Differences between straw varieties in rumen degradability therefore appear to be due to the quality of leaf and stem, rather than the stem to leaf ratio (Ramanzin et al. 1986). Even though there were no significant differences in immediately soluble material, insoluble but degradable material, rate of degradation and potential degradability between varieties, this does not indicate that the varieties in the current study had similar nutritive value, because Orskov et al. (1988) observed degradation at 48 h was closely correlated with intake \((r = 0.90)\). Since in the current study, there was an interaction between part and variety on degradation at 48 hours, it appears that the lower part of the Dong variety might give higher intakes than other varieties, and subsequently result in better animal performance.
The effect of urea treatment on DML and degradation characteristics

Previous studies by Walli et al. (1988), Kernan et al. (1979), Hartley et al. (1984), and Nakashima and Orskov (1990) showed that the increased in DML of rice straw after urea treatment was greater in the leaf than in the stem. In the current study, the upper part contained more leaf than the lower part (63.5% vs 47.8%), hence the increase in DML after treatment was greater for the upper part than for the lower part (Fig 4.3). The greater DML in the upper part after urea treatment may be due to the higher HC level (155 vs 142 g/kg) and lignin levels (67.9 vs 61.9 g/kg) in the upper part compared to the lower part. In a secondary lignified cell wall such as that found in straw, the nature of cross linking between structural carbohydrate and lignin means more HC is bound to lignin, and therefore, the HC becomes unavailable for rumen degradation (Chesson, 1988). Alkali treatments, such as urea, disrupt the ester bond between lignin and HC, resulting in the HC becoming available for digestion (Chesson, 1988).

In the present study urea treatment significantly increased washing loss, potential degradability and the rate of degradation. The increase in the rate of degradation constant represents an increase in rumen flow from the gut, resulting in an increase in roughage consumption (Ibrahim et al. 1989). Since rumen degradability and apparent digestibility in vivo are correlated with intake, voluntary intake should be correlated with the rate of degradation constant. However, Hovell et al. (1986) found that the rate of degradation constant of hay did not correlate with intake as the models for the measurement of degradation characteristics only describes degradation after the maximum rate of degradation is reached. Maximum degradation of straw is seldom reached before 90 h of incubation in the rumen, while the intake is governed by rumen retention time. Rumen retention time for straw is likely to vary between 36 and 60 h.

The increased solubilisation of low quality roughage materials is an indication of improved digestibility (Ololade et al., 1970; McManus and Choung, 1976). In this
study, immediately soluble material ("a") and insoluble but degradable material ("b") were not significantly different between untreated and treated straw. However, the dry matter degradability of treated straw was still improved. This is consistent with the findings of Ørskov et al. (1988) who showed no significant correlation between immediately soluble material, insoluble but degradable material and rate of degradation constant. They found that some straw had similar potential degradability, but differed in the degradation rate constant. Coombe et al. (1979) observed that some feeds had different potential degradability but had a similar rate of constant. In the current study the potential degradability of treated straw was increased from 568 g/kg to 632 g/kg. However, this potential degradability was only reached after 72 h incubation since the DML at 72 h was 512 g/kg and 607 g/kg for untreated and urea treated straw, respectively. Since rumen retention time varied from 36 to 60 h incubation, the in vivo digestibility estimates will differ from the potential degradability with larger amounts insoluble but degradable material and a lower rate of degradation constant. Since the rate of degradation value of urea treated straw was higher than untreated straw it would be expected that the intake of treated straw would be higher. The increase in potential degradability and rate of degradation constant resulting from urea treatment has also been reported by Ibrahim et al. (1989).

4.4.5 Conclusion

From the research presented in this chapter, it is can be concluded that

- the DML of the lower part of rice straw was higher than the upper parts.
- DML varied between varieties and parts, with the lower part of the Dong variety having the highest DML.
- urea treatment increased the DML, potential degradability and rate of degradation, and
- there was a greater increase in DML in the upper part of rice straw following urea treatment.
CHAPTER 5

IN VIVO NUTRIENT DIGESTIBILITY OF BASAL DIET CONTAINING UNTREATED AND UREA TREATED RICE STRAW FED TO SHEEP

5.1 Introduction

From the research presented in Chapter 3 and 4 it can be seen that the IVOMD and rumen degradability of the rice straw varieties used in this study were of medium (40%<IVOMD<50%) and low quality (IVOMD<40%). To be used as a ruminant feed these straw should be treated, or supplemented with high quality feeds.

Urea treatment (urea ensilage) increased the IVOMD and DML (Chapter 3 and 4). It increased the nutritive value of rice straw by increasing the protein content and nutrient digestibility and palatability. This, in turn, may increase dry matter intake (Wanapat et al., 1982; Cheva-Isarakul and Potikanond 1985; Wongsrikeao and Wanapat 1985). However, the application of urea treatment by farmers in Asia may be limited due to the fact that it is labour intensive and time consuming (Cloete and Kritzinger, 1983; Cheva-Isarakul and Jaerachi, 1987) and causes management problems (Cheva-Isarakul and Jaerachi, 1987).

Rice straw sprayed with urea at feeding time might be an alternative means of treatment as it eliminates the long process associated with ensiling (Cheva-Isarakul and Jaerachai, 1987). In practice, supplementation with NPN (non protein nitrogen) can stimulate animal performance (Doyle et al., 1986). However, care must be taken to avoid excessive intake of urea, as urea can be toxic when small amounts are consumed rapidly. At present, non protein supplements are not widely used by farmers in Asia because of the uncertainty surrounding the provision of urea (Doyle et al., 1986).
Forage legume supplementation is more applicable, and legumes already are commonly fed to animals on small farms in Asian countries. These include *Leucaena leucocephala* and *Sesbania grandiflora*. This is because legumes are available near farms, are relatively cheap compared to concentrates, and, are easier to use compared to urea treatment. Forage legumes may provide useful supplements to cover the nutrient deficiencies of rice straws (Van Soest 1988).

An increase in protein content of the diet without balancing with energy could result in inefficient protein utilisation by animals (Cloete et al., 1983). Therefore, in the current experiment, isoenergetic and isonitrogenous diets were formulated using untreated rice straw with high legume content, urea ensiled rice straw, and urea supplemented rice straw. Using these diets the objectives of this experiment were to compare these three diets in term of
1. energy intake and apparent digestibility of nutrients, and
2. nitrogen utilisation.

5.2 Materials and Methods

5.2.1 Feed preparations and treatments
Straw (Pelde variety) was obtained from the Yanco Agricultural Institute, N.S.W, and was used in an *in vivo* feeding trial with Merino sheep. The straw was chopped into 5 cm lengths. The diets were based on on the following components (rice straw, RS; oaten hay, OH; lucerne hay, LH; Urea treated rice straw URS; rice straw with added urea, RS+U). Thus all diets contained about 50% RS and all diets some LH.

- Diet 1 49% RS, 32%LH
- Diet 2 55%URS, 25% OH, 11% LH
- Diet 3 49% RS+U, 20% OH, 18% LH

Urea ensiled rice straw was prepared by spraying chopped straw with urea solution to yield treated straw containing 600g DM per kg straw (William *et al.*, 1984a) and 40g urea/kg straw DM (Schiere and Ibrahim, 1989). The treated straw was then kept in air tight polyethylene bags for 6 weeks at ambient temperature (6-20°C). After 6 weeks the bags were opened and the treated straw was aerated on the floor for 24
hours prior to feeding (to reduce the smell of ammonia). The urea supplemented treatment was prepared by spraying untreated rice straw with a urea solution (see table 5.1) at feeding time.

The untreated, ensiled, and urea supplemented straws were mixed with other feed ingredients to provide isoenergetic and isonitrogenous diets. The rations were formulated for diets containing 1.5%N and 7 MJ/kgDM of ME and fed at a rate of 1023 gDM/day. The "Take Away" computer program (Barber, 1992) was used. The diets were formulated to provide a maintenance ration for mature South Australian Merino wethers of 50 kg body weight. The metabolisable energy (ME) of untreated and ensiled rice straw were estimated using the following equation from MAFF (1984): ME = 0.81 (GE x OMD) where:

GE is gross energy (MJ/kgDM), and

OMD is in vitro organic matter digestibility.

ME for other feed ingredients used in the diets was obtained from the "Take-Away" data base. The feed composition of the diets is given in Table 5.2 and the chemical composition of the diets is presented in Table 5.3.

Table 5.1 Composition of the experimental diets ( % dry matter basis)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Untreated rice straw</td>
<td>48.9</td>
</tr>
<tr>
<td>Ensiled rice straw</td>
<td>-</td>
</tr>
<tr>
<td>Lucerne hay</td>
<td>31.6</td>
</tr>
<tr>
<td>Oaten hay</td>
<td>-</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>15.1</td>
</tr>
<tr>
<td>Molasses</td>
<td>2.3</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
</tr>
<tr>
<td>Rockphos</td>
<td>1.7</td>
</tr>
<tr>
<td>Salt</td>
<td>0.1</td>
</tr>
</tbody>
</table>
The untreated basal diet contained the highest proportion of legume compared to other diets in order to adjust the nitrogen and energy content and make it similar to the other diets. Urea was added in the urea supplemented diet to adjust nitrogen levels.

5.2.2 Animals and Management

Twelve mature South Australian Merino wethers (53.62 ± 3.44 kg) were separated into 4 groups based on their live weight with each group assigned three diets (untreated; urea ensiled or urea supplemented diets). Diets were allocated based on a randomised complete block design. Before the experimental period, the wethers were drenched with a Valbezen-broad spectrum (Smith Kline and French Laboratories). During an adaptation period of 15 days, the sheep were placed in individual pens and gradually introduced to their experimental diets. Sheep were offered 1023 g DM daily of their allocated diets at 0830 and 1700 each day. At the conclusion of the adaptation period, the sheep were transferred to metabolism crates, designed to separately collect urine and faeces. Total faecal and urine output were collected for a seven day period. Water was available at all times.

5.2.3 Sample Collection

5.2.3.1 Feed

Feed ingredients were collected and analysed separately. Urea ensiled rice straw was sampled every day, after the ensiled straw has been aerated for 24 hours. Sub-samples were analysed for NDF, ADF, lignin and N. For the first three analyses, the sub-samples were dried in an forced-air oven at 60°C for 48 hours before grinding to pass through a 1mm sieve. Sub-samples were freeze-dried before grinding and sieving, prior to N analyses.

5.2.3.2 Refusals

Feed refusal samples for analysis were collected daily during the collection period, prior to the morning feed. The residues were sub-sampled and dried in a air-forced
oven at 60° C for 48 hours before being ground through a 1mm sieve. The samples were then kept in air tight plastic containers until required for chemical analysis.

5.2.3.3 Faeces

Ten percent of the total faeces from each animal were sampled daily and retained. At the end of the collection period, faecal samples from each animal were bulked and composited. Samples were then dried in an air-forced oven at 60° C for 48 hours, before grinding to pass through a 1mm sieve. These samples were stored in plastic containers for NDF, ADF, and lignin analysis. Fresh faeces were dried in a freeze drier, then ground through a 1mm sieve and stored in air tight containers prior to nitrogen analysis.

5.2.3.4 Urine

Urine was collected in plastic containers containing 50ml of glacial acetic acid (McMeniman et al., 1988) to prevent nitrogen loss during collection and storage. Ten percent of the total urine collection was sub-sampled daily, pooled on an animal basis, and stored at -20° C until required for analysis.

5.2.4 Chemical analysis

Dry matter (DM), organic matter (OM) and nitrogen (N) analysis was determined by the method of AOAC (1991). Neutral detergent fibre (NDF), acid detergent fibre (ADF), permanganate lignin and silica (insoluble ash) were determined using the methods of Goering and Van Soest (1970). Hemicellulose (HC) was calculated by the difference between NDF and ADF. Cellulose was obtained from the weight loss of ADF residue, after being treated with permanganate solution and ashed at 500° C (Goering and Van Soest, 1970). Gross energy (GE) was measured using adiabatic bomb calorimeter.
5.2.5 Measurements

Measurement of apparent digestibility of nutrients and nitrogen balance was facilitated by total urine and faeces collection. The apparent digestibility coefficients of the nutrients were calculated using the equation

\[
\%NAD = \left( \frac{NI - NF}{NI} \right) \times 100\%
\]

where:

NAD = nutrient apparent digestibility,

NI = nutrient intake, and

NF = nutrient in faeces.

The nitrogen balance was calculated by measuring the difference between total N intake and N excretion in faeces and urine.

5.2.6 Statistical Analysis

Digestible energy intake, apparent nutrient digestibility coefficients, and nitrogen balance values were analysed using analysis of variance of a randomised complete block design (Genstat 5; Lawes Agricultural Institute, 1994). Significant differences between treatments were determined using the least significant difference tests.

5.3 Results

The chemical composition of untreated and urea treated straw in Table 5.2 shows that urea treatment increased the nitrogen content, but there were small differences in cell wall components between untreated and urea treated straw.

Table 5.2 Chemical analysis of untreated and urea ensiled rice straw (% dry matter basis)

<table>
<thead>
<tr>
<th>Rice straw</th>
<th>N</th>
<th>NDF</th>
<th>ADF</th>
<th>cellulose</th>
<th>OM</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ensiled</td>
<td>1.43</td>
<td>78.93</td>
<td>60.04</td>
<td>41.08</td>
<td>81.31</td>
<td>18.89</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.53</td>
<td>77.71</td>
<td>59.48</td>
<td>39.40</td>
<td>80.98</td>
<td>18.23</td>
</tr>
</tbody>
</table>

N, nitrogen; NDF, neutral detergent fibre; ADF, acid detergent fibre; OM, organic matter; HC, hemicellulose.
Table 5.3 Chemical composition of the experimental diets (% dry matter basis)

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>85.01</td>
<td>86.41</td>
<td>86.59</td>
</tr>
<tr>
<td>N</td>
<td>1.52</td>
<td>1.51</td>
<td>1.48</td>
</tr>
<tr>
<td>NDF</td>
<td>61.88</td>
<td>65.86</td>
<td>63.71</td>
</tr>
<tr>
<td>ADF</td>
<td>43.13</td>
<td>45.51</td>
<td>43.38</td>
</tr>
<tr>
<td>HC</td>
<td>18.75</td>
<td>20.35</td>
<td>20.33</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30.94</td>
<td>33.20</td>
<td>31.32</td>
</tr>
<tr>
<td>ME (MJ/kg)</td>
<td>7.04</td>
<td>7.00</td>
<td>7.05</td>
</tr>
</tbody>
</table>

OM, organic matter; N, nitrogen; NDF, neutral detergent fibre; ADF, acid detergent fibre; HC, hemicellulose; ME, metabolisable energy.

The nutrient composition, especially ME and N content of the experimental diets, (presented in table 5.3) were relatively similar.

5.3.1 Dry matter intake, digestible energy intake and apparent digestibility of the diets

The voluntary dry matter intake per metabolic weight (DMI/W\(^{0.75}\)) of Diet 2 was significantly higher (P<0.05) than Diet 3 (Table 5.4). However, there was no significant difference in DMI/W\(^{0.75}\) between Diet 2 and Diet 1. DE intake of Diet 2 was significantly higher compared to Diet 3, but not significantly different from Diet 2 (Table 5.4).

The crude protein digestibility of Diet 2 was significantly (P<0.05) lower compared to other diets (Table 5.4). The HC digestibility of Diet 2 (75.6%) was significantly higher (P<0.01) than the other diets (Table 5.4). The cellulose digestibility of Diet 2 (66.1%) was significantly higher (P<0.05) than the other diets. The NDF digestibility of Diet 2 was not significantly different to Diet 1, but was significantly
digestibility of Diet 2 was not significantly different to Diet 1, but was significantly different (P<0.05) to Diet 3 (Table 5.4). There were no significant difference in DM, OM and ADF digestibility between diets (Table 5.4).

Table 5.4 Voluntary intake, DE intake and apparent digestibility of nutrients from untreated rice straw, urea ensiled rice straw and urea supplemented rice straw.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Diets</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>significance</th>
<th>s.e.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI (g/day)</td>
<td></td>
<td>892a</td>
<td>930a</td>
<td>772b</td>
<td>*</td>
<td>32.8</td>
</tr>
<tr>
<td>gDMI/BW0.75/day</td>
<td></td>
<td>45.9ab</td>
<td>47.3a</td>
<td>40.1b</td>
<td>*</td>
<td>2.53</td>
</tr>
<tr>
<td>DE intake (MJ/day)</td>
<td></td>
<td>7.7a</td>
<td>7.2a</td>
<td>6.3b</td>
<td>*</td>
<td>0.441</td>
</tr>
<tr>
<td>Apparent digestibility (% DM):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td>50.4</td>
<td>52.0</td>
<td>50.1</td>
<td>N.S.</td>
<td>2.94</td>
</tr>
<tr>
<td>OM</td>
<td></td>
<td>58.1</td>
<td>59.6</td>
<td>56.9</td>
<td>N.S.</td>
<td>2.65</td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td>62.9a</td>
<td>50.2b</td>
<td>64.7a</td>
<td>**</td>
<td>2.86</td>
</tr>
<tr>
<td>NDF</td>
<td></td>
<td>47.5ab</td>
<td>54.1a</td>
<td>44.3b</td>
<td>*</td>
<td>3.87</td>
</tr>
<tr>
<td>ADF</td>
<td></td>
<td>40.9</td>
<td>44.1</td>
<td>38.3</td>
<td>N.S.</td>
<td>3.91</td>
</tr>
<tr>
<td>HC</td>
<td></td>
<td>62.4b</td>
<td>75.6a</td>
<td>56.0b</td>
<td>**</td>
<td>3.23</td>
</tr>
<tr>
<td>Cellulose</td>
<td></td>
<td>59.8b</td>
<td>66.1a</td>
<td>56.7b</td>
<td>*</td>
<td>2.26</td>
</tr>
</tbody>
</table>

s.e.d., standard error of difference
N.S., non significant; *, P<0.05; **, P<0.01
different letters in the same row indicate significant different (LSD 5%)
DMI, dry matter intake; DE, digestible energy; DM, dry matter; OM, organic matter;
CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; HC, hemicellulose.

5.3.2 Nitrogen Retention of the diets

The N intake was not significantly different between Diet 1 and Diet 2 (Table5.5). However, the N intake of both these diets was significantly greater (P<0.01) than
Diet 3 (Table 5.5). The faecal nitrogen of Diet 2 was significantly (P<0.05) higher compared to the other diets. However, N excretion from urine in animals fed on Diet 2 was significantly (P<0.05) lower compared to other diets (Table 5.5). There was no significant difference between Diet 1 and Diet 3 in N excretion from urine. There was no significant difference in the nitrogen retention between diets (Table 5.5).

Table 5.5 Nitrogen retention of untreated rice straw, urea ensiled rice straw and urea supplemented rice straw diets (g/day).

<table>
<thead>
<tr>
<th>Description</th>
<th>Diets</th>
<th>significance</th>
<th>s.e.d</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Intake</td>
<td>14.2a</td>
<td>14.4a</td>
<td>12.1b</td>
</tr>
<tr>
<td>N faeces</td>
<td>5.3b</td>
<td>7.1a</td>
<td>4.2c</td>
</tr>
<tr>
<td>N urine</td>
<td>9.8a</td>
<td>5.7b</td>
<td>9.2a</td>
</tr>
<tr>
<td>N retention</td>
<td>-0.8</td>
<td>1.5</td>
<td>-1.3</td>
</tr>
</tbody>
</table>

N = nitrogen  
s.e.d. = standard error different  
N.S. = non significant; *, P<0.05; **, P<0.01  
different letters in the same row indicate significant difference (LSD 5%)

5.4 Discussion

The urea ensiled straw diet (Diet 2) and the untreated rice straw diet (Diet 1) had similar effects on DMI/metabolic weight. The apparent digestibility of cell wall components, particularly cellulose and hemicellulose, was improved in the urea ensiled straw diet. Urea treatment significantly increased the N content of all straw varieties.

*Dry matter intake, digestible energy intake and apparent digestibility of the diets*

The DMI of Diet 3 was lower compared to Diet 2 in this experiment (40.1 vs 47.3 g/W0.75). The greater DMI in Diet 2 may have been associated with improved palatability of urea ensiled straw (Lawlor and O’Shea, 1979). A similar result was obtained with wheat straw by Cloete et al. (1983).
Legume supplementation may have promoted an increase in the DMI of Diet 1. In the current study, total DMI and DE intake of Diet 1 (930 g/day and 7.7 MJ/day respectively) was significantly higher (P<0.05) than Diet 3 (772 g/day and 6.3 MJ/day, respectively) (Table 5.4). This is due to the legume supplementation providing rumen fermentable energy (in addition to protein) in the form of available cellulose and hemicellulose, both of which are known to stimulate fibre digestion (Silva and Orskov 1985). The rate of fibre digestion may have been significantly faster in sheep fed on supplemented legume straw. This straw could have stimulated DMI (McMeniman et al., 1988). McMeniman et al. (1988) also observed that supplementation with NPN did not stimulate the rate of rumen degradability of rice straw to the extent achieved with legume straw supplementation, even though the rumen ammonia concentration may be similar in sheep fed on rice straw supplemented with urea, compared to those fed with rice straw supplemented with legumes.

In the current study the DM digestibility of Diet 2 (52.0 vs 50.1%) was not significantly different to Diet 1. Despite this, urea ensiled rice straw may have the advantage of greater fibre digestibility. The NDF, ADF, cellulose and hemicellulose content of the three diets were relatively similar (Table 5.3), however the digestibility of NDF, cellulose and hemicellulose in Diet 2 was higher than the other diets (Table 5.4). This is because the ammonium hydroxide which is released following ensiling with urea may have caused cleavage of the alkali-labile linkages between lignin and the structural carbohydrates (Hartley and Jones, 1978; Buettner et al., 1982), which could have increased the fibre degradability. Data from the current study suggest that the digestibility of cell wall constituents of urea ensiled rice straw increased. This result is comparable to the result obtained by Dias Da Silva et al. (1986) who obtained a hemicellulose digestibility of urea ensiled wheat straw of 726 g/kg. In the current experiment a hemicellulose digestibility of 756 g/kg was obtained. The increased fibre digestibility could have increased the dry
matter intake of Diet 2 which was 18% higher than that of Diet 3. Urea treatment may cause the structural carbohydrate of the cell wall to become more accessible to rumen micro-organisms. Hence urea treatment may increase the digestibility of organic matter and cell wall components. Comparable results with ammoniated wheat straw have also been reported by Cloete et al. (1983).

The crude protein digestibility of Diet 2 (50.2%) was significantly (P<0.05) lower compared to Diet 1 and Diet 3 (62.9 and 64.7%) respectively. However Diet 2 contained a higher proportion of oaten hay than the other diets. Cloete and Kritzinger (1984b) suggested that supplemental urea is more easily degraded by rumen microbes and hence is associated with a high digestibility of protein compared with urea ammoniated straw. Dias Da Silva and Sundstøl (1986) also observed the lower nitrogen digestibility of ammoniated straw compared to straw supplemented with urea at feeding time. As ammonia is more readily absorbed in the hindgut (Hoover 1978), this could partly account for the high level of faecal nitrogen observed in the ammoniated diet and the enhancement of protein synthesis by microflora in the rumen, caecum and colon. Moreover, Canna et al. (1991) found significantly higher total rectal bacterial counts in sheep fed on ammoniated rice straw diet compared to untreated rice straw (which could contribute to the higher faecal nitrogen). This finding was supported by Hassen and Chenost (1992), who observed faecal nitrogen to be composed mostly of bacterial nitrogen and nitrogen bound to undegraded cell walls. More nitrogen was bound to cell walls in ammoniated straw compared to untreated straw (Hassen and Chenost, 1992). The cell walls that are not degraded in the rumen are fermented in the large intestine. This condition may be responsible, in the current study, for the higher faecal nitrogen excretion from sheep fed on Diet 2 rather than on Diet 1. Hassen and Chenost (1992) suggested that the higher excretion of faecal nitrogen in sheep fed on an ammoniated straw diet, might be due to phenolic nitrogenous compounds generated from the ammonia treatment which could be incorporated into the microbes in the rumen, but which are lost in the faeces in a soluble form. A reduction in CP digestibility on urea ensiled straw diets
compared to untreated straw was also reported by Cloete et al. (1983) and compared to urea supplemented straw by Cheva-Isarakul and Jeerachai (1987), and Cloete and Kritzinger (1984b). Cloete and Kritzinger (1984b) obtained a 22.1% decrease in crude protein digestibility when urea ensiled wheat straw was compared with urea supplemented wheat straw. In the current study, the reduction was 22.4%.

*Nitrogen retention of the diets*

In the current study the nitrogen retention was not significantly different between treatments. Lack of significance in nitrogen retention could have been due to the high individual variations recorded in the feeding trial, as indicated by the high standard error (Table 5.5). Differences would have been more likely to be statistically significant if replication was increased.
5.5 Conclusions

Formulating isoenergetic and isonitrogenous rations based on urea ensiled and untreated rice straws supplemented with legumes had similar effects on DMI, DE intake and digestible cell wall components. This suggests that untreated rice straw basal diets supplemented with legume straws or forage legumes offer an alternative method of improving the nutritional value of rice straw as a ruminant feed.

Urea ensiled straw resulted in higher DMI, DE intake, and digestible cell wall components compared to rice straw which was supplemented with urea at feeding time. This suggests that the urea ensiled rice straw is a superior choice for use in ruminant diets. Although ensiled rice straw had a longer preparation time (42 days), compared to urea supplemented straw, the former had a 15% higher DE intake and resulted in a positive N balance.
CHAPTER 6
RUMINAL FUNGI COLONISATION AND DEGRADATION OF STEM
TISSUE OF UNTREATED AND UREA TREATED
RICE STRAWS

6.1 INTRODUCTION

Differences in digestibility were observed between varieties of rice straw and between untreated and urea treated rice straws in the previous experiment in this study (Chapters 3 and 4). This chapter explores a possible reason for these differences.

The proportion of lignified tissue in the plant structure affects the digestibility of roughage (Akin et al., 1986). Utilisation of poor quality, high fibre crop residues by ruminants is enhanced by ruminal fungi (Gordon and Phillips, 1995). Fungi have the ability to colonise lignified cell walls and to weaken fibrous plant tissues in the rumen (Akin et al., 1990b; Akin and Borneman, 1990) and the ability to degrade the structural components of plant cell walls (Gordon and Phillips 1989). When the diet is high in plant cell walls, the ruminal fungi play an important role in the digestibility of fibre in the rumen (Gordon and Phillips, 1995).

Although there are many studies of the fungal colonisation of grass and the straws of other cereals, little attention has been given to rice straw. Therefore the objectives of this study were to investigate the following, using the medium (30%<IVOMD<50%) and low quality (IVOMD<50%) rice straw; (i) ruminal fungi colonisation (ii) tissue stem degradability, and (iii) the effect of urea treatment on fungal colonisation and tissue stem degradability.
6.2 Materials and methods

6.2.1 Animals and diet

Two rumen fistulated South Australian Merino sheep of 50 kg live weight were used for the experiment. The sheep were fed a maintenance ration (1.2 kg DM/day) which consisted of 50% oat chaff and 50% lucerne chaff supplemented with minerals. This was fed twice daily in equal amounts at 0900 and 1700 hours.

6.2.2 Ruminal degradation of test feeds

The stems of the lower part of three varieties of rice straw from Experiment one (Chapter 3) were chosen, representing the highest (Dong variety), the lowest (Yrl variety) and an intermediate (Ilb variety) IVOMD. Urea treatment of rice straw was carried out as described in Chapter 3. Approximately 5mm of 10 cross sections of untreated and urea treated rice straw stem internodes were taken from the central point of the internode below the flag leaf. The material was placed in nylon bags and incubated in the rumen for 24, 48 or 72 hours. Bags were placed in the rumen before feeding at 0900. All bags were withdrawn simultaneously and gently washed under running tap water, prior to the samples being removed with forceps. Control samples were prepared as above, placed in bags, and soaked in distilled water for 30 minutes.

6.2.3 Preparation of samples for SEM

SEM (scanning electron microscopy) moves electrons across the specimen with scanning coils. The electrons then enter a collector and form a magnified image on a TV screen. Sample preparation was according to the method of Akin et al. (1984). The material from each bag was placed in separate tubes containing glutaraldehyde (4% v/v) in cacodylate buffer (0.1 M; pH 7.4). After 6-8 days of fixation at 4°C, samples were rinsed with the same buffer mixed with 4% sucrose. Samples were then fixed with 1.5% OsO4 (in 0.1M cacodylate buffer pH 7.4) for 4 hours and dehydrated serially in acetone (70, 90, 95, 100% for 30 minutes each).
6.2.4 Counting of ruminal fungi

Longitudinal sections of samples were observed using a Philips XL 20 scanning electron microscope (SEM), and printed on video print. All video images were captured at the same magnification with a data bar of 500 μm. At least two samples per incubation time and two areas per sample were evaluated. Sporangia that were attached to plant material within the delineated areas were counted. The presence of sporangia was verified by comparing incubated and unincubated materials. The ruminal fungi population per cm² was calculated by dividing the total number of sporangia by the total area and multiplying by 100.

6.3 Results

6.3.1 Ruminal fungi colonisation

The number of fungi colonising treated and untreated rice straw internodes are presented in Table 6.1. These results show that, for untreated samples sporangia of ruminal fungi had colonised the untreated Dong and Yrl varieties after samples had been incubated for 48 and 72 hours. Fungi were absent or were present in extremely small numbers, in samples of both varieties after incubation for 24 hours. However, in the intermediate IVOMD variety, Ilb, sporangia were present after 24 and 72 hours incubation, but not after 48 hours.

Urea treatment reduced the time required for fungi to colonise the stem tissue. Urea treated samples of all varieties had been colonised by ruminal fungi after 24 hours of incubation. Urea treatment increased the fungal population of the Dong variety. The internode of treated Dong straw had the highest fungal population at 24 and 48 hours incubation. In 2 of 6 cases fungal population decreased after 72 hours of incubation. Ruminal fungi colonised the thick cell walls of sclerenchyma and small vascular bundles of rice straw internodes (Fig 6.1). Lack of ruminal fungi colonization of Yrl variety are shown in Fig 6.2, in contrast to colonisation in Dongara and Illabong varieties.
Table 6.1 Fungal sporangia/cm² on rice straw stems before and after treatment with urea (mean of at least two observations)

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Treatments</th>
<th>Incubation time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Dong</td>
<td>untreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>urea treated</td>
<td>2.1</td>
</tr>
<tr>
<td>Ilb</td>
<td>untreated</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>urea treated</td>
<td>0.22</td>
</tr>
<tr>
<td>Yrl</td>
<td>untreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>urea treated</td>
<td>0.5</td>
</tr>
</tbody>
</table>

6.3.2 Digestion of stem tissues

The structure of stem tissues of the three varieties of rice straw which were not incubated in the rumen were similar. The stem tissue structure consisted of two circles of vascular bundles (Fig 6.3). Fig. 6.3 shows the rigid lignified tissues of the epidermis, the vascular bundles and sclerenchyma tissue.

After 8 hours incubation in the rumen there were no apparent differences in the stem tissue structure between untreated and treated straw of Ilb variety (Fig. 6.4). On the other hand, the parenchyma tissue of treated Dong straw was under attack by rumen micro-organisms. In untreated Yrl, the inner epidermis and parenchymal tissue were beginning to be digested by rumen micro-organisms whereas digestion had not yet begun in the treated form (Fig. 6.4).

After 24 hours of incubation the parenchymal tissue of untreated rice straw of all varieties was completely degraded, leaving the tissue residue made up of vascular bundles, the sclerenchymal ring and the epidermis (Fig. 6.5). For treated straw, parenchymal and lignified tissues of large vascular bundles were also completely
Fig. 6.1 Scanning electron microscopy of ruminal fungi colonisation on the internodes of rice straw

A. Ruminal fungi (arrow) colonised rice straw after incubation in the rumen for 24 hours (magnification 70x).

B. Ruminal fungi (arrow) colonised substrate after incubation in the rumen for 72 hours (magnification 234x).

C. Ruminal fungi (arrow) colonised lignified tissue of the small vascular bundles and schlerenchyma (magnification x162).

(S, schlerenchyma; OV, outer vascular bundles)
Fig. 6.2  Scanning electron microscopy of a longitudinal section of the internodes of rice straw varieties after incubation in the rumen for 24 hours, with the presence (arrow) or absence of ruminal fungi

A. Ruminal fungi (arrow) present in extremely small numbers on untreated Dongara variety.

B. Ruminal fungi (arrow) colonisation of urea treated Dongara variety

C. Ruminal fungi (arrow) colonisation of untreated Illabong variety

D. Ruminal fungi (arrow) colonisation of urea treated of Illabong variety

E. Ruminal fungi absent on untreated Yrl variety.

F. Ruminal fungi (arrow) colonisation of urea treated Yrl variety.
degraded by rumen micro-organisms. The remaining tissue residues were small vascular bundles, sclerenchyma and cutinised epidermis.

After 48 hours incubation there was no difference in the extent of degradation of stems between untreated and urea treated straw (Fig. 6.6). The parenchymal tissue and large vascular bundles had been completely degraded by rumen micro-organisms. The remaining tissue residues were small vascular bundles, sclerenchymal rings and epidermis. After 72 hours incubation, those residues were still undegraded.

6.4 Discussion

*Ruminal fungi colonisation*

Of the varieties tested (Chapter 3 and 4), the lower part of the straw from Dong had the highest IVOMD and DM degradability. The number of ruminal fungi colonising untreated Dong and IIb samples was also higher than that for Yrl, which has a lower IVOMD than either of these varieties. In addition, the fungal population was higher in the treated, than the untreated, straw. These results suggest that there is a tendency for ruminal fungi to colonise the more digestible straw in higher numbers. The results on ruminal fungi population in this study could not be statistically analysed because there was no sample replication for sheep. Even though the sheep were fed on a similar diet it appeared that there were differences in their preferences for different parts of the diet. The differences in the ruminal fungal population could therefore be due to differences between sheep or to the substrate. Therefore, more work needs to be done to make comparisons between sheep and between diets.

The ruminal fungi population in the untreated Dong variety was extremely small after the straw had been incubated in the rumen for 24 hours, while that of urea treated IIb was higher after the same incubation time (Table 6.1), even though the IVOMD and DM degradability of Dong were higher than those of IIb (Chapter 3 and 4).
Fig. 6.3 Scanning electron microscopy of a cross section of the internode of rice straw varieties before incubation in the rumen

A. Dongara variety.

B. Illabong variety.

C. Yrl variety.

(E, epidermis; IV, inner vascular bundles; OV, outer vascular bundles; P, parenchyma; S, schlerenchyma)
Fig. 6.4  Scanning electron microscopy of a cross section of stem internodes of untreated and urea treated rice straw varieties after incubation in the rumen for 8 hours

A. Untreated Dongara variety
B. Treated Dongara variety
C. Untreated Illabong
D. Treated Illabong
E. Untreated Yrl
F. Treated Yrl

(E, epidermis; IV, inner vascular bundles; OV, outer vascular bundles; P, parenchyma; S, sclerenchyma)
Fig. 6.5  Scanning electron microscopy of a cross section of stem internodes of untreated and urea treated rice straw varieties after incubation in the rumen for 24 hours

A. Untreated Dongara variety

B. Treated Dongara variety

C. Untreated Illabong

D. Treated Illabong

E. Untreated Yrl

F. Treated Yrl

(E, epidermis; IV, inner vascular bundles; OV, outer vascular bundles; P, parenchyma; S, schlerenchyma).
Fig. 6. Scanning electron microscopy of a cross section of stem internodes of untreated and urea treated rice straw varieties after incubation in the rumen for 48 hours

A. Untreated Dongara variety

B. Treated Dongara variety

C. Untreated Illabong

D. Treated Illabong

E. Untreated Yrl

F. Treated Yrl

(E, epidermis; OV, outer vascular bundles)
This indicates that tissue degradation may have occurred in the presence or absence of ruminal fungi. This might indicate that the fungal population was not as active in fibre digestion as bacteria, and agrees with the conclusion of Windham and Akin (1984). They observed that the most active fibre-digesting micro-organisms in the rumen were the bacteria. With a higher population of sporangial fungi in the rumen fluid, following the addition of streptomycin and penicillin to eliminate rumen bacteria, the digestibility of fibre was lower than that of untreated rumen fluid. In an in vitro study, however, Akin et al. (1983) observed that ruminal fungi could remove 62% of the forage material in the absence of bacteria.

Ruminal fungi preferentially colonised lignified tissues of sclerenchyma and the small vascular bundles (Fig. 6.1). Similar results have been observed by Grenet and Barry (1988) and Horn et al. (1989). In spite of this, these walls did not degrade significantly, as can be observed in Figs. 6.5 and 6.6. Akin et al. (1986) also reported similar results for the leaf tissues of grass. Therefore, the development of large numbers of sporangia on fibre may not indicate that ruminal fungi have a substantial role as a forage digester.

Urea treatment reduced the time required for fungi to colonise stem tissue. All urea treated samples had been colonised by ruminal fungi after 24 hours of incubation. However, only with the Dong variety was the number of fungi in the treated straw higher at all incubation times compared to untreated straw (Table 6.1). On the other hand, the IVOMD and DML (at all incubation times) of all rice straw varieties was increased after urea treatment (Chapters 3 and 4). These results indicate that digestibility increased even in the absence of ruminal fungi colonisation. Grenet and Barry (1988) also observed that colonisation by anaerobic rumen fungi did not appear to be modified by ammonia treatment, the untreated samples being colonised in the same manner as the treated samples.
**Stem tissue degradation**

No differences were observed in the stem tissue structures between rice straws of different varieties. Even though the IVOMDs of the three untreated varieties in this study were significantly different (Fig. 3.6), there were no visual differences in tissue structure (Fig. 6.1). Similar results have been observed by Akin et al. (1984) in the stem tissues of *panicum* species and by Akin et al. (1986) in the leaf of Normal-12 and Brown midrib-12 sorghum. The latter had higher digestibility because it had a lower content of *p*-coumaric acid and lignin than the normal species. Differences in digestibility can not be explained by the tissue structure, because the lignin component is the most important factor that determines digestibility of the cell wall (Jung, 1989). In the current study, the lignin components of the straw varieties were not measured. Thus further work needs to done to determine whether visual differences between varieties in the components of the lignin could explain the differences in digestibility that were observed in the IVOMD study.

There were no distinct differences between untreated and urea treated straws in the degradation of stem tissues after 24, 48 or 72 hours incubation. In contrast, Nakashima et al. (1991) found differences in degradation between untreated and NaOH treated straw: stem tissues of NaOH treated material were degraded faster than those of untreated straw. Because tissue degradation was similar between treated and untreated tissues in the current study, urea treatment appears to be weaker than NaOH treatment. However, the stem tissue of treated straw was degraded more extensively than untreated straw after 24 hours incubation. This could be due to chemical treatment resulting in a cleavage of lignin-polysaccharide bonds, which, in turn, would aid the solubilisation of exposed lignin and would act to build up an inert layer, allowing more of the wall to be degraded (Chesson 1988).

After untreated samples had been incubated in the rumen for 48 hours, parenchyma and large vascular bundle tissues were completely degraded. In contrast Akin et al. (1984) studied 8 varieties of grass and observed that the sclerenchymal ring, part of
the parenchyma and the vascular bundles remained undegraded after samples were incubated \textit{in vitro} for 6, 24 and 48 hours. These results indicate that rice straw stem may be more digestible than grass stem.

Evaluation using SEM showed that small vascular bundles, sclerenchymal tissue and the epidermis of all varieties were not degraded after 72 hours of incubation. Similar results were obtained by Nakashima \textit{et al.} (1991). This lack of degradation is because these tissues are highly lignified, and therefore resistant to degradation by rumen micro-organisms (Akin, 1988).

6.4. Conclusions
Stem tissue degradation was similar for all varieties. Urea treatment increased the extent of degradation after 24 hours incubation, but not after 48 or 72 hours.
CHAPTER 7
GENERAL DISCUSSION

This chapter presents a summary of the results from the series of experiments performed to assess the nutritive value of the straw of various rice varieties and the methods for improving rice straw quality. The experiments included studies of chemical composition and digestibility (in vitro, in sacco and in vivo), and observations using scanning electron microscopy (SEM) to obtain visual evidence of stem tissue degradation by rumen micro-organisms and of ruminal fungi colonisation on the stem tissue.

7.1 Chemical composition

Chemical composition (N, NDF, ADF, HC, lignin, silica) varied widely between parts and varieties (Chapter 3). The variation within each rice straw variety (between upper and lower parts) could have been caused by differences in the proportion of botanical fractions (leaves and stem). The nitrogen content in the upper part was higher than in the lower part in all varieties, due to the higher proportion of leaves in the former (63.5% compared to 57.8%). In addition the variation in chemical composition between rice straw varieties was probably due to genetic differences. Urea treatment consistently increased the nitrogen content of both parts in all varieties and was more effective in varieties with lower initial N. The increase in N content after urea treatment was higher in the lower part. The effect of urea treatment on other chemical components was not consistent between parts and varieties. This evidence suggests that chemical composition has a limited usefulness in assessing the nutritive value of rice straw and the effect of urea treatment. However, when the main effect of urea treatment was considered, urea treatment significantly decreased NDF and HC contents, and increased those of ADF and lignin. This is because urea treatment causes partial solubilisation of HC (Kiangi et al., 1981; Givens et al., 1988; Mason et al., 1988). The HC is probably
rendered soluble and enters the neutral detergent solution (Van Soest et al., 1983; Mason et al., 1988). The decrease in HC content results in increased cellulose and lignin contents (Givens et al., 1988; Mason et al., 1988). The increase in ADF and the decrease in NDF in urea treated straw resulted in a drop in apparent HC (Van Soest et al., 1983).

7.2 IVOMD
For untreated straw, IVOMD varied within and between varieties. Most of the lower parts had a higher IVOMD than the upper parts for all varieties except IIb and Yrl. The higher IVOMD in the lower part is caused by a higher proportion of stem than in the upper part (42.3% vs 14.5% respectively). The lower parts of all varieties except, Yrl and Pld, are classified as medium quality (40%<IVOMD<50%), whereas, the upper parts of all varieties, except IIb, are classified as low quality (30%<IVOMD<40%) (Ibrahim et al., 1989). The IVOMD of both parts in all varieties increased after treatment with urea. This is due to the ammonium hydroxide released during urea treatment causing the cleavage of the alkali-labile linkages between lignin and structural carbohydrate. This causes subsequent increase in fibre digestibility (Hartley and Jones, 1978). The increase in IVOMD after urea treatment was higher in the upper part than the lower part, and the response was higher when the original straw quality was low. This suggested that the digestibility of lower quality rice straw, as well as its N content, could be improved efficiently by ensiling with urea. The ability of IVOMD measurements to distinguish between the nutritive value of parts and varieties, and subsequent improvement after urea treatment, suggest that an IVOMD value is a more reliable means than chemical analysis of assessing the nutritive value of rice straw.

An attempt was made to correlate IVOMD and chemical composition in order to determine whether any chemical components had a close relationship with the IVOMD. The linear regressions between IVOMD and N, NDF, ADF, HC, lignin and silica resulted in low determinant coefficients. This indicates that none of these
chemical components could be used as a reliable parameter to predict digestibility, even though the N content and IVOMD consistently increased after urea treatment. Multiple regression analysis indicated that the IVOMD was a function of N, HC, lignin and silica contents, with a determinant coefficient of 0.6. This suggests that only 60% of IVOMD was accounted for by these components.

7.3 Rumen degradability
The differences in the degradability of rice straw within and between treatments (Chapter 4) showed a similar trend to the IVOMD values, in that the degradability of the lower part was higher than that of the upper part except, for IIb, and the lower part of the Dong variety had the highest degradability. Urea treatment increased the dry matter loss (DML) at each incubation time. The improvement in DML, due to urea treatment, was indicated by the higher potential degradability and the higher rate of degradation constant in the treated straw than untreated straw. The increase in degradability due to urea treatment was greater in the upper part than the lower part. The results suggest that varieties containing more leaves might have a greater response to urea treatment than varieties containing more stem.

7.4 Feeding trial
Isoenergetic and isonitrogenous diets were formulated using untreated rice straw, urea ensiled rice straw and urea supplemented rice straw. Diets were compared to determine the best way to improve the feeding value of rice straw (Chapter 5). Urea ensiled rice straw diets had higher DMI per metabolic body weight than the urea supplemented diet. This was due to an improvement in the palatability of urea ensiled rice straw (Lawlor and O'Shea, 1979; Cloete and Kritzinger, 1983). Urea ensiled rice straw had a similar DMI per metabolic body weight, DE intake and apparent digestibility of NDF, to untreated rice straw diet. The supplementation of rice straw with legumes contributed protein and fermentable energy to the rumen in the form of available cellulose and hemicellulose both of which are known to stimulate fibre digestion (Silva and Orskov, 1985). Legumes also stimulated dry
matter intake of the diet. This result suggests that forage legume supplementation had a similar effect to urea ensilage on the feeding value of rice straw.

There were no significant differences in nitrogen retention between the three diets. The lack of significant difference between treatments in nitrogen retention in the present study could be due to a high individual variation between sheep during the feeding trial.

7.5 Ruminal fungi colonisation and stem tissue structure

No clear tendency was observed in the populations of ruminal fungi colonising untreated straw internodes (Table 6.1). In contrast, there were differences in the IVOMD and dry matter degradability between the three varieties studied (Chapters 3 and 4). However, urea treatment increased fungal population only in the Dong variety. In contrast, urea treatment increased the digestibility of all varieties. This result suggests that the fungal population was not as active as the rumen bacteria in fibre digestion. Even though rumen fungi colonised the lignified tissue of the schlerenchyma and small vascular bundles, these tissues were not degraded significantly. Therefore, the development of large numbers of sporangia on fibre may not indicate that ruminal fungi had a substantial role as a forage digester.

There were no differences in the structure of stem tissue between the rice straw varieties studied (Fig. 6.3), even though the IVOMD of the three untreated straw varieties was significantly different. Thus, these differences in digestibility could not be explained by the tissue structure. This could be explained by the fact that the most important factor determining digestibility is the phenolic component of lignin (Jung, 1989). The stem tissue of treated straw degraded more extensively than that of untreated straw after 24 hours incubation. This could be due to chemical treatment resulting in a cleavage of lignin-polysaccharide bonds, which, in turn,
would aid the solubilisation of exposed lignin and would act to build up an inert layer, allowing more of the wall to be degraded (Chesson, 1988).

Evaluation by SEM after 8, 24, 48 or 72 hour incubation showed that small vascular bundles, sclerenchyma tissue and epidermis were not degraded in any varieties. This finding relate the report by Akin (1988) that these tissues are highly lignified.

The following conclusions can be drawn from the above experiments:

- the chemical composition, IVOMD and rumen degradability of rice straw varied between part and between varieties. The N content of the upper part was higher than in the lower part in all varieties. There were no consistent results with other chemical components. *In vitro* organic matter and rumen degradability of the lower part was higher than in the upper part, except for the Ilb variety.

- urea treatment consistently increased the N content, IVOMD and dry matter degradability. The increase in IVOMD due to urea treatment was higher in the original low quality rice straw than medium quality, therefore, urea treatment is more beneficial for use with low quality rice straw to improve its nutritional quality. Rice straw varieties with higher nutritive value are better used as a ruminant feed in the untreated form because the increase after treatment was not as great as that observed for low quality straw.

- IVOMD is a more appropriate method than chemical composition for evaluating the nutritive value of rice straw varieties and the effect of urea treatment.

- The variation in the IVOMD between rice straw varieties could not be explained by using chemical composition as a single factor, nor by ruminal fungi colonisation or from the structure of stem tissue degraded in the rumen.

- Supplementation of untreated rice straw with forage legume or urea ensiled rice straw is able to improve the feeding value of rice straw for ruminants.
In order to fully assess the nutritive value of rice straw, a number of areas remain to be addressed further. These are outlined below:

- It appears from the above experiment and literature review that lignin and the lignin carbohydrate complex have a significant influence on the digestibility of rice straw. The lignin components of the straw varieties were not determined, due to time and financial constraints. Further work needs to be carried out to determine whether lignin components can explain the differences in digestibility between varieties that were observed in the IVOMD study.

- The ruminal fungi colonisation result may have been influenced by the variations between individual sheep. However, the study was not replicated in the number of sheep used. This should be carried out in order to validate the results obtained.
Appendix I  The chemical composition and IVOMD of the upper and lower part of rice straw varieties either untreated or treated with urea (g/kg dry matter).

<table>
<thead>
<tr>
<th>Variety</th>
<th>Part</th>
<th>Treatment</th>
<th>N</th>
<th>NDF</th>
<th>ADF</th>
<th>Lignin</th>
<th>Silica</th>
<th>HC</th>
<th>IVOMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dongara</td>
<td>upper</td>
<td>Untreated</td>
<td>10.3</td>
<td>738.6</td>
<td>534.2</td>
<td>59.6</td>
<td>138.4</td>
<td>204.4</td>
<td>357.4</td>
</tr>
<tr>
<td></td>
<td>lower</td>
<td>Untreated</td>
<td>7.4</td>
<td>653.2</td>
<td>484.8</td>
<td>49.5</td>
<td>101.5</td>
<td>168.4</td>
<td>498.4</td>
</tr>
<tr>
<td></td>
<td>upper</td>
<td>Treated</td>
<td>18.6</td>
<td>705.5</td>
<td>548.9</td>
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N, nitrogen; NDF, acid detergent fibre; ADF, acid detergent fibre; HC, hemicellulose; IVOMD *in vitro* organic matter digestibility
Appendix 2  Dry matter degradability and degradation characteristics constant of the upper and lower parts of four varieties rice straw either untreated or treated with urea

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a, immediately soluble material; b, insoluble but degradable material; c, the rate of degradation of b
REFERENCES


