# THE USE OF MULTIVARIATE STATISTICS TO EVALUATE THE RESPONSE OF RICE STRAW VARIETIES TO CHEMICAL TREATMENT

J. Vadiveloo

Australian Matriculation Programme, MARA Institute of Technology Section 2, 40000 Shah Alam, Malaysia

## Summary

Multivariate statistical procedures were used to analyse data on the chemical composition and *in vitro* digestibility of four varieties of rice straw after treatment with 4% NaOH solution, 4% urea solution or distilled water (control) for 48 hours. For each treatment, stepwise discriminant analysis identified the variables which maximised differences between varieties and the eigenvectors from principal component analysis quantified the contribution of these criterion variables to varietal differences. The overall response of varieties to chemical treatment was demonstrated qualitatively, by cluster analysis, and quantitatively, from the magnitude of the principal component scores. The analyses revealed that the urea and control treatments elicited the same response whereas NaOH had the greatest effect on the poorest straw variety. Similar analyses conducted on the botanical fractions of the varieties showed that the relative response of the inflorescence, stem, leaf blade and leaf sheath fractions was not altered by chemical treatment.
(**Key Words** : Multivariate, Rice Straw, Urea, NaOH)

## Introduction

Multivariate statistical analysis is a technique in which the effect of several criterion variables are evaluated simultaneously (Bray and Maxwell, 1985). Multivariate analysis thus accomodates correlations between criterion variables if they exist or are expected. A range of multivariate statistical procedures are available, including procedures which investigate variables without designating some as dependent or independent, procedures which treat data with one classification variable and several quantitative variables and procedures for grouping variables or observations based on their similarities to each other.

Varietal differences in the nutritive value of rice straw (Bainton et al., 1991; Vadiveloo, 1992) provide an opportunity for employing varietal selection as a method of improving straw quality. To compare this method with chemical treatment, the nutritive value of four rice straw varieties were compared before and after treatment with sodium hydroxide and urea. Sodium hydroxide and urea were selected because these chemicals are commonly used to upgrade the quality of rice straw (Vadiveloo, 1986; Tuen et al., 1991; Mgheni et al., 1993). Data were analysed using univariate and multivariate procedures to demonstrate the value of multivariate analysis as an experimental tool.

## Materials and Methods

### Rice straw

Four varieties of rice straw, MR 1, MR 71, MR 84 and MR 27, hand-harvested, field dried and separated into inflorescence, stem, leaf blade and leaf sheath were used. Their relative proportions by weight in the whole straw had been previously estimated (Vadiveloo, 1992).

Each fraction was cut into 2-3 cm portions and 50 gm of each fraction was placed inside a plastic bag to which was added either 200 ml of distilled water (control), 200 ml of 4% (w/w) sodium hydroxide solution or 200 ml of 4% (w/w) urea solution. Each plastic bag was then sealed and left for 48 hours at room temperature after which the samples were washed with water, oven-dried at 60℃ and ground through a 1 mm sieve in a hammer mill.

### Chemical composition and *in vitro* digestibility

The ground samples of each fraction were analysed in duplicate for crude protein, CP (AOAC, 1984) and neutral

[1]Address reprint requests to Dr. J. Vadiveloo, Australian Matriculation Programme, MARA Institute of Technology Section 2, 40000 Shah Alam, Malaysia.

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detergent fibre (NDF), hemicellulose, cellulose, lignin, insoluble ash and silica (Goering and Van Soest, 1970). *In vitro* digestibility (IVD) was estimated by the pepsin-cellulase method of Jones and Hayward (1975). Enzymes were obtained from the Sigma Chemical Co (pepsin, 1: 10,000) and Yakult Biochemicals Co. (cellulase, Onozuka 3S). The chemical composition and IVD of each variety was then calculated from the relative proportion, composition and IVD of its component fractions.

### Univariate statistical analysis

One-way analysis of variance (Snedecor and Cochran, 1967) was used to compare the effect of chemical treatment between and within straw varieties and fractions.

### Multivariate statistical analyses

Stepwise discriminant analysis (Bray and Maxwell, 1985) was carried out to identify the variables (IVD and seven chemical components) which maximised differences between the 4 varieties and between the 4 fractions within each treatment ($n = 8$). A significance level of 15% and a squared partial correlation of 0.85 were the selection criteria for entry into, or removal from, the discriminant model. Stepwise discriminant analysis also provided tests of significance for multivariate analysis of variance. Pillai's Trace was the test of significance selected to examine the overall hypothesis of no difference between means when all variables are considered simultaneously.

Cluster analysis (Aldenderfer and Blashfield, 1984) was used to classify varieties and fractions which were similar, the variables selected from stepwise discriminant analysis being the criterion variables. Data were standardized (mean = 0, standard deviation = 1) and no outliers were trimmed. Clusters were formed by agglomerative hierarchical clustering and combined at each step by the method of complete linkage. The clustering solution was summarized in a tree diagram from which a two-cluster solution of the data, representing a 50% reduction in the number of varieties and fractions, was tabulated.

Principal component analysis (Marriott, 1974) was carried out to quantify the contribution of each selected variable to the differences between varieties and between fractions and to ascertain the rank order of varieties and fractions before and after chemical treatment. The magnitude of the eigenvectors and the sign and magnitude of the principal component scores were examined to determine contribution and ranking, respectively.

All analyses were run on a SAS Version 6 (1987) statistical package.

## Results

### Chemical composition and *in vitro* digestibility

The effects of chemical treatment on the composition and IVD of the straw varieties are shown in table 1. Compared to the control, NaOH treatment significantly reduced the content of all chemical components but significantly increased IVD. However, the effect of urea treatment on composition and IVD was small. Between varieties within treatments, differences were generally significant ($p < 0.01$ or $p < 0.001$).

The effects of chemical treatment on the composition and IVD of straw fractions are shown in table 2. Compared to the control, the effect of NaOH was large but the effect of urea was small. Between fractions, CP and lignin contents differed significantly ($p < 0.01$) only after treatment.

### Multivariate statistical analyses

Multivariate analysis of variance rejected the null hypothesis of no differences between varieties and fractions; Pillai's Trace was significant ($p < 0.001$) for all treatments. The variables selected by stepwise discriminant analysis are shown in table 3. Between varieties, the only common discriminant variable for all treatments was CP; for urea and control treatments, silica and IVD were also common discriminants.

An examination of the selected variables in table 1 for the NaOH treatment showed that MR 27 had the lowest NDF and lignin contents but the highest CP content amongst the four varieties. Consequently, MR 27 clustered separately in the two cluster solution (table 3 and figure 1). Similarly, an examination of the selected variables in table 1 showed that MR 1 had the lowest silica and highest CP and IVD values for the urea and control treatments. Consequently, MR 1 clustered separately from the remaining three varieties (table 3 and figure 1).

Between fractions, the only common discriminant variable for all three treatments was silica (table 3). For the NaOH treatment, the inflorescence and stem had high NDF and lignin and low CP contents; for the urea treatment, these fractions had the lowest hemicellulose and silica and the highest lignin contents, and for the control, the lowest ash and silica contents, intermediate values for IVD, and extreme values for NDF. The two-cluster solution (table 3 and figure 2) reflected the relative characteristics of the inflorescence and stem compared to the blade and sheath.

The results of principal component analysis are shown in table 4. Between varieties, the first component accounted for 63%, 93% and 84% of the standardised

TABLE 1. COMPOSITION (% IN DM) AND IVD (%) OF TREATED AND UNTREATED RICE STRAW VARIETIES

| Parameter | Treatment | Variety | | | | |
|---|---|---|---|---|---|---|
| | | MR 84 | MR 27 | MR 1 | MR 71 | SE diff |
| Neutral detergent fibre | NaOH | 43.1 | 40.1 | 45.5 | 44.8 | 0.25*** |
| | Urea | 66.4 | 66.5 | 67.3 | 68.2 | 0.54NS |
| | Control | 68.1 | 66.5 | 67.1 | 68.2 | 0.43** |
| | SE diff | 0.18*** | 0.25*** | 0.77*** | 0.19*** | |
| Hemicell | NaOH | 11.7 | 10.7 | 12.1 | 11.9 | 0.19** |
| | Urea | 26.8 | 26.7 | 25.5 | 27.3 | 0.22** |
| | Control | 28.3 | 26.9 | 26.6 | 27.3 | 0.17** |
| | SE diff | 0.15*** | 0.24*** | 0.15*** | 0.21*** | |
| Cellulose | NaOH | 27.9 | 27.6 | 31.5 | 29.3 | 0.46** |
| | Urea | 32.4 | 32.8 | 37.4 | 32.8 | 0.56** |
| | Control | 32.7 | 32.2 | 35.8 | 32.8 | 0.10NS |
| | SE diff | 0.13*** | 0.11* | 0.70** | 0.57* | |
| Lignin | NaOH | 3.5 | 1.8 | 2.2 | 3.7 | 0.34* |
| | Urea | 7.1 | 7.0 | 6.0 | 8.2 | 0.42* |
| | Control | 7.2 | 6.1 | 4.9 | 8.2 | 0.39** |
| | SE diff | 0.10*** | 0.30*** | 0.46** | 0.53** | |
| Insoluble ash | NaOH | 3.2 | 5.4 | 1.5 | 3.3 | 0.11*** |
| | Urea | 5.9 | 7.3 | 4.1 | 6.6 | 0.51* |
| | Control | 6.0 | 7.5 | 4.0 | 6.6 | 0.13*** |
| | SE diff | 0.10*** | 0.13*** | 0.58* | 0.14*** | |
| Silica | NaOH | 2.9 | 4.0 | 1.1 | 3.1 | 0.08*** |
| | Urea | 5.6 | 6.6 | 2.9 | 6.4 | 0.12*** |
| | Control | 5.7 | 6.8 | 2.7 | 6.4 | 0.25*** |
| | SE diff | 0.05*** | 0.21** | 0.17** | 0.19*** | |
| Crude protein | NaOH | 4.4 | 5.2 | 2.6 | 4.4 | 0.04*** |
| | Urea | 6.2 | 6.0 | 3.7 | 5.6 | 0.12*** |
| | Control | 7.4 | 6.1 | 3.5 | 5.0 | 0.04*** |
| | SE diff | 0.12*** | 0.05*** | 0.04*** | 0.04*** | |
| IVD | NaOH | 74.1 | 69.7 | 83.6 | 76.0 | 0.19*** |
| | Urea | 32.0 | 28.6 | 39.9 | 34.8 | 0.19*** |
| | Control | 28.0 | 23.6 | 36.9 | 32.9 | 0.05*** |
| | SE diff | 0.21*** | 0.09*** | 0.09*** | 0.20*** | |

NS = not significant, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
Hemicell = hemicellulose.

variance, respectively, for the NaOH, urea and control treatments. An examination of the magnitude of the eigenvectors for the NaOH treatment showed that CP and NDF were relatively more important than lignin in discriminating between varieties. An examination of the principal component scores for the NaOH treatment showed that only MR 27 had a negative score; the ranking of varieties based on the magnitude of the scores was MR 27 > MR 84 > MR 71 > MR 1. The magnitude of the eigenvectors for the urea and control treatments was similar; the principal component score for MR 1 was negative and the ranking beween varieties for both these treatments was the same, MR 1 > MR 71 > MR 84 > MR 27.

Between fractions, the first principal component accounted for 83%, 63% and 55% of the standardised variance for the NaOH, urea and control treatments, respectively. The magnitude of the eigenvectors for the

TABLE 2. COMPOSITION (% IN DM) AND IVD (%) OF TREATED AND UNTREATED RICE STRAW FRACTIONS

| Parameter | Treatment | Fraction | | | | |
|---|---|---|---|---|---|---|
| | | Inflor | Stem | Leaf blade | Leaf sheath | SE diff |
| Neutral detergent fibre | NaOH | 44.3 | 47.1 | 40.9 | 43.9 | 2.14$^{NS}$ |
| | Urea | 62.3 | 72.3 | 65.7 | 67.7 | 3.38* |
| | Control | 65.0 | 72.5 | 65.8 | 67.8 | 3.58$^{NS}$ |
| | SE diff | 5.74** | 1.29*** | 1.27*** | 1.49*** | |
| Hemicell | NaOH | 14.3 | 10.3 | 11.5 | 12.0 | 0.72*** |
| | Urea | 25.1 | 24.8 | 28.7 | 26.5 | 1.34* |
| | Control | 25.5 | 25.9 | 29.7 | 26.5 | 1.55* |
| | SE diff | 2.13*** | 0.91*** | 0.77*** | 0.57*** | |
| Cellulose | NaOH | 26.5 | 34.5 | 26.0 | 30.2 | 2.28** |
| | Urea | 29.3 | 39.8 | 30.2 | 36.5 | 2.28*** |
| | Control | 31.8 | 39.2 | 30.2 | 34.9 | 2.26** |
| | SE diff | 3.64$^{NS}$ | 1.86* | 0.95*** | 1.74** | |
| Lignin | NaOH | 3.5 | 2.2 | 3.5 | 1.9 | 0.49** |
| | Urea | 7.6 | 7.7 | 6.9 | 6.0 | 0.60* |
| | Control | 7.7 | 7.4 | 6.0 | 6.4 | 0.85$^{NS}$ |
| | SE diff | 0.59*** | 0.36*** | 0.67*** | 0.91*** | |
| Insoluble ash | NaOH | 0.6 | 0.5 | 3.5 | 6.1 | 0.74*** |
| | Urea | 2.3 | 3.4 | 6.7 | 8.2 | 0.87*** |
| | Control | 2.8 | 2.8 | 6.8 | 8.4 | 0.81*** |
| | SE diff | 0.64** | 0.30*** | 1.17* | 0.87* | |
| Silica | NaOH | 0.5 | 0.3 | 3.3 | 4.6 | 0.58*** |
| | Urea | 2.0 | 2.8 | 6.3 | 6.8 | 0.76*** |
| | Control | 2.2 | 2.4 | 6.4 | 7.1 | 0.79*** |
| | SE diff | 0.58* | 0.32*** | 1.18* | 0.48*** | |
| Crude protein | NaOH | 4.1 | 2.8 | 4.8 | 4.1 | 0.56** |
| | Urea | 6.6 | 4.6 | 6.3 | 4.2 | 0.68** |
| | Control | 6.1 | 5.0 | 6.1 | 5.0 | 1.23$^{NS}$ |
| | SE diff | 0.71** | 1.14$^{NS}$ | 0.61* | 0.93$^{NS}$ | |
| IVD | NaOH | 77.0 | 78.4 | 74.5 | 75.8 | 3.44$^{NS}$ |
| | Urea | 30.5 | 33.6 | 33.0 | 34.7 | 4.03$^{NS}$ |
| | Control | 27.4 | 33.1 | 26.1 | 33.3 | 4.41$^{NS}$ |
| | SE diff | 6.53*** | 2.79*** | 3.14*** | 2.09*** | |

$^{NS}$ = not significant, $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
Hemicell = hemicellulose. inflor = inflorescence.

NaOH treatment was similar; inflorescence and stem recorded negative scores. An examination of the magnitude of the eigenvectors for the urea and control treatments showed that CP and IVD were respectively less important than the other selected variables in discriminating between fractions. Inflorescence and stem also recorded negative scores for these treatments.

## Discussion

The objective of this study was to compare the response of straw fractions and varieties to each treatment. Several parameters were measured and first evaluated singly by univariate analysis of variance (table 1 and table 2). This procedure is useful for providing basic information on the mean response of varieties and fractions but is limited by the inherent assumption that each variable is independent and has equal discriminating value. Since no single parameter can adequately explain the nutritive value of feeds (Vadiveloo and Fadel, 1992), multivariate procedures were explored to resolve these concerns.

Significance in multivariate analysis of variance indicated that it was valid to consider all variables simultaneously. Stepwise discriminant analysis then went on to identify the more important discriminating variables (table 3) and principal component analysis to rank them based on the magnitude of their eigenvectors. It was thus possible to assess the relative importance of these variables in explaining differences between varieties and fractions.

TABLE 3. VARIABLES (X) AND TWO-CLUSTER SOLUTIONS OF TREATED AND UNTREATED RICE STRAW VARIETIES AND FRACTIONS

| Class | Treatment | Variable | | | | | | | | Cluster | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NDF | Hemi | Cell | Lignin | Ash | Silica | CP | IVD | 1 | 2 |
| Variety | NaOH | × | | | × | | | × | | MR 84 MR 71 MR 1 | MR 27 |
| | Urea | | | | | | × | × | × | MR 27 MR 71 MR 84 | MR 1 |
| | Control | | | | | × | × | × | × | MR 27 MR 84 MR 71 | MR 1 |
| Fraction | NaOH | × | | | | | × | × | | Inflor Stem | Blade Sheath |
| | Urea | | × | | × | | × | | | Inflor Stem | Blade Sheath |
| | Control | × | | | | × | × | | × | Inflor Stem | Blade Sheath |

Hemi = hemicellulose.
Inflor = inflorescence.
Cell = cellulose.

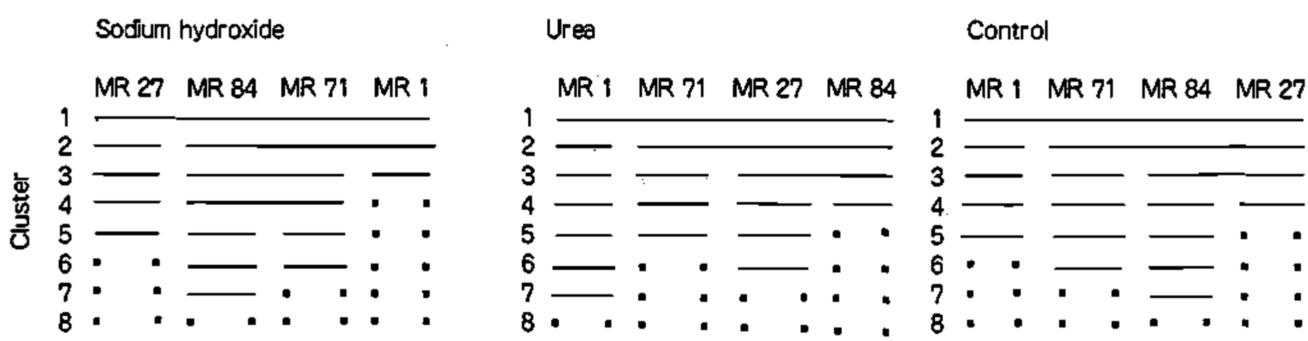
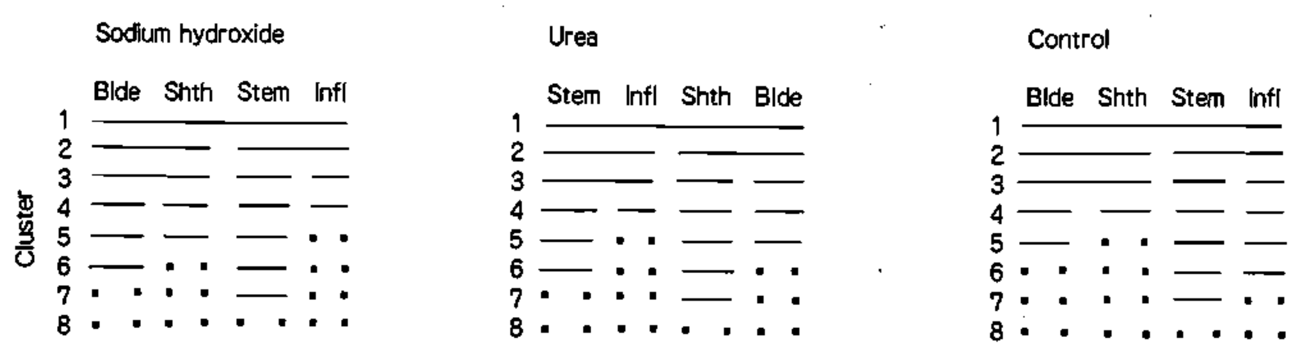



Figure 1. Hierarchical cluster analysis of treated and untreated rice straw varieties.




Blde = blade.
Shth = sheath.
Infl = inflorescence.

Figure 2. Hierarchical cluster analysis of treated and untreated rice straw fractions.

TABLE 4. PRINCIPAL COMPONENT ANALYSIS OF TREATED AND UNTREATED RICE STRAW VARIETIES AND STRAW FRACTIONS : STATISTICS ON THE FIRST PRINCIPAL COMPONENT

| Class | Treat-ment | Variance (%) | Eigenvector | | | | | | Scores | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CP | NDF | Lignin | IVD | Silica | Ash | MR 84 | MR 27 | MR 1 | MR 71 |
| Variety | NaOH | 63.2 | −0.62 | 0.72 | 0.30 | | | | 0.00 | −1.38 | 0.96 | 0.42 |
| | Urea | 92.5 | 0.58 | | | −0.57 | 0.57 | | 0.43 | 0.82 | −1.45 | 0.21 |
| | Control | 84.1 | 0.46 | | | −0.51 | 0.52 | 0.52 | 0.44 | 0.91 | −1.41 | 0.06 |

| Class | Treat-ment | Variance (%) | Eigenvector | | | | | | | Scores | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Hemi | CP | NDF | Lignin | IVD | Silica | Ash | Inflor | Stem | Blade | Sheath |
| Fraction | NaOH | 82.6 | | 0.61 | −0.61 | | | 0.51 | | −0.24 | −1.39 | 1.11 | 0.52 |
| | Urea | 63.0 | 0.48 | −0.21 | | −0.58 | | 0.63 | | −1.04 | −0.80 | 0.72 | 1.11 |
| | Control | 54.5 | | | −0.45 | | −0.24 | 0.61 | 0.60 | −0.38 | −1.35 | 0.95 | 0.78 |

Hemi = hemicellulose.
Inflor = inflorescence.

Cluster analysis provided a qualitative, and principal component scores a quantitative, summary of the response of varieties and fractions to chemical treatment. The results showed that NaOH treatment altered the relative relationship between varieties but not between fractions, the latter because varietal differences within fractions were not extracted in the analysis of the data. However, it is the overall varietal effect (NB varietal parameters were computed from fraction data), which is of practical relevance.

A comparison of the ranking of varieties for the control and NaOH treatments showed that the poorest variety, MR 27, responded best to alkali treatment. Similar responses have been reported with rice cultivars following ammonia treatment by Walli et al. (1988) and by Nakashima and Orskov (1990). Although the overall effect of urea treatment was similar to the control due probably to the short treatment time of 48 hours, MR 27, the poorest variety, recorded the largest improvement in IVD (5% units). Similar observations were made by Colucci et al. (1992) following urea treatment of barley and oat straw varieties.

In conclusion, multivariate statistical methods can be a very useful experimental tool because the methods facilitate objective and unequivocal interpretation of data. They are available in most major statistical programmes such as SAS, SPSS and GENSTAT and are thus readily accessible to researchers.

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### Literature Cited

Aldenderfer, M. S. and R. K. Blashfield. 1984. Cluster analysis. Sage University Paper No 44. Sage Publications, Beverly Hills.

Association of Official Analytical Chemists. 1984. Official methods of analysis. 14th Edn AOAC, Washington, D. C.

Bainton, S. J., V. E. Plumb, B. O. Juliano, C. M. Perez, D. B. Roxas, G. S. Khush, J. C. de Jesus and K. A. Gomez. 1991. Variation in the nutritional value of rice straw. Anim. Feed. Sci. Technol., 34:261-277.

Bray, J. H. and S. E. Maxwell. 1985. Multivariate analysis of variance. Sage University Paper No 54. Sage Publications, Beverly Hills.

Colucci, P. E., D. Falk, G. K. Macleod and D. G. Grieve. 1992. In situ organic matter degradability of untreated and urea-treated varieties of spring barley and oat straws, and of untreated varieties of winter wheat straws. Anim. Feed. Sci. Technol., 37:73-84.

Goering, H. K. and P. J. Van Soest. 1970. Forage fibre analyses. Apparatus, reagents, procedures and some applications. USDA Agricultural Handbook No 379.

Jones, D. I. H. and M. V. Hayward. 1975. The effect of pepsin pretreatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase. J. Sci. Fd. Agric., 26:711-718.

Marriott, F. H. C. 1974. The interpretation of multiple observations. Academic Press, London.

Mgheni, D. M., A. E. Kimambo, F. Sundstol and J.

Madsen. 1993. Influence of urea treatment or supplementation on degradation, intake and growth performance of goats fed rice straw diets. Anim. Feed. Sci. Technol., 44:209-220.

Nakashima, Y. and E. R. Orskov. 1990. Rumen degradation of straw. 9. Effect of cellulase and ammonia treatment on different varieties of rice straws and their botanical fractions. Anim. Prod., 50:309-317.

SAS Institute, 1987. SAS Users Guide : Statistics (Version 6). SAS Institute, Cary NC.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods (6th ed). Iowa State University Press, Ames.

Tuen, A. A., M. M. Dahan, B. A. Young and P. Vijchulata. 1991. Intake and digestion of urea-treated, urea-supplemented and untreated rice straw by goats. Anim. Feed Sci. Technol., 32:333-340.

Vadiveloo, J. 1986. The effect of alkali treatment of straw and dried palm-oil sludge on the intake and performance of goats of varying genotype. Agric. Wastes, 18:233-245.

Vadiveloo, J. 1992. Varietal differences in the chemical composition and *in vitro* digestibility of rice straw. J. agric. Sci. Camb., 119:27-33.

Vadiveloo, J. and J. G. Fadel. 1992. Compositional analyses and rumen degradability of selected tropical feeds. Anim. Feed Sci. Technol., 37:265-279.

Walli, T. K., E. R. Orskov and P. K. Bhargava. 1988. Rumen degradation of straw. 3. Botanical fractions of two rice straw varieties and effects of ammonia treatment. Anim. Prod., 46:347-352.