# Feeding Value of Ammoniated Rice Straw Supplemented with Rice Bran in Sheep : I. Effects on Digestibility, Nitrogen Retention and Microbial Protein Yield

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**ABSTRACT :** *In vivo* digestibility, nitrogen retention and microbial protein yield from diets of 100% ammonia treated rice straw (ARS) ($D_1$); 65% untreated rice straw (URS)+30% rice bran (RB)+5% SBM ($D_2$) and 85% ARS+15% RB ($D_3$) were determined using three Japanese Corriedale wethers in a 3×3 Latin Square Design. Results showed that DM consumption and organic matter digestibility were highest in $D_3$; but did not promote high protein digestibility, which RB+SBM had effected in URS based-diet. Dry matter intake and OM digestibility were the same for $D_1$ and $D_3$. Solubility of fiber bonds was increased by ammoniation, resulting in higher NDF digestibility. Nitrogen retention and microbial protein yield of rice bran supplemented groups was higher than ARS, but supplementation did not significantly increase efficiency of microbial protein synthesis from ARS which did occur when RB+SBM was added to untreated straw. The quality of ammoniated rice straw could be improved through RB supplementation because of its positive effects on DM digestibility, nitrogen retention and microbial protein yield. However, the addition of RB+SBM to URS resulted to more efficient N utilization. *(Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 4 : 490-496)*

**Key Words :** *In Vivo* Digestibility, Untreated Rice Straw (URS), Ammoniated Rice Straw (ARS), Nitrogen Balance, Microbial Nitrogen Yield

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## INTRODUCTION

There is an abundant supply of cereal straw in the Third World countries. Asia alone produces about 478.7 million tons, or 92% of the world's rice straw production (FAO, 1990). The vast supply of straw represents a potential source of animal feed, particularly for ruminants, but its full potential has not been harnessed. Rice straw has a high degree of lignification and silica content that makes it resistant to microbial digestion, hence, nutrient availability is poor (Theander and Aman, 1984). It is in this context that the problem of poor quality and scarcity of feed supply still remains in spite of the vast supply of straw.

Increasing nitrogen concentration through ammoniation has been found to be the most practical and economical method (Silva et al, 1989) to improve the feeding quality of straw. Optimum results on dry matter digestibility (DMD) could be attained when ammoniated rice straw (ARS) is supplemented with soybean meal (SBM), alfalfa hay or citrus by-products (Sundstol, 1984; Maeng and Kim, 1984; Han et al., 1989; Zorilla-Rios et al., 1985), but other reports suggest that inclusion of supplements in straw based-diets tend to reduce dry matter (DM) intake and digestibility (Castrillo et al., 1995; Mgheni et al., 1993).

The effect of ammoniation is dependent on the kind of basal diet and the type of supplement (Jarrige et al., 1986; Fahmy et al., 1984; Henning et al., 1980). Rolled barley and sugar beet pulp as supplements for ammoniated barley straw in sheep have a more pronounced effect on fiber digestibility than on protein digestibility because of their readily fermentable sugars (Castrillo et al., 1995). Rice bran (RB), a by-product of rice milling, could also supply easily degradable carbohydrates for rumen microbes (Williams et al., 1984). When used at the rate of 30% in urea-supplemented or urea-treated rice straw diets, rice bran provides an optimum rumen environment for straw degradation even without the addition of fish meal (Mgheni et al., 1994). In this study, rice bran was used because it is produced in a large quantity in rice producing countries, and the presence of readily soluble carbohydrates from this material could provide energy for rumen microbes and carbon chain needed in protein synthesis.

In this study the nutrient digestibility, nitrogen (N) retention and microbial N yield of untreated rice straw and ARS based-diets supplemented with rice bran in sheep were determined. This was undertaken to assess the effects of ammonia treatment and rice bran supplementation in improving the quality of locally available rice straw.

## MATERIALS AND METHODS

### Rice straw treatment

About 5 kg of chopped rice straw (2-3 cm length) were placed in polyethylene sacks. Air was removed

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from each sack using a vacuum pump, then a 25% ammonia solution was infused at the rate of 3 g nitrogen/kg DM using a pipe. The bags were then tightly secured with a rope. After 30 days curing, the bags were opened and treated straw was spread on a concrete floor to allow drying for 2-3 days, and to ensure evaporation of excess ammonia.

### Animals, diet and experimental design

A digestibility trial was conducted using three rumen cannulated Japanese Corriedale sheep with mean body weight of 32 kg. The animals were kept individually in stainless metabolic crates (40×90×80 cm) with plastic feeder, and fecal and urine collecting equipment. The three experimental diets contained 100% ammoniated rice straw ($D_1$); 65% untreated rice straw (URS)+30% RB+5% SBM ($D_2$), and 85% ARS+ 15% RB ($D_3$); these were fed to the animals in a 3× 3 Latin Square Design. Each segment of the trial was composed of 14 d adjustment and 5 d collection periods. Animals were given free access to a multi-mineral salt lick (Nippon Zenyaku Kogyo Co. Ltd., Fukushima, Japan).

Total fecal and urine excretion were determined during the five day-collection period. Daily samples of feeds, orts, feces, and urine were collected, pooled and stored at -10℃ for further analysis. During the last day of each collection period, rumen fluid samples were taken before the morning feeding and 1, 2, 4, 6, and 8 hours after feeding.

### Laboratory analysis

Representative samples of feed, orts and feces were analysed for DM and organic matter (OM) contents following the AOAC (1984) procedures. Nitrogen content of the samples was determined by the Kjeldahl method. Fiber fractions such as NDF, ADF, hemicellulose and cellulose were analyzed following the Goering and Van Soest methods (1970). The ammonia-nitrogen concentration of rumen fluid was measured by the procedure of Oser (1965).

Urinary purine derivative (PD) was analyzed as the sum of allantoin, xanthine-hypoxanthine and uric acid excretions. Allantoin was determined by the method of Young and Conway (1942), while xanthine, hypoxanthine and uric acid were analyzed following the method of Fujihara et al. (1987).

### Calculations

Microbial purines absorbed (X) was calculated from total urinary PD based on the quantitative relationship defined by Chen and Gomes (1992) as:

$$Y=0.84X+0.150\ W^{0.75}e^{-0.25x}$$

Where: 0.84 represents the slope (recovery of absorbed purines in the urine), and 0.150 $W^{0.75}e^{-0.25x}$ is the contribution of endogenous PD to total excretion based on metabolic body weight.

From the equation, X was calculated from Y following the Newton-Raphson iteration process as:

$$X_{(n+1)}=X_n[f(X_n)/-f(X_n)]$$

Where: $f(X)=0.84X+0.150\ W^{0.75}e^{-0.25x}-Y$
$f'(X)=0.84-0.038\ W^{0.75}e^{-0.25x}$ is the derivative of $f(X)$.

Microbial N yield (g N/d) was computed as:

$$\text{Microbial N yield (g N/d)} = \frac{\text{Microbial purine absorbed (mmol/d)}\times 70}{0.116\times 0.83\times 1000}$$

given the following assumptions: 830 g/kg DM digestibility for microbial purine; 70 mg N/mmol as N content of purines; and the ratio of purine-N: total N in mixed microbes as 11.6:100 (Chen and Gomes, 1992).

### Statistical analysis

All data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of Statistica for Windows™ Release 4.3 (StatSoft, Inc., Tulsa, OK., 1993). Mean comparison was done using Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

### Chemical composition

The chemical composition of URS, ARS and other experimental diets is shown in table 1. The treatment of rice straw with ammonia increased the total N content by almost 2.5 times from 0.64 to 1.43%; however, only a small proportion of N was attached to the cell walls (0.30% NDF-N) as a result of the treatment. It is possible that most of the nitrogen incorporated is present as free $NH_3$-N (Oliveros et al., 1993). Results confirm the earlier report of Maeng and Chung (1989) that only 32.4% of the added $NH_3$-N was retained in rice straw after ammoniation.

There was a slight decrease in the NDF fraction due to partial solubilization of hemicellulose (Klopfenstein, 1978), while the ADF component remained more or less constant. The increase in N content and the reduction in cell wall fractions, as indicated by NDF and hemicellulose levels, are consistent with the earlier findings of Djajanegara and Doyle (1989), Mgheni et al. (1994) and Pradhan et al. (1996).

**Table 1.** Chemical composition of feed stuffs and experimental diets (% DM)

| Nutrients | RB | SBM | URS | $D_1$ | $D_2$ | $D_3$ |
|---|---|---|---|---|---|---|
| Dry matter | 86.48 | 87.66 | 88.87 | 88.16 | 89.36 | 87.84 |
| Organic matter | 89.68 | 91.76 | 77.93 | 81.65 | 82.52 | 82.76 |
| Crude ash | 10.31 | 8.24 | 22.06 | 18.35 | 17.48 | 17.23 |
| Nitrogen | 2.0 | 7.29 | 0.64 | 1.43 | 1.46 | 1.51 |
| ADF-N |  |  | 0.26 | 0.30 |  |  |
| NDF | 21.69 | 11.23 | 63.96 | 60.68 | 49.86 | 54.31 |
| ADF | 9.37 | 9.00 | 40.74 | 39.27 | 31.00 | 37.39 |
| Hemicellulose | 12.32 | 2.23 | 23.22 | 21.41 | 18.86 | 16.92 |
| Cellulose | 6.70 | 8.70 | 35.23 | 35.09 | 25.88 | 33.20 |
| Lignin | 2.67 | 0.30 | 5.51 | 4.59 | 5.12 | 4.19 |
| Silica | 0.12 | 0.23 | 15.89 | 10.96 | 9.86 | 10.49 |
| ME MJ/kg* | 10.15 | 11.87 | 4.18 | 6.60 | 7.02 | 7.11 |

* Calculated based on Standard Tables of Feed Composition in Japan (1995).

$D_1$=100% ARS; $D_2$=65% URS+30% RB+5% SBM; $D_3$=85% ARS+15% RB.

**Table 2.** Dry matter intake, *in vivo* digestibility and digestible nutrient intake of sheep fed with URS and ARS based-diet supplemented with rice bran

| Nutrients | $D_1$ | $D_2$ | $D_3$ | SEM | Level of significance |
|---|---|---|---|---|---|
| DM intake (g/kg $W^{0.75}$) | 42.51$^{a}$ | 47.19$^{a}$ | 54.82$^{b}$ | 2.88 | * |
| Nutrient digestibility (%) |  |  |  |  |  |
| DM | 50.72$^{a}$ | 55.22$^{b}$ | 61.33$^{c}$ | 1.60 | ** |
| OM | 53.14$^{a}$ | 54.75$^{a}$ | 65.24$^{b}$ | 2.20 | ** |
| N | 54.49$^{a}$ | 70.65$^{b}$ | 57.74$^{a}$ | 3.45 | * |
| NDF | 71.06$^{a}$ | 50.85$^{b}$ | 73.34$^{a}$ | 3.73 | ** |
| ADF | 64.24$^{a}$ | 58.58$^{b}$ | 71.37$^{a}$ | 2.88 | * |
| Digestible nutrient intake (g/kg $W^{0.75}$) |  |  |  |  |  |
| DDMI | 21.56$^{a}$ | 26.06$^{a}$ | 33.62$^{b}$ | 2.07 | * |
| DOMI | 18.13$^{a}$ | 20.83$^{a}$ | 29.52$^{b}$ | 1.86 | * |
| DNI | 0.33$^{a}$ | 0.679$^{b}$ | 0.58$^{b}$ | 0.056 | * |
| DNDFI | 17.94$^{a}$ | 11.08$^{b}$ | 21.48$^{a}$ | 1.62 | * |

$^{a,b,c}$ Means with common letter superscript within rows are not significant.

$D_1$=100% ARS; $D_2$=65% URS+30% RB+5% SBM; $D_3$=85% ARS+15% RB.

* $p<0.05$; ** $p<0.01$.

The diets ($D_1$, $D_2$ and $D_3$) used in the trial had almost the same N and OM contents, which are significant improvements compared with the N and OM contents of URS. Ammoniation and the inclusion of rice bran allowed for the formulation of diets with almost the same protein and energy levels (calculated ME kcal/kg).

**Dry matter intake (DMI) and nutrient digestibility**

Table 2 presents the DMI and nutrient digestibility of URS and ARS based-diets supplemented with rice bran. Animals fed with $D_3$ had higher ($p<0.05$) DMI than those fed with $D_1$ and $D_2$. No significant difference was observed between DMI in $D_2$ and $D_1$. Results indicate that the increased consumption in $D_3$ is a result of rice bran inclusion (over $D_1$) and the use of ARS (over $D_2$). The addition of rice bran to ARS improved DM digestibility of the diet resulting in higher intake. Higher digestibility values of DM and OM in $D_3$ resulted in higher daily intake of digestible DM (DDMI) and OM (DOMI) compared with $D_1$. The marked improvement in the overall digestibility of $D_3$ could be attributed to the extremely high detergent fiber digestibility of ARS in combination with RB.

Dry matter digestibility of $D_3$ was significantly greater than with $D_1$ and $D_2$ ($p<0.05$). The 61% DM digestibility (DMD) is higher, but the 65% OM digestibility is almost the same as the corresponding mean values for ammoniated barley straw (ABS)+300 g rolled barley grains reported by Castrillo et al. (1995). However, DMD in this study is lower than those earlier reported, when ARS was supplemented with either soybean meal (Pradhan et al., 1996), alfalfa hay (Han et al., 1989) or as reported by Maeng et al. (1989), and when ARS was supplemented by either SBM or alfalfa hay. Result tends to emphasize the differential effects of protein supplementation on nutrient digestibility of ammoniated straw diet.

Conversely, the N digestibility in $D_3$ was significantly lower than in $D_2$, but was not significantly different from $D_1$. The 54% N digestibility of $D_1$ and 57% of $D_3$ support the conclusion of Males (1989) that only about 50% of N from ammoniated or urea treated cereal or grain straw is available for digestion. Not even the addition of 15% RB effected a significant increase in N digestibility of ARS based-diet. The low CP digestibility of $D_1$ and $D_3$ emphasizes the importance of true protein supplementation in straw based-diets to correct nutrient deficiencies in order to increase nutrient availability (Silva et al., 1989; Leng, 1990; Oosting, 1993). Results also indicate that the inclusion of a small amount of SBM in RB supplemented URS diet ($D_2$) provided a better N source, which eventually yielded higher N digestibility.

The significantly higher digestibility of cell wall components of ARS based-diets than the untreated straw is also depicted in table 2. Ammonia treatment of straw improved digestibility of NDF ($p<0.01$) and ADF ($p<0.05$) by about 20% in agreement with the previous works of Garrett et al. (1974, 1979), Klopfenstein (1978), Sundstol (1981, 1984), and Maeng and Kim (1984). The higher digestibility of fiber fractions in ARS based-diets than the URS based-diet indicates the apparent loosening of bonds between lignin and cellulose or hemicellulose as a result of ammonia treatment. The 15% inclusion of RB to ARS ($D_3$) did not reduce the cellulolytic activities in the rumen as indicated by the insignificant differences in NDF and ADF digestibility between $D_1$ and $D_3$. The level of RB in this study did not cause depressing effect on fiber digestibility, confirming earlier reports that more than 30% incorporation of RB (Zhao et al., 1996) or rice mill feed (Chowdhury, 1997) reduced cell wall digestibility when fed to cattle. The 20% higher ($p<0.01$) NDF digestibility is in agreement with Mgheni et al. (1994) that the feeding of urea-supplemented rice straw and RB (70:30) could provide optimum rumen environment for straw degradation, without necessarily affecting cell wall digestibility.

### Rumen $NH_3$-N, nitrogen balance, PD excretion, and microbial nitrogen yield

Table 3 presents the N balance, rumen $NH_3$-N concentration, PD excretions, and microbial N yields

**Table 3.** Nitrogen balance (g), rumen $NH_3$-N (mg/dl), purine derivative (PD) excretions (mmol/d) and microbial N yield (g/d) in sheep fed with URS and ARS diet supplemented with RB

| | $D_1$ | $D_2$ | $D_3$ | SEM | Level of significance |
|---|---|---|---|---|---|
| N intake | $8.80^a$ | $13.36^b$ | $13.09^b$ | 0.88 | * |
| Fecal N excretion | $3.99^a$ | $3.93^a$ | $5.45^b$ | 0.37 | * |
| Urinary N excretion | 4.37 | 4.02 | 4.46 | 0.40 | * |
| Retained N | $0.44^a$ | $5.41^b$ | $3.18^b$ | 0.78 | * |
| Rumen $NH_3$-N | $8.63^a$ | $14.81^b$ | $13.70^b$ | 1.40 | * |
| Total PD | $3.83^a$ | $4.83^b$ | $6.17^b$ | 0.38 | * |
| Allantoin | $2.62^a$ | $3.50^b$ | $4.15^b$ | 0.29 | * |
| Xanthine+hypoxanthine | 0.258 | 0.280 | 0.426 | 0.01 | NS |
| Uric acid | 1.05 | 1.12 | 1.25 | 0.09 | * |
| Microbial N | $2.54^a$ | $4.9^b$ | $5.04^b$ | 0.26 | * |
| DOMI | $0.419^a$ | $0.457^a$ | $0.39^b$ | 0.02 | * |
| RDOM (%) | $52.01^b$ | $48.17^b$ | $57.51^c$ | 1.42 | * |
| RDOM (kg/d) | $0.17^a$ | $0.19^a$ | $0.25^b$ | 0.02 | * |
| EMNS | $18.26^a$ | $27.63^b$ | $20.48^a$ | 1.57 | |

DOMI: Digestible OM intake (kg/d).

RDOM (%) calculated from actual *in-situ* nylon bag disappearance using the equation $p=a+b\ (1-e^{-ct})$ (Orskov and McDonald, 1979).

RDOM: Rumen degradable OM (from nylon bag data using outflow rate of 0.02) × DOMI.

EMNS: Efficiency of microbial N synthesis=g microbial N/kg RDOM.

$D_1$=100% ARS; $D_2$=65% URS+30% RB+5% SBM; $D_3$=85% ARS+15% RB.

$^{a,b,c}$ Means with common superscripts within rows are not significant; NS=not significant ($p>0.05$); * ($p<0.05$).

in sheep fed with URS and ARS supplemented with rice bran. All the animals showed positive N-balance in response to the dietary N intake from the experimental diets. $D_2$ and $D_3$ had significantly higher N-balance than $D_1$ due to the improvement in protein source brought about by supplementation. $D_2$ had the highest retained N among all treatments indicating that the addition of SBM and RB to a URS diet has a positive effect on N retention. $D_1$ had the lowest retained N because of high fecal and urinary excretion relative to the N intake. The lesser amount of N added by ammoniation was made available to the animals as protein source, but supplementation of rice bran provided a better N source.

The level of $NH_3$-N that supports maximum digestibility and N utilization of straw in the rumen varies depending on the type of substrate. The critical level of $NH_3$-N as reported by Leng (1990) is between 5 and 25 mg/100 ml of rumen liquor. Although $NH_3$-N concentration in sheep fed with $D_1$ alone was significantly lower ($p<0.05$) than $D_2$ and $D_3$, its mean value is above the minimum concentration of 5 mg/100 ml necessary for promoting desirable rumen fermentation rate (Balcells et al., 1993) and microbial protein synthesis of alkali treated straw (Satter and Slyter, 1974). Animals fed with $D_1$ had low microbial synthesis in spite of the 8.63 mg $NH_3$-N /100 ml of rumen fluid. This could indicate that excess N (more than 5 mg N/100 ml) from ammoniated rice straw did not influence uptake by microbes or increase protein production (Alawa and Hemingway, 1986).

The significantly higher ($p<0.05$) N retention and OM digestibility of the RB supplemented groups resulted in higher urinary PD excretions of animals fed with $D_2$ and $D_3$ promoting higher microbial N yields when compared with the control group. However, when it is expressed as a proportion of RDOM, microbial N yield was the same for $D_1$ and $D_3$. Both supplemented diets ($D_2$ and $D_3$) had better microbial synthesis than the unsupplemented diet. Although there was a higher digestibility of OM in $D_3$ (table 2) this did not result in better microbial protein synthesis compared with $D_2$. This result is contrary to the findings of Moller and Hvelplund (1982) that supplementing urea or SBM in cows receiving $D_1$ had no positive effect on the microbial protein synthesis. In spite of the significantly higher OM digestibility of rice straw brought about by ammoniation, Herrera-Saldana et al. (1982) recommended that supplemental energy in the form of RB is needed for maximum utilization of added N.

Results of this study indicate that minimal supplementation of RB can improve N utilization of either ARS or URS based-diets when fed to sheep. Furthermore, it proved that energy supplementation can be more beneficial than protein supplementation in improving microbial yield of ammoniated rice straw.

## CONCLUSION

The quality of rice straw could be improved by ammonia treatment, but the improvement is only minimal compared with the improvement that could be achieved by supplementing it with rice bran. The *in vivo* trial revealed that ammonia treatment could increase dry matter intake, and supplementation with rice bran could further improve DM and OM digestibility of rice straw which is attributable to the extremely high detergent fiber digestibility of ARS with RB. However, ammoniation alone is insufficient to improve protein digestibility, which the supplementation of RB+SBM had provided to URS. Nevertheless, ammoniation resulted in higher NDF digestibility due to improved ruminal degradability of fiber bonds. Rice bran supplementation improved nitrogen retention and microbial protein yield of ammoniated rice straw, but the addition of RB+SBM in URS provided a more efficient microbial-N synthesis.

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