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# Mode Of Action Of Ammoniating In Increasing Nitrogen Content And In Vitro Apparent Organic Matter Digestibility Of Forages

Dede Kardaya, Deden Sudrajat

**Abstract**— An experiment was conducted in order to reveal mode of action of ammoniating in increasing nitrogen content and in vitro apparent organic matter digestibility of forages. The experiment was accomplished under completely randomized design with two factors (2 x 4) and three replicates. The collected data were analysed with a two-factor ANOVA and, if applicable, continued with Tukey's tests. Regression analyses were applied to reveal the incubation rate. Results showed that ammoniating treatment used 3% of urea increased crude protein content and in vitro apparent OM digestibility of rice straw significantly ( $P < 0.05$ ). Nitrogen originated from urea retained in rice straw materials during the consecutive incubation period could be best predicted with a cubical regression  $Y = 0,447 + 0,114 t - 0,52 t^2 + 0,004 t^3$  ( $R = 0.849$ ;  $p = 0.002$ ) and the highest rate of ammoniating process occurred in week 2 of the incubation period. It was an implication that disruption of lignocellulosic fractions occurred as a result of retaining N originated from urea in  $C_1$  site of reduced sugar.

**Index Terms**— urea, ammoniation, rice straw, in vitro digestibility.

## INTRODUCTION

Urea might improve the nutritive value of low quality forages although the improvement depended on the dry matter content of the forages [1][2]. The improvement of low quality forages included in crude protein and organic matter digestibility [3][4][5][6][7]. However, all of the invention above did not explain either the mode of action or chemical reaction occurred in ammoniating in such a way that crude protein and organic matter digestibility of low quality forage increased. The study attempted to reveal mode of action of ammoniating in increasing crude protein content and organic matter digestibility of low quality forages.

## MATERIAL DAN METHOD

The experiments were conducted at the Animal Science Laboratory of Bogor Djuanda University in two phases. The first phase aimed to reveal the highest reaction rate of ammoniating process and the second phase to reveal mode of action of ammoniating in increasing crude protein content and organic matter digestibility of low quality forages.

### Experiment 1

**Sampling and laboratory analyses.** Dried rice straws (DM 89%) were chopped in 5 cm in length and sprayed with urea solution (3% urea/kg DM; [8]). The sprayed rice straws were sampled (5 kg), packed in polyethylene bags, pressed, and fixed tightly with plastic wrap. A similar procedure was applied to dried rice straw without urea solution spraying. All samples were then incubated at room temperature with incubation periods of 2, 4, 6, and 8 weeks. At the end of each

incubation period, the rice straw samples were sub sampled and analysed for DM [9]; OM determined by ash [9]; CP obtained by total N determination using the micro-Kjeldahl technique [9] and calculated as  $N \times 6.25$ ; NDF [24]; ADF [9]; and sulfuric acid lignin [25]. In vitro apparent digestibility of DM and OM were analysed according to [10] for each of the consecutive incubation period.

**Statistical analyses.** The experiment was conducted under completely randomized design with two factors (2 x 4) and three replicates. The collected data were analysed with a two-factor ANOVA and, if applicable, continued with Tukey's tests. Regression analyses were applied to reveal the incubation rate [11]. Samples showed the highest incubation rate would be used as samples for experiment 2.

### Experiment 2

**Sampling and laboratory analyses.** Samples showed the highest incubation rate in experiment 1 were sub sampled (100 g), ground through a 1-mm screen in a Willey mill, and analysed for total sugar determined with Anthron method, and reduced sugar determined with Molisch reagents [12]. Nitrogen content of ammoniated rice straw originated from urea was calculated as N ammoniated rice straw - N nonammoniated rice straw.

## RESULT AND DISCUSSION

### Quality Of Ammoniated Rice Straw

**NDF.** NDF contents of rice straw were not affected by ammoniating treatment but were affected by an incubation period ( $P < 0.05$ ; Table 1). It was a tendency that ammoniating resulted in decreasing NDF contents. This result was similar to the result reported by many researchers [13] [14] [15] [16] [17] [18]. Apparently, ammoniating treatment tended to increase solute materials in NDF solution and resulted in decreasing NDF content of the straw. Among incubation periods, rice straw of week 8 showed the lowest NDF value. It was

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implicated that microbial activity increased following consecutive incubation periods.

Table 1. NDF content of rice straw

| Incubation (weeks) | Control (0% urea) | Ammoniated (3% urea) | Mean               |
|--------------------|-------------------|----------------------|--------------------|
| .....NDF (%) ..... |                   |                      |                    |
| 2                  | 75.24             | 73.60                | 74.42 <sup>b</sup> |
| 4                  | 72.11             | 74.79                | 73.45 <sup>b</sup> |
| 6                  | 73.79             | 73.36                | 73.57 <sup>b</sup> |
| 8                  | 71.70             | 70.72                | 71.21 <sup>a</sup> |
| Mean               | 73.38             | 72.89                |                    |

Different superscript within a similar column differed significantly (P < 0.05)

**ADF.** ADF contents of rice straw were not affected either by ammoniating treatment or by an incubation period (Table 2). Else, it was a tendency that ammoniating resulted in decreasing NDF contents. This result was similar to the result reported by many researchers [13] [14] [15] [16] [17] but was lower than the result reported by [18]. Apparently, similar to NDF fractions, ammoniating treatment tended to increase solute materials in ADF solution and resulted in decreasing ADF content of the straw. In addition, the ADF fraction of rice straw in consecutive week of incubation was similar. It indicated that prolong the incubation period did not increase microbial access to cellulose fraction and resulted in unchanged ADF content.

Table 2. ADF contents of rice straw

| Incubation (weeks) | Control (0% urea) | Ammoniated (3% urea) | Mean  |
|--------------------|-------------------|----------------------|-------|
| .....ADF (%) ..... |                   |                      |       |
| 2                  | 52.85             | 54.25                | 53.55 |
| 4                  | 52.15             | 54.48                | 53.32 |
| 6                  | 54.26             | 54.38                | 54.32 |
| 8                  | 54.67             | 53.32                | 54.00 |
| Mean               | 53.13             | 52.83                |       |

**Cellulose and hemicellulose.** Either cellulose or hemicellulose content of rice straw was not affected by ammoniating treatments but was affected by the incubation period (P<0.05; Table 3 and 4). It was a tendency that ammoniating resulted in decreasing NDF contents. In order that cellulose and hemicellulose fractions could be accessed by microbe, lignocellulose fractions must be degraded microbiologically or solubilized chemically [19]. It seemed that ammoniating treatment with 3% of urea in recent research did not show an optimal result in degrading lignocellulose matrix. This result was supported by [20] finding which reported that the effect of urea treatment on cell wall fractions showed inconsistent results.

Table 3. Cellulose contents of rice straw

| Incubation (weeks)       | Control (0% urea) | Ammoniated (3% urea) | Mean                |
|--------------------------|-------------------|----------------------|---------------------|
| .....cellulose (%) ..... |                   |                      |                     |
| 2                        | 33.47             | 33.63                | 33.55 <sup>ab</sup> |
| 4                        | 28.83             | 33.77                | 31.30 <sup>a</sup>  |
| 6                        | 34.60             | 33.63                | 34.11 <sup>b</sup>  |
| 8                        | 32.47             | 31.37                | 31.92 <sup>ab</sup> |
| Mean                     | 32.42             | 33.01                |                     |

Different superscript within a similar column differed significantly (P < 0.05)

Table 4. Hemicellulose contents of rice straw

| Incubation (weeks)           | Control (0% urea) | Ammoniated (3% urea) | Mean                |
|------------------------------|-------------------|----------------------|---------------------|
| .....hemicellulose (%) ..... |                   |                      |                     |
| 2                            | 22.39             | 19.35                | 20.87 <sup>b</sup>  |
| 4                            | 19.96             | 20.31                | 20.14 <sup>ab</sup> |
| 6                            | 19.53             | 18.98                | 19.25 <sup>ab</sup> |
| 8                            | 17.03             | 17.40                | 17.21 <sup>a</sup>  |
| Mean                         | 20.25             | 20.06                |                     |

Different superscript within a similar column differed significantly (P < 0.05)

**Lignin.** Lignin contents of rice straw were not affected by ammoniating treatment, but were affected by an incubation period (Table 5). This result was lower (4.56%) than the result (10.0%) reported by [15] but was in the range (3.4 - 8.1%) reported by [17]. This result was inconsistent with previous result reported that the lignin content of forages decreased with ammonia or urea treatment [21][22]. Likely, ammoniating treatment with 3% of urea in recent research did not strong enough to solubilize or degrade lignocellulose matrix.

Table 5. Lignin contents of rice straw

| Incubation (weeks)    | Control (0% urea) | Ammoniated (3% urea) | Mean               |
|-----------------------|-------------------|----------------------|--------------------|
| .....lignin (%) ..... |                   |                      |                    |
| 2                     | 4.93              | 4.17                 | 4.55 <sup>ab</sup> |
| 4                     | 4.34              | 4.58                 | 4.46 <sup>a</sup>  |
| 6                     | 4.73              | 4.31                 | 4.52 <sup>a</sup>  |
| 8                     | 4.85              | 5.22                 | 5.04 <sup>b</sup>  |
| Mean                  | 4.73              | 4.56                 |                    |

Different superscript within a similar column differed significantly (P < 0.05)

**Crude protein.** Crude protein content of rice straw was increased (P<0.05) with ammoniating treatment but was decreased (P<0.05) with an incubation period (Table 6). This result was similar to [16] and [15] who reported that either urea or ammonia treatment increased crude protein content of rice straw. The increase in crude protein content of ammoniated rice straw occurred as a direct effect of nitrogen addition originated from urea used in ammoniating.

Table 6. Crude protein content of rice straw

| Incubation (weeks)           | Control (0% urea) | Ammoniated (3% urea) | Mean               |
|------------------------------|-------------------|----------------------|--------------------|
| .....crude protein (%) ..... |                   |                      |                    |
| 2                            | 1.76              | 3.33                 | 2.54 <sup>c</sup>  |
| 4                            | 1.19              | 1.90                 | 1.54 <sup>ab</sup> |
| 6                            | 0.73              | 1.50                 | 1.12 <sup>a</sup>  |
| 8                            | 1.37              | 1.78                 | 1.57 <sup>ab</sup> |
| Mean                         | 1.25 <sup>a</sup> | 2.16 <sup>b</sup>    |                    |

Different superscript within a similar row or column differed significantly (P < 0.05)

**In vitro DM and OM apparent digestibility.** In vitro DM and apparent digestibility of rice straw were increased (P<0.05) with ammoniating treatment but did not change with an incubation period (Table 7 and 8). This result supported the previous research [16] [18] reported that DM apparent digestibility of rice straw increased with urea or ammonia treatment. The increase in DM or OM apparent digestibility of ammoniated rice straw indicated that ammoniating treatment improved low quality forages. This result was consistent with the result reported in previous researches [18] [4] [7]. Unfortunately, the improvement in DM or OM digestibility did not occur in NDF or ADF fraction. Rahat et al. [26] claimed that ADF fraction of rice straw treated with ammonia might contain components showed a lower degradability rate than components contained in NDF fractions.

Table 7. In vitro DM apparent digestibility of rice straw

| Incubation (weeks)                            | Control (0% urea)  | Ammoniated (3% urea) | Mean  |
|---|--------------------|----------------------|-------|
| ...In vitro DM apparent digestibility (%) ... |                    |                      |       |
| 2   | 12.25              | 12.71                | 12.48 |
| 4   | 13.69              | 14.32                | 14.00 |
| 6   | 11.20              | 15.54                | 13.37 |
| 8   | 11.78              | 16.07                | 13.93 |
| Mean  | 12.09 <sup>a</sup> | 14.25 <sup>b</sup>   |       |

Different superscript within similar row differed significantly (P < 0.05)

Table 8. In vitro OM apparent digestibility of rice straw

| Incubation (weeks)                            | Control (0% urea) | Ammoniated (3% urea) | Mean  |
|---|-------------------|----------------------|-------|
| ...In vitro OM apparent digestibility (%) ... |                   |                      |       |
| 2   | 65.32             | 72.24                | 68.78 |
| 4   | 63.35             | 67.15                | 65.25 |
| 6   | 65.25             | 68.56                | 66.90 |

Table 9. Total sugar and reduced sugar contained in rice straw

| Sampel  | Total sugar, ppm    |                       | Reduced sugar, ppm  |                     | Reduced sugar (% of total sugar) |                    |
|---------|---------------------|-----------------------|---------------------|---------------------|----------------------------------|--------------------|
|         | Nonammoniated       | Ammoniated            | Nonammoniated       | Ammoniated          | Nonammoniated                    | Ammoniated         |
| U1      | 296.78              | 2,137.85              | 168.62              | 534.93              | 56.82                            | 25.02              |
| U2      | 287.48              | 1,874.79              | 156.12              | 444.36              | 54.30                            | 23.70              |
| U3      | 269.19              | 1,674.95              | 182.51              | 359.88              | 67.80                            | 21.49              |
| Average | 284.48 <sup>a</sup> | 1,895.86 <sup>b</sup> | 169.08 <sup>a</sup> | 446.39 <sup>b</sup> | 59.64 <sup>a</sup>               | 23.40 <sup>b</sup> |

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|      |                    |                    |       |
|------|--------------------|--------------------|-------|
| 8    | 64.94              | 67.78              | 66.36 |
| Mean | 65.02 <sup>a</sup> | 68.60 <sup>b</sup> |       |

Different superscript within similar row differed significantly (P < 0.05)

**Ammoniating Rate**

The Ammoniating rate of rice straw was predicted by a cubical regression model as follows:  
 $Y = 0.447 + 0.114t - 0.52t^2 + 0.004t^3$ ; R = 0.849, p = 0.002, Ynj = N contained in ammoniated straw; t = incubation period (week). The result revealed that the highest ammoniating rate occurred at week two of the incubation period (Figure 1).

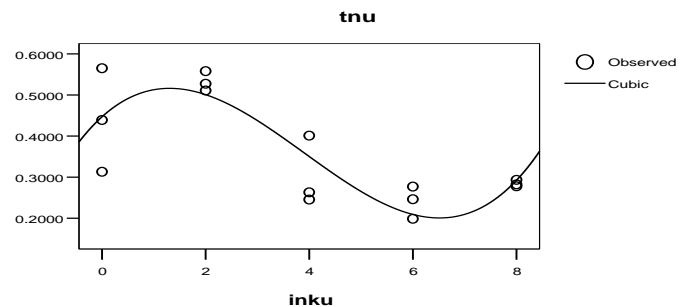


Figure 1. The Ammoniating rate of rice straw predicted with a cubical regression curve. Inku = incubation period (week), tnu = total of nitrogen contained in ammoniated rice straw originated from urea (mg/mg).

**Disruption Of Lignocellulose Linkage**

Ammoniating treatment increased (P<0.05) both total sugar and reduced sugar but decreased (P<0.05) reduced sugar percentage (Table 9). A similar result was also reported by [23] used cellulase to release reduced sugar from cellulose substrates in in vitro study. It indicated that ammoniating treatment degraded lignocellulosic fractions effectively and allowed microbial cellulase to attack cellulose fraction of rice straw. On the contrary, percentage of reduced sugar relative to total sugar decreased significantly. The decrease in percentage of reduced sugar indicated that retaining nitrogen with reduced sugar occurred, most likely in C<sub>1</sub> site of reduced sugar. This retained reduced sugar could not be detected in determination procedure of reduced sugar content and resulted in decreasing reduced sugar content of ammoniated rice straw. This presumes was supported with the previous finding that nitrogen originated from urea was retained quantitatively in ammoniated rice straw materials (Table 6 and Figure 1).

## CONCLUSION

Ammoniating treatment used 3% of urea improved quality of rice straw by increasing crude protein content and in vitro apparent digestibility of ammoniated rice straw. Nitrogen originated from urea retained in rice straw materials could be best predicted with a cubical regression  $Y_{nj} = 0,447 + 0,114 t - 0,52 t^2 + 0,004 t^3$  and the highest rate of ammoniating process occurred in week 2 of the incubation period. It was an implication that disruption of lignocellulosic fractions occurred as a result of retaining N originated from urea in C<sub>1</sub> site of reduced sugar.

## ACKNOWLEDGEMENT

The author would like to thank the Directorate General of Higher Education (DIKTI) of Indonesia Government, for financial support for the experiment under Fundamental Research Program.

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