Effect of different level of urea addition for rice straw fermentation application: in vitro evaluation

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Abstract. Rice straw is an agricultural by-product that generally used as a roughage. The fermentation process could increase the nutrient availability of rice straw. Urea addition in the fermentation process is expected to increase the digestibility. The current study aimed to evaluate the nutrient content and in vitro digestibility of fermented rice straw by different levels of urea addition. Microstar LA2 was used for the fermentation process in rice straw by 21 days. Completely randomized design with four treatments and five replications was applied in the current study. The treatments were K (fermented rice straw without urea addition), U1 (fermented rice straw with 0.15% urea addition), U2 (fermented rice straw with 0.3% urea addition) and U3 (fermented rice straw with 0.45% urea addition). The observed parameters were the nutrient content, in vitro gas production, in vitro true digestibility (IVTD) and rumen fermentation product. The results showed that there was no significant difference in ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) for all treatments. However, adding urea on the rice straw fermentation process could increase total gas production after 24h time of incubation (P<0.05). Adding 0.15% urea could increase gas production caused by fermentation of the insoluble fraction (GPNSF) by 31.62% (P<0.05). There was no significant difference between U1, U2, and U3 on GPNSF variables. Adding a minimum 0.15% urea on the rice straw fermentation process also could increase metabolizable energy (ME), organic matter digestibility (OMD), microbial protein (MP) and single-chain fatty acid (SCFA) production. It could be concluded that adding 0.15% urea on the fermentation process is enough to increase the nutrient availability and in vitro digestibility of rice straw.

1. Introduction
Rice straw is an agricultural by-product with abundant availability in Indonesia. Rice straw is often used as a ruminant roughage by small farmers. As a byproduct, it has many constraints in the nutrient absorption. Yulistiani et al. [1] reported that rice straw is characterized by low mineral, protein, and energy levels. High levels of lignin and complex carbohydrates are the primary limiting factors in rice straw [2]. The pretreatment process needs to be applied to increase the availability of energy source from rice straw. Urea is the most popularly used chemical pretreatment of rice straws due to the non-hazardous component [2].

Two main factors affect the quality of rice straw fermentation: 1) the nature of feedstuffs material, and 2) the mechanism of pretreatment[2]. Lunsin et al. [3] reported that pretreatment with 5% urea...
and 5% molasses could improve the nutritive value and in vitro fermentation of sugarcane bagasse. Yulistiani et al. [1] stated that urea treatment on rice straw could produce high dry matter intake and digestible cell wall. Urea pretreatment could be combined with microbes starter addition to accelerate the fermentation process. Samadi et al. [4] had showed that urea pretreatment combination with Trichoderma harzianum could improve the in vitro fermentation process of sugarcane bagasse. There is not much information related to the effect of different level urea addition on the digestibility of rice straw. This study aimed to evaluate the nutrient content and in vitro digestibility of fermented rice straw by different levels of urea addition.

2. Material and methods

2.1. Sample preparation
Sidenuk variety rice straw was collected from the field of Agricultural division, Center for Isotope and Radiation Application (CIRA), National Nuclear Energy Agency of Indonesia (BATAN) in January 2019. Urea, rice bran, and molasses were obtained from a local market. Rice straw was chopped to 2-3 cm, dried at 55°C for 48 h and used for fermentation substrate. MikostarLA2 was used for fermentation starter. MikostarLA2 was obtained from selected culture collections from Animal Production Laboratory, CIRA, BATAN.

2.2. Experiment design
The experiment followed a completely randomized design with four treatments and four replications. The treatments were K (fermented rice straw without urea addition), U1 (fermented rice straw with 0.15% urea addition), U2 (fermented rice straw with 0.3% urea addition) and U3 (fermented rice straw with 0.45% urea addition).

2.3. Rice straw fermentation
A total of 400 g of rice straw was put into 1 kg plastic boxes fermentor and adding 0.75% of MikostarLA2, 0.4% of rice bran, 0.2% of molasses and urea (depends on each treatment). Aquades was adding to initial the 65% moisture content, then incubated at room temperature for 21 days. Samples were collected with the respective urea treatment. All samples were dried at 55°C for 48 h and used for nutrient content analysis and in vitro incubation.

2.4. Nutrient content analysis
All samples were analyzed for ash, organic matter (OM), ether extract (EE) and crude protein (CP) using a proximate analysis procedure[5]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using a detergent fiber analysis methods [6].

2.5. In vitro incubation and analysis
The method used for in vitro evaluation was based on the gas production technique described by Menke et al [7]. Rumen liquor was obtained from 2 local cattle freshly slaughtered at a local abattoir (Ciputat slaughterhouse, South Tangerang, Banten, Indonesia). A sample of fresh rumen was filled into pre-warmed thermost flasks, sealed and transported to animal production laboratory, CIRA, BATAN. The slurry was filtered through 4 layers of nylon cloth and flushed with carbon dioxide (CO2). Rumen liquor was kept at approximately 39°C before use. The glass syringe containing a total of 200 mg DM samples was added with 30 ml rumen liquor-buffer (2:1) following Menke et al. [7]. The incubation was carried out at 39°C for 48 h. Total gas production was performed at 0, 3, 6, 9, 12, 24 and 48 incubation time. At 48 h post-incubation, samples were taken to determine the in vitro true digestibility (IVTD). Samples were heated with neutral detergent soluble (NDS) at 100°C, rinsed with acetone and filtered through pre-weighed Gooch crucibles and residual DM was estimated. The percentage loss in weight was presented as IVTD.

The gas production caused by fermentation of soluble fraction (GPSF) and insoluble fraction (GPNSF) were calculated by total gas produced at 3 and 24 h incubation time, calculated as
described by Van Gelder et al.[8]. Metabolizable energy (ME) and organic matter digestibility (OMD) was calculated from the gas production at 24 h of incubation, CP, EE and ash value, according to Menke et al. [7]. Microbial protein (MP) was calculated as described by Czerkawski[9]. Short-chain fatty acids concentration was calculated as follows Menke et al. [7].

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\begin{align*}
\text{GPSF (ml)} &= \text{(gas at 3 h x 0.99 x 5)} - 3 \\
\text{GPNSF} &= (1.02 x ((\text{gas at 24 h x 5}) - (\text{gas at 3 h x 5}))) + 2 \\
\text{ME (MJ/kg DM)} &= 2.2 + (0.136 x \text{gas at 24 h}) + (0.057 x \text{CP}) + (0.0029 x \text{EE}) \\
\text{OMD (%)} &= 14.88 + (0.889 x \text{gas at 24 h}) + (0.45 x \text{CP}) + (0.0651 x \text{ash}) \\
\text{MP (g/kg OMD)} &= \text{OMD} x 19.3 x 6.25 \\
\text{SCFA (mmol/200 mg DM)} &= (0.0222 x \text{gas at 24h}) - 0.00425
\end{align*}
\]

2.6. Statistical analysis

Ørskov and Mcdonald[10] was used to determine kinetics gas, as follows: \( p = a + b (1-e^{-ct}) \). where, \( p \) is total gas production at t time, \( a \) is gas production from soluble fraction (ml/200 mg DM). \( b \) is gas production from insoluble fraction (ml/200 mg DM). \( c \) is gas production rate constant (ml/h), \((a+b)\) is potential gas production (ml/200 mg DM) and \( t \) is the time of incubation (h). Data were analyzed using NAWAY® software.

Data were statistically analyzed using SPSS 22.00. All multiple comparisons among means were performed using Duncan Multiple Range Test (DMRT)[11].

3. Results and discussion

3.1. Nutrient content

The chemical composition of rice straw fermented with or without urea is presented in Table 1. Organic matter content of U1 and U3 were higher than control (K) (P<0.05). Otherwise, U1 and U2 had lower ash content than control treatment (P<0.05). There were no differences for EE, NDF and ADF contents among all treatments.

| Treatments | OM (%) | Ash (%) | CP* (%) | EE (%) | NDF (%) | ADF (%) |
|------------|--------|---------|---------|--------|---------|---------|
| K          | 59.08a | 40.92b  | 9.24    | 2.66   | 86.91   | 80.59   |
| U1         | 61.20b | 38.80a  | 9.51    | 2.50   | 87.74   | 81.59   |
| U2         | 60.20ab| 39.80ab | 10.41   | 3.73   | 85.38   | 79.01   |
| U3         | 61.15b | 38.85a  | 7.37    | 3.37   | 84.91   | 78.86   |
| SEM        | 0.271  | 0.271   | 0.638   | 0.253  | 0.625   | 0.828   |

Fermented rice straw without urea addition (K), fermented rice straw with 0.15% urea addition (U1), fermented rice straw with 0.3% urea addition (U2) and fermented rice straw with 0.45% urea addition (U3). Organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF). Different superscripts in the same row indicate significant differences (P<0.05). Standard Error of the Means (SEM). *CP values are without replications.

The increase in OM content could be influenced by the increase in microbial population due to the addition of MikostarLA2 supplemented by urea. The growth of bacteria, fungi, and yeast in the substrate could improve the degradation of DM to OM [12]. Otherwise, Salman et al. [12] also reported that OM could decrease due to a decrease in crude fiber content. In the previous study, there is no reduction in fiber profile (NDF and ADF) after urea addition in the rice straw fermentation process. U1 and U2 treatment had higher CP than control by 9.51 and 10.41% respectively. These results agree with Samadi et al. [4] who found that an increase in CP content probably due to the degradation of other organic materials, thus resulted in improving the concentration of CP content. Different arguments expressed by Salman et al. [12], who stated that the increase in CP was mainly
due to increasing of nitrogen content after urea addition. Yulistiani et al. [1] also reported that urea pretreatment could increased the nitrogen content. The lowest CP content performed by U3. This due to the toxic effects of urea which affect microbial activity. Samadi et al. [4] reported that the microorganism population reduced in the fermentation process due to poisonous NH$_3$ and it harmed cellulose activities.

There was no reduction of fiber content, expressed in NDF and ADF, after urea addition in the fermentation process. Yulistiani et al. [1] stated that there were small differences in cell wall components between untreated and urea treated in rice straw. This probably due to a strong and very complex bonding system in fiber components such as lignocellulose[4]. However, this needs to be observed in the following digestibility parameters.

### 3.2. In vitro gas production

Cumulative gas production, gas characteristics and gas kinetics of fermented rice straw are presented in Table 1. Gas produced after 24 h time incubation and gas produced caused by fermentation of insoluble fraction in the fermented rice straw without urea addition were significantly lower than urea treatments (U1-U3) (P<0.05). The highest gas produced caused by fermentation of soluble fraction (GPSF) was performed by U2, but not significant different with U1. The U3 treatment was also produced the highest rate of gas production (c) with a value of 0.029. However, there was no significant difference between urea and non-urea treatment on the potential extent of gas production (a+b).

| Treatments | Total gas production (ml/200 mg DM) | Gas kinetics |
|------------|------------------------------------|--------------|
|            | 3        | 6        | 9        | 12       | 24       | 48       | GPSF | GPNSF | a+b | c |
| K          | 3.24$^a$ | 5.58$^a$ | 7.02$^a$ | 8.46$^a$ | 15.13$^a$ | 23.96$^a$ | 13.05$^a$ | 62.63$^a$ | 42.07 | 0.017$^a$ |
| U1         | 3.94$^{ab}$ | 7.16$^{ab}$ | 9.48$^{ab}$ | 11.27$^{ab}$ | 19.68$^{b}$ | 30.06$^{b}$ | 16.49$^{ab}$ | 82.31$^{b}$ | 45.15 | 0.023$^{ab}$ |
| U2         | 4.89$^b$ | 8.16$^b$ | 10.33$^b$ | 13.04$^b$ | 21.02$^b$ | 30.62$^b$ | 21.22$^b$ | 84.24$^b$ | 40.49 | 0.029$^b$ |
| U3         | 3.25$^a$ | 6.68$^{ab}$ | 8.85$^{ab}$ | 11.20$^{ab}$ | 20.42$^{b}$ | 30.72$^{b}$ | 13.10$^{a}$ | 89.54$^{b}$ | 43.03 | 0.026$^b$ |
| SEM        | 0.279 | 0.411 | 0.487 | 0.621 | 0.863 | 1.048 | 1.382 | 3.615 | 0.889 | 0.002 |

Fermented rice straw without urea addition (K), fermented rice straw with 0.15% urea addition (U1), fermented rice straw with 0.3% urea addition (U2) and fermented rice straw with 0.45% urea addition (U3). The gas production caused by fermentation of soluble fraction (GPSF) and insoluble fraction (GPNSF). Different superscripts in the same row indicate significant differences (P<0.05). Standard Error of the Means (SEM). Potential gas production (ml/200 mg DM) (ml/h). Gas production rate constant (ml/h) (c).

In vitro gas production represent the energy source degradability value of each treatment. Cumulative gas production mostly results in carbohydrate source fermentation [13]. Cumulative gas production at 24 and 48 h were highest after fermentation of rice straw with urea added (P<0.05). This was caused by an increase in digestibility in structural carbohydrates due to the effect of urea addition during fermentation. This could be observed in the increasing value of GPNSF. The calculation of GPNSF is obtained from gas values produced at 24 h. This represents an increase in gas production due to fermentation from insoluble starts from 24 h incubation time. These results were in agreement with Lunsin et al. [3] who found that cumulative gas production at 24, 48 and 96 h were highest after fermentation of the sugarcane bagasse with urea and molasses added.

The value of potential gas (a+b) which is not significantly different is thought to be caused by a short incubation time. In Lunsin et al. [3] and Salem et al. [14], observation of gas production was carried out at least until 72 h incubation time. High fibrous feedstuff need longer observation to determine accurate gas kinetics, even up to 96 h incubation [15]. Urea addition could increase the fermentation rate. This could be observed by high c value in U1-U3 treatment. High c constants demonstrate that the incubated substrated had a faster fermentation rate[15].
3.3. *In vitro* digestibility, predicted of energy utilization and microbial protein

It is clear from Table 3 that urea addition in the fermentation process had a significant effect on microbial protein (MP), ME, SCFA and OMD predicted (P<0.05). However, there were no significant differences between all urea treatments. In this study, urea addition not significantly affect IVTD value.

**Table 3. In vitro** digestibility and predicted of rumen fermentation product of fermented rice straw in different urea level

| Treatments | IVTD (%) | MP (g/kg OMD) | ME (MJ/kg DM) | SCFA (mmol/200 mg DM) | OMD (%) |
|------------|----------|---------------|---------------|------------------------|---------|
| K          | 48.06    | 42.40<sup>a</sup> | 4.79<sup>a</sup> | 0.33<sup>a</sup> | 35.15<sup>a</sup> |
| U1         | 49.71    | 47.26<sup>b</sup> | 5.43<sup>b</sup> | 0.43<sup>b</sup> | 39.18<sup>b</sup> |
| U2         | 51.29    | 49.26<sup>b</sup> | 5.66<sup>b</sup> | 0.46<sup>b</sup> | 40.84<sup>b</sup> |
| U3         | 50.32    | 46.89<sup>b</sup> | 5.41<sup>b</sup> | 0.45<sup>b</sup> | 38.88<sup>b</sup> |
| SEM        | 0.837    | 0.931          | 0.119         | 0.019                  | 0.772   |

Fermented rice straw without urea addition (K), fermented rice straw with 0.15% urea addition (U1), fermented rice straw with 0.3% urea addition (U2) and fermented rice straw with 0.45% urea addition (U3). In vitro true digestibility (IVTD), microbial protein (MP), metabolizable energy (ME), single-chain fatty acid (SCFA), organic matter digestibility (OMD), dry matter (DM). Different superscripts in the same row indicate significant differences (P<0.05). Standard Error of the Means (SEM).

There are contradictory results between IVTD and OMD values. This due to OMD is calculated from cumulative gas production at 24 h incubation time, while IVTD is determined after 48 h incubation time. Blummel et al. [16]reported that IVTD from NDS treated measurement could predicted the DM intake of roughages. *In vitro* true digestibility that not differ represents an imbalance between energy and nitrogen sources to support microbial growth [4]. An increase in urea addition must be balanced with an increase in molasses percentage component. Lunsin et al. [3]found that a combination of 5% urea and 5% molasses treatment of sugarcane bagasse could improve DM and OM digestibility. However, increasing microbial activity in the present study represented by MP and SCFA values. In this study, the nutrient value of rice straw obtained by indirect mechanisms. Urea addition would provide fermentable nitrogen for stimulating rumen microbial fermentation [14]. Single chain fatty acids are representations of the energy conversion from feedstuffs into energy required by microbes [16].

4. Conclusion

The results of the current study indicated that adding 0.15% urea on the fermentation process could improve the nitrogen value and *in vitro* digestibility with increase OM content, cumulative gas production, fermentation rate, microbial protein, metabolizable energy, and SCFA value. Further study should be done to investigate the effects of urea treated for rice straw fermentation in ruminant production performance (*in vivo* study).

5. References

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