The effects of fermentation using gamma-irradiated 
*Aspergillus niger* and adding rice bran on rice straw digestibility: *in vitro* study

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**Abstract.** This study aimed to evaluate the *in vitro* digestibility of rice straw fermented with gamma-irradiated *Aspergillus niger*. This study was also determined to evaluate the *in vitro* digestibility of the combination fermented rice straw and rice bran as ruminant rations. A 500 Gy gamma-irradiated *A. niger* was used for the fermentation process in rice straw by 21 days. Completely randomized design with six treatments and four replications was applied in this study. The treatments were: JNF (rice straw), JF (fermented rice straw), JNF 85 (85% rice straw + 15% rice bran), JF 85 (85% fermented rice straw + 15% rice bran), JNF 70 (70% rice straw + 30% rice bran), JF 70 (70% fermented rice straw + 30% rice bran). The observed variables were the nutrient content, *in vitro* gas production and *in vitro* true digestibility (IVTD). The results showed that fermentation treatment by gamma-irradiated *A. niger* could reduce ADF content of rice straw (P<0.05). however, there was no significant difference in NDF content. JF 70 treatment produced the highest *in vitro* gas production (P<0.05). JF 70 also produced the highest IVTD (P<0.05). in conclusion, fermentation treatment by gamma-irradiated *A. niger* could reduce ADF content and increase IVTD. Adding rice bran in ruminant rations was also increase the availability of soluble nutrients for rumen microbes.

1. Introduction

Rice straw is a source of carbohydrate commonly used as ruminant ration ingredients in tropical countries. However, the complexity of carbohydrate bonds in rice straw was also inhibiting the conversion of energy from plants to animals [1]. Pretreatments are necessary to increase energy conversion by increasing the nutrients utility. Malik et al. [2] reported that chemical, physical and biological pretreatments are used to increase nutrient availability, thus increasing daily feed intake and digestion rate of straw. Using fibrinolytic and ligninolytic fungi may be one potential alternative to provide a more practical approach for enhancing the nutritive value of rice straw [3].

Sasongko *et al.* [4] reported that the fermentation process by *Trichoderma viride* and *Phanerochaete chrysosporium* could increase the availability of glucose compounds from rice straw. Additives, especially *Lactobacillus Plantarum* are necessary to improve the quality of rice straw silage and reducing *in vitro* ruminal CH₄ production [5]. Fungal treatment (*Cyathus stercoraceus*) increased fiber digestibility by increasing the availability of cellulose [6]. Wahyono *et al.* [7] reported
that gamma-irradiated *A. niger* fermentation in fine particle size rice straw could *increase in Sacco* degradability. Some previous studies stated that *A. niger* is a cellulolytic microorganism that has the potential to be used as a fermentation agent to increase energy utilization of rice straw. Gamma irradiation treatment could increase some enzyme activities in microorganism. Kusumaningrum *et al.* [8] reported that 500 kGy irradiation dose was the best dose to increase cellulose activity of *A. niger* inoculant. A 500 kGy gamma irradiation treatment also affected glucose substrate production [9]. Therefore, gamma-irradiated *A. niger* can be utilized as fermentation able that able to improve the digestibility of rice straw.

The addition of soluble carbohydrate in the ruminant rations is necessary to increase the availability of energy. Rice bran is a commonly non-structural carbohydrate source in ruminant rations. Rice bran supplementation in treating rice straw could increase buffalo live weight gain [10]. There is not much information about the degradability of fermented rice straw and rice bran combination as ruminant rations. Therefore, this study aimed to evaluate the *in vitro* digestibility of rice straw fermented with gamma-irradiated *A. niger*. The second object was to evaluate the *in vitro* digestibility of fermented rice straw and rice bran as ruminant rations.

### 2. Material and Methods

#### 2.1 Sample preparation

Rice straw was collected from the experiment field in Center of Isotope and Radiation Application, South Jakarta, Indonesia. Rice bran was collected from the local feed store in South Jakarta, Indonesia. The materials were dried at 60°C for 48 h and ground to 2 mm size and stored in plastic bags at 4°C for further nutrition analyses and fermentation treatment.

#### 2.2 Rice straw fermentation

Rice straw as substrate, nutrient solution, and *A. niger* inoculum were prepared for the fermentation process. Nutrient solution was prepared using procedures in Wahyono *et al.* [8]. *Aspergillus niger* inoculum was irradiated using $^{60}$Co source gamma irradiator at a dose of 500 Gy. Fermentation processed using procedures in Wahyono *et al.* [11]. A total of 200 ml nutrient solution and 10 g *A. niger* inoculum were dissolved into 200 ml distilled water. The solution was mixed with 200 g rice straw substrate, thus incubated into a 5 kg plastic bag for 21 d. After the fermentation process, the materials were dried at 60°C for 48 h and ground to 1 mm size for nutrient analyses and *in vitro* incubation. Dry matter (DM), organic matter (OM), ash and ether extract (EE) of fermented and non-fermented rice straw were analyzed by AOAC [12] methods. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed using Van Soest *et al.* [13] procedure.

#### 2.3 Rice straw fermentation

The experimental design used was completely randomized design with six treatments and four replications. The treatments were: JNF (rice straw), JF (fermented rice straw), JNF 85 (85% rice straw + 15% rice bran), JF 85 (85% fermented rice straw + 15% rice bran), JNF 70 (70% rice straw + 30% rice bran) and JF 70 (70% fermented rice straw + 30% rice bran).

#### 2.4 In vitro analysis

The *in vitro* gas production and digestibility were determined according to Menke *et al.* [14] procedure, modified by Blümmel *et al.* [15]. A 380 mg of each sample was weighed into a 100 ml calibrated glass syringe (Model Fortuna, Germany). Media included 33% rumen fluid from fistulated buffalo given a 50:50 native grass: concentrate diet. The glass syringe containing the sample was incubated at 39°C for 48 h. Gas production was measured after 2, 4, 6, 8, 10, 12, 24 and 48 h of incubation.

#### 2.5 Chemical analysis

Once the incubation was finished, rumen fermentation products (pH, NH$_3$ and VFA) were measured immediately. The pH was measured immediately using a pH meter Hanna instrument. The NH$_3$ was
measured according to microdiffusion Conway methods [16]. Analysis of VFA production performed by steam distillation methods [12]. In vitro true digestibility also measured after 48 h incubations. The sample was extracted in 100 ml neutral detergent solution (NDS) for 75 minutes and rinsing with aquades (90°C), thus rinsing with enough acetone. When extraction was complete, dried for 24 h at 105°C to estimate IVTD.

2.6 Statistical analysis
Data were analyzed using One Way ANOVA procedure of SPSS 16.00. All comparisons among means were performed using Duncan’s Multiple Range Test (DMRT) [17].

3. Result and discussion
3.1 Nutrient and fiber content of rice straw
Nutrient and fiber content parameters were observed to determine differences in nutrient composition due to fermentation treatment by irradiated A. niger. There was no significant difference between fermented and untreated rice straw on organic matter, ash and NDF content (Table 1). However, the fermentation process by irradiated A. niger could change the content of dry matter, EE and ADF. Fermentation treatment could increase dry matter level (P<0.05). Otherwise, the level of ADF was degreased (P<0.05).

| Nutrient content (%) | Substrate | Non-fermented rice straw | Fermented rice straw | SEM |
|----------------------|-----------|--------------------------|----------------------|-----|
| Dry matter*          |           | 93.27<sup>a</sup>        | 95.72<sup>b</sup>    | 0.579 |
| Organic matter (OM)  |           | 78.09                    | 78.52                | 0.709 |
| Ash                  |           | 21.91                    | 21.48                | 0.709 |
| Ether extract (EE)   |           | 0.35<sup>b</sup>         | 0.34<sup>a</sup>     | 0.003 |
| Neutral detergent fiber (NDF) | | 77.69 | 77.25 | 0.158 |
| Acid detergent fiber (ADF) | | 70.00<sup>b</sup> | 68.28<sup>a</sup> | 0.949 |

*dry matter after sample dried in 60°C oven at 48 h. Different superscripts in the same column indicate significant differences (P<0.05). Standard Error of the Means (SEM).

The increased of DM content post-fermentation treatment due to the water content reduction after the fermentation process. Water content used as a critical component medium for A. niger growing. Wahyono et al. [11] reported that DM content increases as a result of cellulose degradation by A. niger fermentation process. The same results were reported by Azhari et al. [18] that DM content of millet flour was increased after the fermentation process. Munier et al. [19] also stated that the DM level of cocoa pod husk was increased after the fermentation process by A. niger. On the contrary, Jahromi et al. [20] reported that biological treatment using white-rot fungi could reduce DM composition as the result of a long incubation period. This difference could be caused by incubation periods and different treatments in the incubation process. Goeser et al. [21] stated that the DM losses had a relationship with the fermentation-length.

The EE content was decreased after the fermentation process (Table 1). This due to EE content has been used for reservation of energy source [19]. Reducing ADF content represent the reduction of structural carbohydrates component. Aspergillus niger as a fermentation agent consumed structural carbohydrates and converting to soluble sugars. Munier et al. [19] reported that A. niger produced many enzymes, such as mannanase, cellulose, and other carbohydrate degradation enzymes. These enzymes were useful to decompose fiber fraction. The fermentation process by A. niger could improved the nutritive value by decreasing the fiber fractions (NDF and ADF) [22]. Zhao et al. [5] reported that structural carbohydrate components reduced by fermentation process and changed to nonstructural carbohydrate contents (water-soluble carbohydrates, glucose, fructose, xylose, and sucrose). The fermentation process does not affect the NDF levels. This could due to A. niger plays a
specific role in degrading cellulose, not hemicellulose. Sarnklong et al. [3] reported that A. niger had cellulase enzyme to increase degradability of grass hay.

3.2 In vitro total gas production and digestibility

The initial hypothesis of this experiment is that changes in nutrient content in rice straw (after fermentation) and the addition of soluble carbohydrate sources (rice bran) will affect the digestibility of feed. The information of rice bran nutrient content could be seen in Table 2. The in vitro total gas production (Table 3), rumen fermentation products and IVTD (Table 4) represent the digestibility of the substrate.

There were not many differences between total gas production in JNF and JF treatments at 2-48 h incubation time. However, fermentation pretreatment could increase total gas production in rations based 15% rice bran after 6 h incubation. While in rations based 30% rice bran just increased after 24 h incubation time. The treatments of JF and JNF was not significant different until 48 h incubation time due to there was a fraction that has not been degraded. The differences may be seen if observations are continued until > 48 incubation time. This due to the treatment containing fermented rice straw contains lower ADF content (Table 1). The higher total gas production on rations based fermented rice straw also indicates that this treatment did not produce an inhibitor that inhibits the fermentation mechanism by rumen microbes [23].

Table 2. Nutrient content of rice bran

| Nutrient content (%) | Dry matter* | Organic matter | Ash | Ether extract | NDF | ADF |
|----------------------|-------------|----------------|-----|---------------|-----|-----|
| Rice bran            | 93.10       | 81.06          | 18.94| 3.36          | 76.54| 50.49|
|*dry matter after sample dried in 60°C oven at 48 h. Neutral detergent fiber (NDF), acid detergent fiber (ADF). |

Table 3. In vitro total gas production of treatments (ml/380 mg DM)

| Treatment     | Incubation periods (h) | 2 | 4 | 6 | 8 | 10 | 12 | 24 | 48 |
|---------------|------------------------|---|---|---|---|----|----|----|----|
| JNF           | 1.82bc                 | 3.65h | 3.78h | 4.69h | 5.60h | 6.25h | 10.55h | 22.93h |
| JF            | 1.04a                  | 2.22a | 2.35a | 2.87a | 3.52a | 3.91a | 10.56a | 26.07ab |
| JNF 85        | 2.46bc                | 4.02bc | 5.58bc | 6.61bc | 7.65bc | 8.69bc | 16.47bc | 28.01bc |
| JF 85         | 3.13a                | 5.34cd | 8.21e | 10.69d | 12.77d | 14.96d | 26.85c | 39.89a |
| JNF 70        | 3.26cd               | 5.60d | 7.82c | 10.55d | 13.16d | 16.42e | 31.66d | 48.86d |
| JF 70         | 2.68bc               | 4.68bc | 6.82d | 10.17d | 12.71d | 15.92de | 34.25d | 54.59d |
| SEM           | 0.134                | 0.200 | 0.367 | 0.432 | 0.574 | 0.665 | 1.357 | 1.811 |

JNF (rice straw), JF (fermented rice straw), JNF 85 (85% rice straw + 15% rice bran), JF 85 (85% fermented rice straw + 15% rice bran), JNF 70 (70% rice straw + 30% rice bran) and JF 70 (70% fermented rice straw + 30% rice bran). Different superscripts in the same row indicate significant differences (P<0.05). Standard Error of the Means (SEM).

The insoluble components mainly contribute to the in vitro total gas production after the high contribution from soluble components [23]. After 6 h, the treatments with rice bran addition produced higher total gas than rice straw as a single feed. It was demonstrated that the degradation process of substrates by rumen microbials was effective due to soluble carbohydrate (rice bran) addition. Munier et al. [19] reported that the substrate contains low fiber content that could accelerate the degradation process, faster than high fiber content. On the contrary, another study reported that the addition of purified starch source had negatively affected the in vitro microbial fermentation of straw [24]. The main factors were: 1) competition with amylolytic bacteria and 2) depression of fibrinolytic bacteria in the presence of high glucose concentrations. In this experiment, it could be seen in under 4 h incubation periods. In vitro total gas produced through 4 h incubation time has been considered to fermentation of soluble/non-structural carbohydrate [25]. Furthermore, the free sugars
produced by the fungal degradation of the structural carbohydrates might contribute to the soluble fraction in the straw [23].

The highest in vitro total gas production (at 48h) produced by JF 70 treatment. This indicated that JF 70 rations had highly degradability. This was confirmed by the highest IVTD value of JF 70 (Table 4). Kondo et al. [26] reported that total gas production had a significant correlation with single chain fatty acids (SCFA) (r = 0.96). Rumen fermentation characteristics depend on pH value. Ruminant rations with low pH significantly reduce the activity of ruminal microorganisms (pH < 6) [20]. In the previous study, the ruminal pH between 6.96 – 7.09, thus they are still within the acceptable range of ruminal microorganisms environment.

The JNF 70 and JF 70 treatments had higher NH₃ concentration than other treatments. Ammonia (NH₃) value depends on protein content and its degradability. Tampoebolon et al. [27] also reported that the protein degradation reflected in organic matter digestibility. Degradability mechanism is used for microbial protein synthesis. There was no significant difference between fermented and non-fermented rice straw on NH₃ concentration, both as single feed or as ingredients in the rations. This due to the possibility of an inhibitor for protein degradability that is produced during the fermentation process. Niu et al. [23] reported that some nitrogen in fungal cell was incorporated in chitin. Furthermore, it was reported that chitin and its derivative could reduce the NH₃ concentration in rumen fluid.

### Table 4. Rumen fermentation products and IVTD of treatments

| Treatment | pH   | NH₃ (mM) | VFA (mM) | IVTD (%) |
|-----------|------|----------|----------|----------|
| JNF       | 7.04<sup>bc</sup> | 3.66<sup>a</sup> | 136.12<sup>a</sup> | 69.59<sup>a</sup> |
| JF        | 7.09<sup>d</sup> | 4.02<sup>a</sup> | 155.92<sup>b</sup> | 70.53<sup>b</sup> |
| JNF 85    | 6.96<sup>a</sup> | 5.28<sup>bc</sup> | 163.35<sup>b</sup> | 71.95<sup>ab</sup> |
| JF 85     | 7.07<sup>cd</sup> | 5.10<sup>b</sup> | 168.30<sup>b</sup> | 74.71<sup>cd</sup> |
| JNF 70    | 7.03<sup>bc</sup> | 5.58<sup>cd</sup> | 153.45<sup>b</sup> | 73.01<sup>bc</sup> |
| JF 70     | 6.99<sup>ab</sup> | 5.76<sup>d</sup> | 160.87<sup>b</sup> | 75.79<sup>d</sup> |
| SEM       | 0.011 | 0.143    | 2.610    | 0.464    |

JNF (rice straw), JF (fermented rice straw), JNF 85 (85% rice straw + 15% rice bran), JF 85 (85% fermented rice straw + 15% rice bran), JNF 70 (70% rice straw + 30% rice bran) and JF 70 (70% fermented rice straw + 30% rice bran). Ammonia concentration (NH₃), volatile fatty acids (VFA), in vitro true digestibility (IVTD). Different superscripts in the same row indicate significant differences (P<0.05). Standard Error of the Means (SEM).

The optimum VFA production is needed to support ruminant productivity [27]. Volatile fatty acids also directly reflects the digestibility of feed [23]. The fermentation process and rice bran addition significantly increased VFA production (Table 4). This reflects that the fermentation process and the addition of soluble carbohydrates could increase the digestibility of carbohydrates. Niu et al. [23] reported that the biological treatment of straw not only increased total gas production but also VFA production. The treatment of JF 70 produced the highest IVTD (P<0.05). This related to in vitro total gas value (Tabel 3) and the lower structural carbohydrate (ADF) content after fermentation process (Table 1). Wahyono et al. [11] also stated that fermentation treatment by A. niger could increase in Sacco degradability of rice straw. Malik et al. [2] stated that rice straw pretreatment results in degrading cellulose content which improve its nutrient value.

### 4. Conclusion

The results of this study demonstrated that fermentation treatment by gamma-irradiated A. niger could reduce ADF content of rice straw. In vitro digestibility was also increased due to the availability of easily degradable carbohydrates. Thus, adding rice bran in ruminant ration based fermented rice straw could increase the availability of soluble nutrients for rumen microbes.
5. References

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6. Acknowledgments

The authors wish to acknowledge funding provided by the Animal Nutrition Laboratory, Center for Isotope and Radiation Application, National Nuclear Energy Agency of Indonesia. We also thank to Ir. Suharyono, M.Rur. Sci for technical support.