Fiber Content and Relative Feed Value Estimation of Gamma Irradiated Rice Straw

Firsoni¹, Shintia Nugrahini Wahyu Hardani¹ and Teguh Wahyono¹*

¹Agricultural Division, Center for Isotopes and Radiation Application, National Nuclear Energy Agency, Lebak Bulus 49, Jakarta 12440, Indonesia

*Corresponding author: teguhwahyono@batan.go.id

Abstract. Ionizing irradiation is an alternative treatment to reduce the cell wall constituents and improve the nutritional value of substrates. The purpose of this research was to evaluate the fiber content and relative feed value of rice straw after gamma irradiation treatment. Rice straw was obtained from Sidenuk rice variety. Two polyethylene packages of samples were irradiated in Irradiator Karet Alam (IRKA), Indonesia at doses of 5 and 10 kGy in the presence of air. Treatments includes untreated/unirradiated, 5 kGy and 10 kGy irradiated rice straw. The observed parameters were neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter digestibility (DMD), dry matter intake (DMI) and relative feed value (RFV) estimation. The change of fiber content was also qualitatively observed by Fourier Transform Mid-Infrared (FTIR). This research was arranged into a completely randomized block design with five replications. The results showed that there was no difference in NDF and ADF content between all treatments. There was also no difference in RFV due to the results of NDF and ADF measurements. Interestingly, there was a strong absorption with a wide and strong band centred at 3331 cm⁻¹ (O-H stretch) only observed in the untreated sample. Meanwhile, a peak signal was observed at 1722 cm⁻¹ only in 5 kGy and 10 kGy irradiated rice straw spectra. This could affect in the increased of hemicellulose compounds after irradiated treatments. It can be concluded that gamma irradiation treatment at doses of 5 and 10 kGy has not been able to influence the fiber content and relative feed value of rice straw. However, there was a tendency for hemicellulose compounds increasingly due to irradiation treatment.

Keywords: Fiber content, FTIR, gamma irradiation, relative feed value, rice straw.

1. Introduction

The high level of lignification and the slow ruminal degradation of carbohydrates fraction are the main deficiencies of rice straw as ruminant’s feed [1]. The complexity of carbohydrate bonds was also inhibiting the conversion of energy from plants to livestock. Wanapat et al. [2] reported that the lack of nutrients utilization in rice straw due to the low passage rate through rumen, the low disappearance rate and the low intake. Based on those findings, there were several studies have been reported on the physical, biological and chemical treatments in rice straw to improve the utilization as ruminant’s feed in past-present years.

The physical processing methods which material exposed to ionizing radiation has been recognized as a safe method and reliable to improve the nutritive value and digestibility of sweet sorghum bagasse [3,4], sorghum straw [5], soybean meal [6], corn stalk [7] and some agricultural residues[8,9]. The aim of irradiation treatment in several previous studies was to break the ligno-cellulose or ligno-
hemicellulose bond to increase the digestibility and nutritional value of feed stuffs. There was only little information about the effects of gamma irradiation on fiber fraction and RFV estimation in rice straw. There was also only little literature about qualitative measurements by FTIR on rice straw after pre-treatment. Therefore, the purpose of this research was to evaluate the fiber content and relative feed value of rice straw after gamma irradiation treatment.

2. Material and Methods

2.1 Sample Preparation
Rice straw was harvested at the field station of Agricultural Division, Center for Isotopes and Radiation Application (CIRA), National Nuclear Energy Agency (BATAN) in October 2018. All samples were chopped to 2-3 cm, and then dried at 60°C for 48 hours to obtain dry samples. Afterward, samples were ground to pass a 1 mm screen. Gamma irradiation was carried out in a cobalt-60 irradiator in CIRA, BATAN. Two polyethylene packages of samples were irradiated in Irradiator Karet Alam (IRKA) at doses of 5 and 10 kGy in the presence of air.

2.2 Fiber Measurements
Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were observed in this study. The NDF and ADF compound were analyzed using the procedure in Van Soest et al. [10]. 250 mg samples was dissolved into ± 100 neutral detergent soluble (NDS) (30 g sodium dodecyl sulfate; 18.61 g ethylenediaminetetraacetic/EDTA; 6.81 g sodium borate; 4.56 sodium phosphate dibasic and 10 ml triethylene glycol in 1 L destilled water) for 60 minutes at 100°C. Afterwards, samples rinsed using acetone and hot water (70-90°C). The samples then filtered using Robu® crucible filter glass (50 ml-Por. 1, Germany) to obtain NDF fraction.

Samples of 250 mg also dissolved into ±100 acid detergent soluble (ADS) (20 g cetyltrimethylammonium bromide in 1 L H₂SO₄ 1 N) for 60 minutes at 100°C. Afterwards, the samples were rinsed using acetone and hot water (70-90°C). The samples then filtered off using Robu® crucible filter glass (50 ml-Por. 1, Germany) to obtain ADF fraction. Hemicellulose fraction obtained from calculation of NDF – ADF.

2.3 FTIR Measurements
KBr disc was prepared with the concentration of ± 2 mg sample and 200 mg KBr, mixed quickly and homogeneously using mortar. The sample was measured using IRPrestige-21 Fourier Transform Mid-Infrared (FTIR) Shimadzu® Japan within the range of 4000-500 cm⁻¹ in 60 seconds. Shimadzu IR solution 1.50 (Shimadzu Corporation) was used to determine the peak positions. The results were then processed using OriginLab® software.

2.4 RFV Estimation
Relative feed value of rice straws was calculated as following [11]:

\[ \text{Dry matter digestibility (\% DMD)} = 88.9 - (ADF\% \times 0.779) \]
\[ \text{Dry matter intake (\% live weight, DMI)} = 120/(NDF\%) \]
\[ \text{Relative feed value (RFV)} = (\text{DMD x DMI})/1.29 \]

RFV scores were assessed according to the Quality Grading Standard (The Hay Marketing Task Force of the American Forage and Grassland Council) [12], based on class: prime (>151); 1(premium, 151-125); 2 (good, 124-103); 3 (fair, 102-87); 4 (poor, 86-75); 5 (reject, <75).

2.5 Data Analysis
Data of NDF, ADF, DMD and DMI were analysed using a completely randomized block design with five replications. Data were analysed using analysis of variance (ANOVA) by SPSS 22.0 with the following statistical model of \( Y_{ij} = \mu + \alpha_i + e_{ij} \), where \( Y \) is the dependent variable, \( \mu \) the overall mean,
The gamma irradiation effect [13] and \( \varepsilon_{ij} \) is the residual error. Differences among treatments were separated using Duncan Multiple Range Test.

3. Result and Discussion

3.1. Fiber Content

Table 1 shows the results of NDF, ADF and hemicellulose compounds in rice straw. There was no effect of gamma irradiation on NDF and ADF content. However, the dose levels of 5 and 10 kGy was able to increase hemicellulose content percentage by 24.04 and 19.26 % (P<0.01). FTIR spectra for fiber content after gamma irradiation treatments are shown in Fig. 1 and Table 2, respectively. FTIR measurements were carried out to determine spectrum suspected be related to cellulose, hemicellulose and lignin. A band due to free esters and acids C=O stretch are observed at 1722.43 and 1728.22 cm\(^{-1}\) in 5 and 10 kGy treatments, respectively. A weak signal at 1159.22 and 1157.29 are observed and related to C–O–C asymmetrical stretching.

Table 1. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose compounds.

| Parameters   | 0 kGy | 5 kGy | 10 kGy | SEM |
|--------------|-------|-------|--------|-----|
| NDF          | 68.67 | 74.05 | 73.61  | 0.988|
| ADF          | 42.56 | 41.62 | 42.46  | 1.149|
| Hemicellulose| 26.12\(^a\) | 32.42\(^b\) | 31.15\(^b\) | 0.872|

Standard error mean (SEM); Means with different superscripts within column in same parameters were different.

The expectation in present study was to change the fiber fraction (NDF and ADF) after 5-10 kGy doses of irradiation treatments. However, the dose of 5-10 kGy has not been able to change the lignin-cellulose-hemicellulose bond that represented in NDF and ADF content. In previous study, there is general agreement that the metabolizable energy of macronutrients (carbohydrate) is unaffected by radiation doses up to 10 kGy [5]. Shawrang et al. [6] reported that there were no effect on chemical composition of soybean meal after gamma irradiation treatment for 25-75 kGy. Previous study indicated that gamma irradiation processing at doses of 10 kGy has not been able to influence the NDF, ADF and acid detergent lignin (ADL) of rice straw [7]. In previous study, the gamma dose of 10 kGy was only effective in reducing microbial contamination and then proceed with ammoniation treatment to increase fiber content degradation. Wahyono and Firsoni [8] stated that NDF and ADF changed significantly.
after gamma irradiation at doses > 50 kGy. Based on those findings, the attempt that must be done include: 1) increasing the dose of radiation and 2) combined gamma irradiation (based on microbial sterilization) with enzymatic or chemical process to increase the fiber digestibility and glucose yield.

The appearance of 1722 and 1159 cm\(^{-1}\) spectrum were obtained due to the changes of ligno-hemicellulose or ligno-cellulose matrix structures after radiation (Fig. 1). This has not affected the NDF and ADF fractions but could increase the percentage of hemicellulose (Table 1). In some spectrums, gamma irradiation doses at 5 and 10 kGy have no effect on functional groups O-H in plane bending, C-H vibration, C – O, C = C, and C – C – O stretching. This could be seen from the peak of 1431-1423, 1321-1319 and 1072-1062 cm\(^{-1}\) spectrum which are consistent after the radiation treatment. Peaks determining during 1431-1423, 1321-1319 and 1072-1062 cm\(^{-1}\) are characteristics of cellulose-hemicellulose-lignin polymer compounds [6]. There were also no changes or shifts near 1514 and 1637 cm\(^{-1}\) wavenumbers which associated with lignin compounds [6]. Those findings showed no degradation of cell wall matrix, especially in lignocellulose complex. Further study is required to understand this observation.

### Table 2. Assignments of wavenumber bands of the FTIR measurement in irradiated rice straw.

| Functional Group | Polymer                     | Wavenumber (cm\(^{-1}\)) | Ref |
|------------------|-----------------------------|---------------------------|-----|
| O-H              | Hemicellulose               | 3331.07 / 3282.84 / 3277.06 | [5] |
| Free esters and acids C=O | Lignin | 1637.56 / 1653.00 / 1658.78 | [6] |
| C=O stretching (unconjugated) | Lignin | 1516.05 / 1514.12 / 1514.12 | [6] |
| O-H in-plane bending | Cellulose, hemicellulose, lignin | 1423.47 / 1427.32 / 1431.18 | [6] |
| C–H vibration, O–H in-plane bending | Cellulose, hemicellulose, lignin | 1321.24 / 1319.31 / 1321.24 | [6] |
| C–O asymmetrical stretching | Cellulose, hemicellulose | 1062.78 / 1060.85 / 1072.42 | [6] |
| Glycosidic linkage | Cellulose, hemicellulose | 904.61 / 902.69 / 902.69 | [7] |

### 3.2. Relative Feed Value (RFV) Estimation

Table 3 shows the estimation results of DMD, DMI and RFV of rice straw. There were no significant differences between all treatments in DMD and DMI. RFV represents digestibility (from % ADF) and intake potential (from % NDF) from feed stuffs. RFV is a simple empirical prediction system that fundamentally predict DMI and DMD that designed to help ranking of varying classes of feed stuffs [12]. Untreated rice straw had the higher RFV than irradiation treatments. However, all treatments are included in poor – reject class (≤ 75 value).

### Table 3. Dry matter digestibility (DMD), dry matter intake (DMI) and relative feed value (RFV) estimation.

| Parameters          | 0 kGy | 5 kGy | 10 kGy | SEM |
|---------------------|-------|-------|--------|-----|
| DMD (% DM)          | 55.75 | 56.47 | 55.83  | 1.155 |
| DMI (% live weight) | 1.75  | 1.62  | 1.63   | 0.030 |
| RFV                 | 75.94 | 71.11 | 70.78  | 2.573 |

Standard error mean (SEM)
The dose of 5 and 10 kGy could increase DMD estimation numerically but no significant difference. This due to no significant difference in ADF content (Table 1). The DMI estimation was also no significant due to no difference in NDF content. In present study, the DMD of rice straw was around 55.75-56.47 % DM. Meanwhile, DMI value was around 1.62-1.75 % live weight). Wanapat et al. [2] reported that the average of DMD and DMI of rice straw are 49% and 1.43 % live weight, respectively. This different was due to some limitations in the calculations include: 1) NDF and ADF are the only fractions used in this calculation, 2) there are no calculations affected by animal performance and 3) there are no calculation that consider the lignin content.

Al-Masri and Zarkawi [8] reported that there was no significant difference in in vitro organic matter digestibility (IVOMD) in barley straw and sorghum straw after gamma irradiation processing at doses of 5 and 20 kGy. The IVOMD increased after treatments at > 50 kGy doses. In previous study, the high dose of gamma irradiation could change the degradation of NDF, ADF and other fiber contents in substrates. Gamma irradiation treatments (100 kGy) could increase DM, OM and NDF degradation of sorghum bagasse due to the break down mechanism of β-1,4-cellulose branch chain [14]. Wahyono et al. [4] reported that the dose of 150 kGy gamma irradiation could increase the DMD of sorghum straw. The RFV in all treatments are around 70.78-75.94. According to the RFV index, it was found that the utilization of rice straw as feed stuffs must be combined with the energy and protein source ingredients to supply the ruminant’s fed requirements [15,16]. Sarnklong et al. [1] also stated that feeding only rice straw does not provide enough nutrients for high level production ruminant’s due to the low nutritive value. The higher of RFV, the fewer amounts will be needed to feed up based on nutrient requirements for ruminant’s [17].

4. Conclusion
The results of present study indicated that gamma irradiation processing at doses of 5 and 10 kGy has not been able to influence the fiber content of rice straw. There was also no difference in RFV due to the results of NDF and ADF measurements. Based on FTIR analysis, there were two strong peaks obtained after gamma irradiation treatments (1728-1722 and 1159-1157 cm⁻¹). This proves, there is a tendency for increasing hemicellulose compounds due to irradiation treatment. Subsequently, further study to determine the effect of ionizing radiations (>10 kGy) combination with enzymatic process is required to decreased the fiber compounds.

Acknowledgments
Authors are grateful to the National Nuclear Energy Agency of Indonesia (BATAN) for the irradiation operations. Authors also acknowledges Mr. Dedi Ansori for preparing rice straw samples and Mr Dian Priyadi Perkasa, M.Biotech for laboratory assistance. The research work was financially supported by Center of Isotope and Radiation Application (CIRA), Indonesia.

References
[1] C. Sarnklong, J.W. Cone, W. Pellikaan, and W.H. Hendriks. 2010. Asian-Australasian J. Anim. Sci. 23 680.
[2] M. Wanapat, S. Kang, N. Hankla, and K. Phesatcha. 2013. African J. Agric. Res. 8 1677.
[3] T. Wahyono and Firsoni. 2016. J. Ilmu Apl. Isot. Rad. 12 69.
[4] T. Wahyono, N. Lelananingtyas, and Sihono. 2017. Atom Indones. 43 35.
[5] T. Wahyono, W. Apriliani, A. Muawanah, and Sihono. 2017. J. Ilmu Apl. Isot. Rad. 13 87.
[6] P. Shawrang, A. Nikkhah, and A. Zare-shahneh. 2007. Anim. Feed Sci. Technol. 134 140.
[7] S. Bancomrdhevakul. 2002. Radiat. Phys. Chem. 64 417.
[8] M.R. Al-Masri and M. Zarkawi. 1999. Appl. Radiat. Isot. 50 883.
[9] D.L. Sills and J.M. Gossett. 2012. Biotechnol. Bioeng. 109 353.
[10] P.J. Van Soest, J.B. Robertson, and B.A. Lewis. 1991. J. Dairy Sci. 74 3583.
[11] D.A. Rohlweder, R.F. Barnes, and N. Jorgensen. 1978. J. Anim. Sci. 47 747.
[12] U. Kilic and E. Guilecyuz. 2017. Open Life Sci. 12 206.
[13] R.G.D. Steel and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw, New York, USA.
[14] M.S. Taipina, M.L. Garbelotti, L.C.A. Lamardo, S. Josefina, and M.A.B. Rodas. 2011. Proceed. Food Sci. 1 1992.
[15] F. Xu, J. Yu, T. Tesso, F. Dowell, and D. Wang. 2013. Appl. Energy 104 801.
[16] D. Fekadu, M. Walegen, and G. Terefe. 2017. J. Biol. Agric. Healthc. 7 57–60.
[17] M.J. Kassim, M.H. Hussin, A. Achmad, N.H. Dahon, T.K. Suan, and H.S. Hamdan, Majalah Farm. Indones. 22 50.