The Effect of Gamma Irradiation on Nutrient Profiles and Total Gas Production *In Vitro* of Two Sorghum Straw Variety

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ABSTRACT

The aim of the current work is to investigate the effects of some levels of gamma irradiation doses on nutrient composition and total gas production *in vitro* of two sorghum straw variety. The level doses 0, 50, 100 and 150 kGy from cobalt-60 gamma rays irradiator was used to treate sorghum straw. Variables measured were nutrient composition, *in vitro* total gas production, *in vitro* gas characteristics and rumen fermentation products after 72 h incubation times. Experimental design of this study was 4 x 2 factorial design with irradiation dose as the first factor and sorghum variety as the second factor. All treatments were analyzed in three replicates. The results show that gamma irradiation doses of 150 kGy reduced neutral detergent fiber (NDF) of Pahat and Samurai 2 sorghum straw by 5.10 and 3.01% ($P<0.05$). Gamma irradiation doses of 150 kGy also reduced acid detergent fiber (ADF) of Pahat and Samurai 2 sorghum straw by 12.56 and 8.74% ($P<0.05$). The dose of 100 and 150 kGy increased total gas production at 72 h incubation of Pahat straw ($P<0.05$). Gamma irradiation had no effect on the total gas production of Samurai 2 straw. Gamma irradiation pretreatment was capable of improving sorghum straw nutrient profiles. Further study is needed to determine degradability and economically benefits.

Keywords: Gamma irradiation, *In vitro*, Nutrient profiles, Sorghum straw, Total gas production

INTRODUCTION

Pahat and Samurai 2 sorghum variety are some varieties produced by Indonesia National Nuclear Energy Agency (BATAN) in 2014. Samurai 2 is derived from breeding by irradiation mutation from Pahat variety seeds. Both varieties is a type of sorghum for food (Sihono *et al*., 2013). The use of sorghum grain as food leaves agricultural residues in the form of straw. Sorghum straw can be used as a roughage source for ruminant, unfortunately, these roughages contain high amounts of lignocellulose and ligno-hemicellulose as other agricultural residues. This makes an effect in low digestibility and poor nutrient elements for feed utility.

Several methods have been used to improves degradability and digestibility of agricultural residues. Dogaris *et al*. (2009) studied using *Neurospora crassa* and *Fusarium oxysporum* fungi to increase the depolymerize of lignocellulosic components on sweet sorghum bagasse (SSB). Some of traditional chemical-based treatments ($Ca(OH)_2$ and NaOH) were dangerous for health, environmental or occupational safety reasons because of chemical-environmental pollution (Bhat and Sridhar, 2008).

There has been another physical method to improve the nutritive value of roughage. Feed processing by irradiation has a high potential for substitution by other methods. Recently studies have been completed using gamma irradiation to treated soybean meal

(Shawrang *et al*., 2007), alfalfa hay (Shahbazi *et al*., 2008), canola seed (Anwar *et al*., 2015) and sweet sorghum bagasse (Wahyono and Firsoni 2016). There were only a little literature and information about the effects of gamma irradiation on nutrient values and *in vitro* ruminal fermentation characteristics of sorghum straw. The aim of the current work is to investigate the effects of some levels of gamma irradiation doses on nutrient composition and total gas production *in vitro* of two sorghum straw variety

MATERIALS AND METHODS

Samples preparation and gamma irradiation

Pahat and Samurai 2 sorghum harvested at 80 days. Parts that used in this study was sorghum straw (leaves and stems). Samples were dried at 60°C for 2 days and ground to pass a fine particle size. Irradiation of sorghum straw was done in gamma irradiation facility in Center for the Application of Isotopes and Radiation, Indonesia National Nuclear Energy Agency. Irradiation was carried out with a Co-60 gamma ray source with the 3.7 kGy/h dose rate. Pahat and Samurai 2 sorghum straw were double-packed in polyethylene bags and exposed at doses of 50, 100 and 150 kGy. Samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE) and ash content by procedures of AOAC (2010). NDF and ADF were analyzed according to a method of Goering and Van Soest (1970).

In Vitro Gas Production Technique

Rumen fluid obtained from fistulated male buffalo fed a concentrate:native grass (40:60 DM). The rumen strained and filtered through nylon net. The glassware infused with CO_2 and kept at approximately 39°C before use. Amounts of 200 mg samples were added with 30 ml of rumen fluid-buffer in 100 ml syringe (Fortuna model, Germany) by following the method of Menke *et al.* (1979). The incubation was carried out in water bath at 39°C for 72 h. All of measurements were repeated three times. Gas production measurements were performed at 0, 3, 6, 12, 24, 48 and 72 h. Methane and carbon dioxide production were measurements after 72 h incubation.

Experimental Methods and Analysis

Experimental design of this study was 4 x 2 factorial design with irradiation dose as first factor and sorghum variety as second factor. Each treatment was analyzed in three replicates. All treatments described as follows: 1) P (untreated Pahat sorghum straw); 2) S (untreated Samurai 2 sorghum straw); 3) P 50 (50 kGy irradiated Pahat sorghum straw); 4) S 50 (50 kGy irradiated Samurai 2 sorghum straw); 5) P 100 (100 kGy irradiated Pahat sorghum straw); 6) S 100 (100 kGy irradiated Samurai 2 sorghum straw); 7) P 150 (150 kGy irradiated Pahat sorghum straw); 8) S 150 (150 kGy irradiated Samurai 2 sorghum straw). Methane and carbon dioxide production were determined using MRU gas analyzer®. The total gas production data were fitted to the ruminant degradation model of Ørskov and McDonald (1979) using software NEWAY® as follows: $P=a+b(1-e^{-ct})$; Where, P is the gas production at time t, a is the gas production from soluble fraction (ml/200 mg DM), b is the gas production from insoluble fraction (ml/380 mg DM), c is the gas production rate constant (ml/h), (a+b) is the potential gas production (ml/200 mg DM) and t is the incubation time (h). Effect of treatment was analyzed using SPSS 16.00 based the test of variance (ANOVA) with the following statistical model: $Yijk = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{ijk}$. Y_{ijk} is the observed value, μ is the overall mean, α_i is the effect of irradiation dose, β_j is the effect of sorghum variety, $(\alpha\beta)_{ij}$ is the interaction effect between irradiation dose and sorghum variety and γ_{ijk} is the random residual error.

RESULTS AND DISCUSSION

The nutrient composition of two sorghum straw variety are listed in Table 1. Gamma irradiation had no effect on DM and ash composition. The NDF and ADF composition of 150 kGy gamma irradiated Pahat sorghum straw were decreased ($P<0.05$) 5.38% and 14.36%, respectively. The NDF and ADF composition of 150 kGy gamma irradiated Samurai 2 sorghum straw also decreased ($P<0.05$) 3.11% and 9.58%, respectively. Protein composition of 50 and 100 kGy gamma irradiated Pahat sorghum straw was decreased ($P<0.05$) by 9.22% and 21.80%, respectively. The interested thing was the protein composition of Samurai 2 sorghum straw increase ($P<0.05$) by 12.93% after 100 kGy gamma irradiation.

Table 1. Nutrient composition of two sorghum straw variety after gamma irradiation (% Dry Matter)

Treatment	Dry matter	Crude protein	Ash	Neutral Detergent Fiber	Acid Detergent Fiber
P	93.23	10.09^{a}	12.02	79.96^{abc}	48.02^{ab}
S	93.28	8.66^{cd}	11.79	78.99^{bc}	50.34^{a}
P 50	93.95	9.16^{bc}	12.03	81.17^{ab}	48.67^{ab}
S 50	93.52	8.23^{de}	12.14	80.13^{ab}	48.25^{ab}
P 100	93.79	7.89^{e}	11.96	78.62^{cd}	48.31^{ab}
S 100	93.82	7.92^{e}	12.19	78.45^{cd}	48.35^{ab}
P 150	94.08	9.65^{ab}	12.44	75.88^{e}	41.99^{c}
S 150	94.10	9.78^{a}	12.38	76.61^{de}	45.94^{b}
SEM	0.107	0.178	0.075	0.466	0.591

P (untreated Pahat sorghum straw); S (untreated Samurai 2 sorghum straw); P 50 (50 kGy Pahat sorghum straw); S 50 (50 kGy Samurai 2 sorghum straw); P 100 (100 kGy Pahat sorghum straw); S 100 (100 kGy Samurai 2 sorghum straw); P 150 (150 kGy Pahat sorghum straw); S 150 (150 kGy Samurai 2 sorghum straw); NDF: neutral detergent fiber; ADF: acid detergent fiber; SEM: standard error of mean; Each value is a mean of four samples; $^{a-c}$: means within a columns followed by a different letter differ significantly ($P < 0.05$).

In agreement with the present study, Chumwaengwapee *et al.* (2013) reported that fiber decreased occurred with a significant increase in available carbohydrate. Gamma rays may destroy physical barriers in form lignocellulosic constituents. Decreased level of crude protein may be caused by structural changes after gamma irradiation. Zand *et al.* (2011) reported that Gamma irradiation of proteins may include structural changes and alter functional properties. Crude protein content of 150 kGy gamma irradiated increased after NDF and ADF decreased. This due to the change of nutrient content that could affect other nutrient percentage. There was no interaction between irradiation dose and sorghum straw variety.

In vitro total gas production of untreated and gamma irradiated sorghum are shown in Table 2. Increasing radiation dose from 50 kGy – 150 kGy for Pahat sorghum straw increased total gas production in 3, 6, 48 and 72 h incubation time ($P<0.05$). Total gas production in gamma irradiated Pahat sorghum straw at 48 h incubation was increased ($P<0.01$) 7.58% at doses of 100 kGy and 7.03% at doses of 150 kGy. Total gas production at 72 h incubation of 100 and 150 kGy gamma irradiated Pahat sorghum straw also increased ($P<0.05$) by 6.19 and 5.92%. Gamma irradiation had no effect on total gas production in 12 and 24 h incubation time on both Pahat and Samurai 2 sorghum straw.

Deocaris et al. (2003) informed that total gas production in irradiated chicken feathers with 50 kGy dose had lower value than untreated samples. The different results from present study due to the different substrate and irradiation effect for NDF and ADF contents. Massive decreased of ADF content in 150 kGy gamma irradiated (Table 1) could increase potential gas production (a+b) of Pahat sorghum straw.

Table 2. *In vitro* total gas production of two sorghum straw variety after gamma irradiation (ml/200 mg DM)

Treatment	Incubation time (h)					
	3	6	12	24	48	72
P	4.19[d]	7.72[d]	14.27	28.03	44.97[b]	46.82[bc]
S	4.69[bc]	8.70[abc]	15.72	29.75	46.97[ab]	48.98[ab]
P 50	4.78[b]	8.41[abcd]	14.68	28.21	45.03[b]	46.68[c]
S 50	4.29[cd]	8.09[cd]	15.53	30.40	47.09[a]	48.57[abc]
P 100	5.17[ab]	9.01[ab]	15.85	30.53	48.38[a]	49.72[a]
S 100	4.17[d]	8.28[bcd]	15.51	30.47	47.24[a]	48.89[ab]
P 150	5.54[a]	9.19[a]	15.52	30.20	48.13[a]	49.59[a]
S 150	5.10[ab]	9.22[a]	15.80	30.29	47.57[a]	48.59[abc]
SEM	0.109	0.133	0.197	0.320	0.314	0.296

SEM (standard error of mean). Each value is a mean of three replicates; [abcd] means within columns followed by a different letter differ significantly ($p < 0.05$).

Table 3. *In vitro* gas characteristics of two sorghum straw variety after gamma irradiation

Treatment	Parameters						
	a+b	c	CH_4 (%)	CO_2 (%)	CH_4 (ml/50 mg IVOMD)	CO_2 (ml/50 mg IVOMD)	CO_2:CH_4
P	53.60[d]	0.033	9.12[c]	42.48[e]	3.48[c]	16.21[c]	4.66[c]
S	55.49[abc]	0.034	8.61[c]	43.33[de]	3.33[cd]	16.76[c]	5.04[c]
P 50	53.49[d]	0.033	13.19[b]	65.67[ab]	4.97[b]	24.75[a]	4.98[c]
S 50	54.44[cd]	0.036	8.59[c]	49.27[c]	3.24[cd]	18.55[b]	5.74[b]
P 100	56.51[ab]	0.034	12.58[b]	63.18[b]	4.89[b]	24.55[a]	5.02[c]
S 100	54.79[bcd]	0.036	7.34[d]	46.91[cd]	2.78[e]	17.75[bc]	6.41[a]
P 150	56.83[a]	0.033	14.27[a]	68.21[a]	5.33[a]	25.50[a]	4.78[c]
S 150	54.87[bcd]	0.035	8.37[c]	50.86[c]	3.09[de]	18.82[b]	6.08[ab]
SEM	0.292	0.000	0.521	2.059	0.198	0.776	0.134

SEM (standard error of mean). Each value is a mean of three replicates; [abc]: means within a columns followed by a different letter differ significantly ($p < 0.05$).

In vitro gas characteristics are shown in Table 3. The irradiation dose of 100 and 150 kGy was able to increase potential gas production (a+b) of Pahat sorghum straw by 5.43 and 6.03% ($P<0.05$). gamma irradiation had no effect on potential gas production of Samurai 2 straw sorghum, but it was able to increase CO_2:CH_4 ratio ($P<0.05$). There where significant effect of gamma irradiation on Pahat straw CH_4 production (ml/50 mg IVOMD) ($P<0.05$). Gamma irradiation treatment was able to increase CH_4 production due to breaking down the polysaccharides and lignin linkage (Wahyono and Firsoni, 2016). Wahyono *et al.* (2016) reported that energy lost was represented by high methane emission.

CONCLUSIONS

Gamma irradiation pretreatment was capable of improving Pahat and Samurai 2 sorghum straw nutrient profiles. Based on the present nutrient analysis and *in vitro* gas characteristics, it is recommended that optimization of gamma irradiation may be done at 150 kGy dose. Further study is needed to determine digestibility and economically benefits.

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