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The Changes of Nutrient Composition and *In Vitro* Evaluation on Gamma Irradiated Sweet Sorghum Bagasse

Perubahan Komposisi Nutrien dan Evaluasi In Vitro Pada Bagas Sorgum Manis Hasil Iradiasi Gamma

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ABSTRACT

The Changes of Nutrient Composition and *In Vitro* Evaluation on Gamma Irradiated Sweet Sorghum Bagasse. *In vitro* rumen fermentation study was done to evaluate the effects of gamma irradiation on nutrient compound changes and rumen fermentation product of sweet sorghum bagasse (SSB). The level doses 0, 50, 100 and 150 kGy from cobalt-60 gamma rays irradiator was used to treate sweet sorghum bagasse (SSB). Variables measured were nutrient values, gas production, methane (CH_4) production, total volatile fatty acid (TVFA), ammonia (NH_3), *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) after 72 h *in-vitro* incubation times. Complete randomized design (CRD) (four treatments and four replications) was used to analyze data. The results showed that gamma irradiation doses of 50, 100 and 150 kGy were able to reduce neutral detergent fibre (NDF) (2.15; 3.29 and 5.44% respectively) and acid detergent fibre (ADF) (3.29; 4.58 and 4.58% respectively) and significantly different ($P<0.05$). Gamma irradiation was capable to increas total volatile fatty acid (TVFA), IVDMD and IVOMD ($P<0.05$). Irradiation doses of 100 and 150 kGy also increased protozoa population and CH_4 production significantly ($P<0.05$). Gamma irradiation improved *in vitro* rumen performance represented in rumen fermentation products.

Keywords : Gamma irradiation, *In vitro* fermentation, Nutrient composition, Sweet sorghum bagasse

ABSTRAK

Perubahan Komposisi Nutrien dan Evaluasi *In Vitro* Pada Bagas Sorgum Manis Hasil Iradiasi Gamma. Studi fermentasi rumen secara *in vitro* dilakukan untuk mengevaluasi pengaruh iradiasi gamma terhadap perubahan nutrien dan produk fermentasi rumen dari substrat bagas sorgum manis (SSB). Dosis iradiasi yang digunakan sebesar 0, 50, 100 dan 150 kGy bersumber dari cobalt-60. Variabel yang diamati adalah komposisi nutrien, produksi gas total, produksi gas metana (CH_4), *total volatile fatty acid* (TVFA), amonia (NH_3), degradasi bahan kering *in vitro* (IVDMD) dan degradasi bahan organik *in vitro* (IVOMD) setelah inkubasi selama 72 jam. Penelitian ini menggunakan rancangan acak lengkap (RAL) empat perlakuan dan empat ulangan. Hasil penelitian menunjukkan bahwa iradiasi sinar gamma dosis 50, 100 dan 150 kGy dapat menurunkan fraksi *neutral detergent fiber* (NDF) masing-masing sebesar 2,15; 3,29 dan 5,44% dibandingkan kontrol ($P<0,05$). Ketiga dosis tersebut juga dapat menurunkan fraksi *acid detergent fiber* (ADF) sebesar 3,29; 4,58 dan 4,58% ($P<0,05$). Iradiasi gamma meningkatkan produk fermentasi rumen berupa produksi TVFA, IVDMD dan IVOMD ($P<0,05$). Dosis 100 dan 150 kGy juga yang meningkatkan populasi protozoa dan produksi CH_4 ($P<0,05$). Iradiasi gamma pada SSB dapat meningkatkan kinerja rumen secara *in vitro* yang direpresentasikan dari produk fermentasi rumen.

Kata kunci : Bagas sorgum, Fermentasi *in vitro*, Iradiasi Gamma, Komposisi nutrien

INTRODUCTION

Bio-ethanol of sorghum was produced from the sweet sorghum stalks after grain harvest. Sweet sorghum bagasse (SSB) is by-product from sweet sorghum stem after extraction. This by-product is suitable to be used for ruminant's diets and also gives economics impact from bio-ethanol production [1]. SSB has high fiber content, low palatability and poor nutrient due to low degradability fiber or complex carbohydrates [2] femained after bio-ethanol process.

Improvement of degradability of by-products feed chemical component have been done recently. Physical and chemical treatments were used to improve nutritional value of sugarcane bagasse [3]. Wright *et al.* [4] reported that heat and lignosulfonate-treated of canola meal able to decrease the protein ruminal degradation and improve protein value for lactating dairy cows. Dogaris *et al.* [5] investigated using *Neurospora crassa* and *Fusarium oxysporum* fungi to increase the depolymerize of lignocellulosic components on SSB. Yan *et al.* [6] investigated the effect of NaOH and $Ca(OH)_2$ on cellulose degradation in SSB. Some of methods above were dangerous for livestock because of chemical-environmental pollution.

The processing methods which material exposed to gamma rays has been recognized as a reliable and safe method to improve the nutritive value of corn stalk [7], soybean meal [8], alfalfa hay [9], canola seed [10] and other agricultural by-product [11]. Feed irradiation with gamma rays emitted from radioisotope Cobalt-60 used for improve nutrient quality and rumen degradation. There were only little literature and information about the effects of gamma irradiation on nutritient values and *in vitro* ruminal fermentation characteristics of SSB. Therefore SSB need to be conducted by evaluating the effects of some levels of gamma irradiation doses on nutrient composition and *in vitro* rumen fermentation.

MATERIAL AND METHOD

Samples Preparation and Treatments

Samurai 1 sweet sorghum variety harvested at 100 days. SSB is by-product from sweet sorghum stem after extraction. Samples were dried at 60°C and ground to pass a fine particle size. Cobalt-60 Gamma irradiation in Center for the Application of Isotopes and Radiation, Indonesia National Nuclear Energy Agency was used to treat the three polyethylene packages of samples at 3 level doses (50, 100 and 150 kGy). Samples were analyze for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content by procedures of AOAC [12]. NDF and ADF were analyzed using Goering and Van Soest [13] procedures.

In Vitro Study and Fermentation Technique

Rumen fluid was collected from fistulated buffalo bull with 330 kg live weight. The diets of Buffalo were containing field grass (*Digitaria sp., Setaria barbata, Axonopus compressus* dominate) (*ad libitum*) and concentrate (0,5% body weight) based on DM. Diet formulation contain 13% CP of DM. Buffalo bull was fed twice daily at 08:30 and 15:30 h. Rumen fluid was collected from middle part of the rumen. The 650 ml rumen liquor was obtained from fistulated buffalo before the morning feeding. Rumen fluid was filtered through four layers of cheesecloth and warmed in incubator at 39°C. Preparation of artificial saliva, fermentation solution and sample incubation were done according to [14]. The 100 ml glass syringe (Fortuna model, Germany) with the mixture sample treatments were pre-warmed in a waterbath at 39°C for 1 h before filling with 30 ml buffer-rumen. Gas production measurements were performed at 0, 2, 4, 6, 8, 10, 12, 24, 48 and 72 h. Rumen fermentation parameters were collected at 72 h incubation.

Determination of Fermentation Parameters

Total gas production, CH_4 concentration and sample inoculum were collected after 72 h incubation. Measurement of CH_4 concentration was determined using MRU gas analyzer. Measurement of TVFA was done using AOAC (2010). NH_3 measurement was using conway microdifusion methods [15]. The DM and OM degradation measurement were determined according to the calculation Blummel *et al.* [16]. Protozoa population was measured by Ogimoto and Imai [17].

Statistical Analyses

Experimental design used completely randomized design (CRD) in four treatments and four replications. SPSS 16 was used to analyzed data, with the following statistical model as follows:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where, Y is the dependent variable, μ is the overall mean, α_i is the gamma irradiation effect and ε_{ij} is the residual error. Cumulative total gas production data are fitted to gas kinetics with software NEWAY® based with Ørskov and McDonald [18] model as follows:

$$P = a + b(1\text{-}e^{-ct})$$

Where, a is the gas production from soluble fraction, b is the gas production from insoluble fraction, c is the gas production rate constant from insoluble fraction, t is incubation time, (a + b) is the potential extent of gas production and P is gas produced at "t" time. Differences between treatment were analyzed using Duncan's Multiple Range Test [19].

RESULTS AND DISCUSSIONS

Nutrient Composition

Nutrient composition of untreated and gamma irradiated SSB are presented in Table 1. There was no effect of Gamma Irradiation on dry matter, crude protein, ether extract and ash composition on SSB. The dose levels of 50, 100 and 150 kGy was able to decrease NDF content by 2.15; 3.29 and 5.44% ($P < 0.05$) respectively. The same dose treatments also decreased ADF content by 3.29; 4.58 and 4.58% ($P < 0.05$) respectively.

Gamma irradiation had no significant effect on dry matter, crude protein, ether extract and ash composition [20, 21]. In other study, chemical composition of soy-bean was unaffected by gamma irradiation [20]. There was no effect occurred on some studies using lower dose of gamma irradiation (< 45 kGy). Anwar *et al.* [11] and Shawrang *et al.* [22] reported that irradiation doses levels 10, 20 and 30 kGy were not affecting on the chemical composition of canola seeds. Ebrahimi-Mahmoudabad and Taghinejad-Roudbaneh [23] also concluded that lower irradiation dose (< 45 kGy) was

Table 1. Nutrient composition untreated and gamma irradiated of SSB (g/kg Dry Matter)

Treatment	Dry matter	Crude protein	Ether extract	Ash	NDF	ADF
Untreated	941	54.0	18.5	84.5	791^a	486^a
50 kGy	931	59.3	17.3	83.9	774^b	470^b
100 kGy	932	59.5	16.7	83.0	765^b	454^c
150 kGy	934	62.4	18.5	83.2	748^c	454^c
SEM	1.9	0.2	0.8	0.8	5	4.3

NDF: neutral detergent fiber; ADF: acid detergent fiber; SEM: standard error of mean; Each value is a mean of four samples; $^{a\text{-}c}$: means within a columns followed by a different letter differ significantly ($P < 0.05$).

not sufficient to change chemical composition of whole cottonseed, soybean and canola seeds. The same effects were also happened at higher doses.

Gamma irradiation dose of 150 kGy could affect in the lowest NDF value content, but the ADF fraction was not significant different compared to 100 kGy dose (Table 1). It was proved that 100 kGy gamma irradiation dose was sufficient to decrease NDF and ADF content. This dose also needed to improve alfalfa hay digestibility [10]. The decrease of NDF and ADF was caused by gamma irradiation where it is able to cut some lignohemicellulose and lignocellulose chains. Increasing the ratio of lignocellulose and crude fiber because of decreasing NDF and ADF. Banchorndhevakul [8] informed that NDF and ADF were decreased after irradiation treatment due to degradation of cellulose and hemicellulose into soluble materials. Decrease of NDF and ADF in irradiated roughages due to depolymerisation and delignification proportional to increase of irradiation dose [24]. This study was similar with the results from Flachowsky *et al.* [25] and Shawrang *et al.* [21]. The NDF and ADF content of wood by-products were decreased after irradiation dose over the range 100 – 2000 kGy [25] and after electron beam irradiation with 250 and 500 kGy dose [21].

Gas Production

Total gas production and kinetics after 72 h incubation are presented in Table 2. Gamma irradiation dose of 150 kGy increased total gas production 11.01; 13.85 and 6.57% on 2, 4 and 6 h incubations respectively ($P<0.05$). On 48 and 72 h incubation, there were no significant effect between untreated and 150 kGy gamma irradiated SSB. There were no significant effect on total gas production during early incubation between dose level at 50 and 150 kGy gamma irradiation. Gas production from soluble fraction (a) were significantly different after 100 and 150 kGy dose level gamma irradiation ($P<0.05$). Nevertheless, gas production from insoluble fraction (b) and potential extent of gas production (a+b) on 100 kGy gamma irradiation treatment were lower than untreated SSB. Gas production rate fraction (c) has no affected by gamma irradiation.

Table 2. Total gas production, a+b and c constant of untreated and gamma irradiated SSB after 72 h incubation

Treatment	Time collection (h)									Gas kinetics			
	2	4	6	8	10	12	24	48	72	a	b	a+b	c
Untreated	4.45^{a}	7.77^{a}	10.34^{a}	12.90	15.09	16.82	28.52	42.70^{b}	51.07^{b}	1.99^{a}	59.13^{b}	61.12^{b}	0.025
50 kGy	4.73^{ab}	7.78^{a}	10.37^{a}	12.82	14.95	16.48	28.30	41.65^{ab}	48.59^{a}	2.28^{ab}	57.58^{ab}	59.85^{ab}	0.025
100 kGy	4.88^{ab}	8.16^{ab}	10.75^{ab}	13.04	15.25	16.70	27.60	40.95^{a}	48.42^{a}	2.68^{b}	54.12^{a}	56.8^{a}	0.026
150 kGy	4.94^{b}	8.36^{b}	11.02^{b}	13.15	15.28	16.87	28.04	41.80^{ab}	49.47^{ab}	2.75^{b}	55.85^{ab}	58.60^{ab}	0.025
SEM	0.079	0.099	0.098	0.095	0.103	0.110	0.176	0.246	0.401	0.097	0.648	0.638	0.000

a: gas production from soluble fraction; b: gas production from insoluble fraction; c: gas production rate constant for the insoluble fraction (b); a+b: potential extent of gas production: SEM: standard error of mean; Each value is a mean of four samples; ^{ab}: means within a columns followed by a different letter differ significantly ($P < 0.05$).

Total gas production in the dose of 150 kGy irradiation was higher than untreated during 2, 4 and 6 h incubation time. This was caused by increasing the potential for carbohydrates degradable fraction after irradiation treatment. This was evidenced by a high fraction of gas production from the soluble fraction (a). Total gas production after 72 h on 50 and 100 kGy gamma irradiation dose were lower than untreated samples ($P<0.05$). This study was similar to Deocaris *et al.* [26] that informed total gas production in irradiated chicken feathers with 25 and 50 kGy dose had lower value than untreated samples. High value of "a" fraction was also obtained at 100 kGy irradiation treatment.

CH_4 Production

The CH_4 concentration and production are presented in Figure 1 and 2. There were significant effect of gamma irradiation on rumen CH_4 concentration and production. Gamma irradiation dose level at 50, 100 and 150 kGy increased CH_4 concentration (%) by 56.39 up to 89.66 and 105.07% ($P<0.05$) respectively (Figure 1). Gamma irradiation dose levels of 50, 100 and 150 kGy increased significantly CH_4 production (ml/100 mg IVOMD) by 42.68 up to 89.43 and 91.06% ($P<0.05$) respectively, but there was no signficant effect on 100 and 150 kGy dose level (Figure 2).

Gamma irradiation treatment was able to increase CH_4 gas production due to breaking down the linkage between polysaccharides and lignin. This was caused by cellulose and hemicellulose more digestible for microbes enzyme activity [27]. Sambusiti *et al.* [28] reported that lignocellulosic substrates, the methane production of roughages mainly depends on their complex structure which limits their degradability. Irradiation pre-treatments

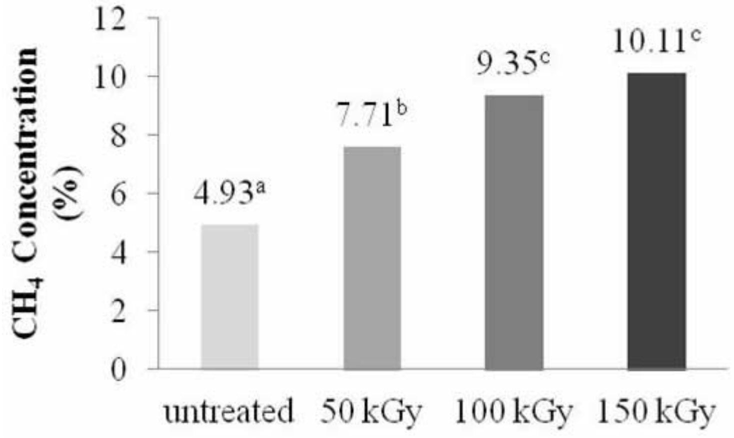

Figure 1. CH_4 concentration of untreated and gamma irradiated SSB on 72 h incubation

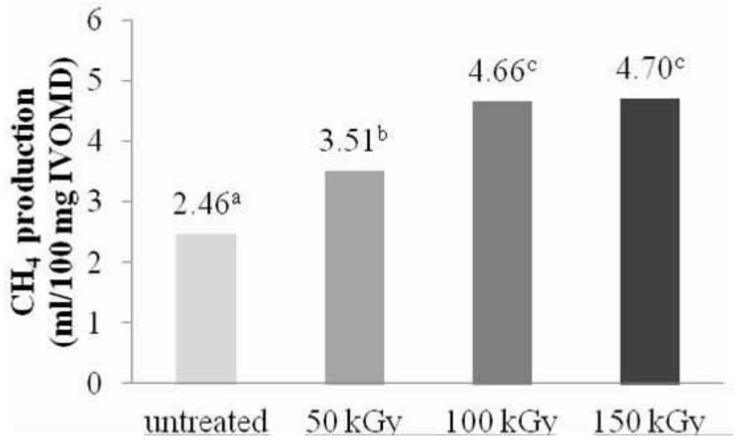

Figure 2. CH_4 production of untreated and gamma irradiated SSB on 72 h incubation

could accelerate the hydrolysis process, therefore was able to improve the final CH_4 gas production.

The increase of CH_4 production have relationship with the decrease of NDF and ADF fraction after gamma irradiation pretreatment (Table 1). The decrease value were followed by plysaccharides and lignin linkage breakdown that increase degradability for microbes activity. Increasing levels of crude fiber biodegradable also resulted in increasing of cellulolytic bacteria activity that increase the availability for CH_4 inhibitor agent. The CO_2 and H_2 are mainly produced during fermentation of hemicellulose as structural carbohydrate [29,30]. CH_4 was produced by CO_2 and H_2 reduction that catalyzed by enzyme which secreted by methanogen microbes. Santoso *et al.* [31] reported that there was a strong relationship between CH_4 production (g/day) and NDF digested (g/day) ($r = 0.88$). Estermann *et al.* [32] also reported that there was a strong relationship between NDF intake and CH_4 production. Another reason for the increasing of fiber hydrolysis after gamma irradiated was a cellulose crystallinity reduction. The crystallinity reduction of cellulose was evident at doses above 100 kGy [33]. Moss and Givens [34] reported that CH_4 production had positive correlated with NDF content ($r = 0.79$). Santoso *et al.* [31] and Singh *et al.* [35] also reported that carbohydrate fractions (NDF and ADF) were better CH_4 predictors than other feed components. Nevertheless, in this study had different result. This was able to increase of NDF degradability after gamma irradiation pretreatment.

Rumen Fermentation Products

In vitro rumen fermentation products of SSB are presented in Table 3. Gamma irradiation had no effect on pH and NH_3 values. Gamma irradiation dose levels of 50, 100 and 150 kGy increased TVFA product ($P < 0.05$). These values were 42.02 up to 65.22 and 84.06%. Gamma irradiation dose levels of 100 and 150 kGy also increased protozoa population by 66.25 and 83.75% respectively ($P < 0.05$). The IVDMD and IVOMD also increased significantly different ($P < 0.05$) due to gamma irradiation on dose levels 50, 100 and 150 kGy. These values were 4.33; 7.20 and 8.15% (IVDMD) and 4.70; 8.29 and 8.36% (IVOMD) respectively.

Gamma irradiation had no effect on NH_3 concentration due to similar crude protein values between treatments (Table 1). Anwar *et al.* [11] reported that Gamma irradiation had significant effect on reducing anti-nutritional factors, increasing protein functional properties and *in-vitro* protein digestibility. The reason of improvement crude protein (CP) digestibility by modificated and denaturated in the three dimensional structure of proteins due to irradiation [23]. Gamma irradiation dose of 50, 100 and 150 kGy was not yet able to improve the digestibility of proteins. It

Table 3. *In vitro* rumen fermentation product of untreated and gamma irradiated SSB after 72 h incubation

Treatment	pH	NH_3 (mg/100ml)	TVFA (mM)	Protozoa (sel/ml)	IVDMD (%)	IVOMD (%)
Untreated	7.11	17.00	46.00^a	0.80×10^{6a}	55.83^a	57.44^a
50 kGy	7.18	18.00	65.33^b	0.80×10^{6a}	58.25^b	60.14^b
100 kGy	7.16	16.67	76.00^{bc}	1.33×10^{6b}	59.85^c	62.20^b
150 kGy	7.15	12.67	84.67^c	1.47×10^{6b}	60.38^c	62.24^b
SEM	0.014	0.857	4.586	0.111	0.569	0.661

NH_3: ammonia; TVFA: total volatile fatty acid; IVDMD: *in vitro* dry matter degradability; IVOMD: *in vitro* organic matter degradability; SEM: standard error of mean; Each value is a mean of four samples; ^{ab}: means within a columns followed by a different letter differ significantly ($P < 0.05$).

represented by no significant concentration of NH_3 obtained. Nevertheless, the concentration of NH_3 on four treatments met the optimum level. The optimal concentration of buffalo rumen microbial fermentation in a closed system culture is 5 mg/100 ml, but also depends on the feed fermentability level [36,37]. High concentration results of NH_3 was caused by fermentation products covered culture techniques which resulted in the absence of NH_3 uptake. It causes high accumulated concentrations of NH_3.

Gamma irradiation was effective to increase TFVA production from SSB. This values had strong relationship with decreasing NDF and ADF fractions value (Table 1), that was caused by microbes enzyme activity on cellulose and hemicellulose digestion after gamma irradiation pretreatment. Untreated SSB had the lowest TVFA because there was exist a lignin matrix associated with cellulose and hemicellulose reduced microbial rumen activity on cell wall fraction [10]. Shawrang *et al.* [9] presented that irradiation treatment could effect to lignocellulosic bonds. The final product of carbohydrate fermentation represented by value of TVFA. The higher irradiation dose, more higher NDF and ADF degradability of barley straw ($P<0.01$). The dose of 100 kGy was capable of increasing the degradability of NDF and to be NDF effectively at rumen outflow rate 0.05 h^{-1} by about 3% [38]. Siddhuraju *et al.* [39] also reported that high levels of irradiation could be used to improve degradability of the crude fiber on various plant residues and cereal straws. CH_4 production (Figure 1) could be increased as long as increasing of TVFA production. Rapidly NDF degradation increased hydrogen-fermented carbohydrates production that initiate CH_4 formation [40].

Gamma irradiated dose levels of 100 and 150 kGy were effective to increase protozoa population (Table 3) and influenced by optimal concentrations of NH_3 and availability fraction of degradable NDF and ADF. Optimal concentration of NH_3 supported the microbial protein synthesis development. Availability fraction of degradable NDF and ADF would support cellulolytic bacteria population growth, but protozoa like easy substrate digest such as starch, sugar and bacteria as a source of food [41]. Carbohydrates fermented by protozoa to produce CO_2, H_2 and VFA, wherever CO_2 and H_2 are a major precursor of CH_4 formation. Increasing of protoza population was accompanied by increasing of CH_4 gas production (Figure 1).

Increase of IVDMD and IVOMD after gamma irradiation were influenced by several factors that have been analyzed previously. These factors included the degradable of NDF, the degradable of ADF, the increase of NH_3, TVFA production and microbial populations especially protozoa. improving of IVDMD on gamma irradiation was similar to others study with *in sacco* methods. Dose of gamma iradiation higher than 50 kGy could increase ruminal DM degradability of feedstuffs [9]. Higher levels of irradiation, up to 600 kGy, can be used to improve the rumen degradability of the dry matter of various plant residues and cereal straws [39]. The increase of protozoa population (Table 3) followed by increasing in CH_4 cincentration and production (Figure 1 and 2). Bhatta et al. [42] reported that methane production related to the protozoa population. Methane was generated by methanogens bacteria that consumed hydrogen.

CONCLUSSION

Gamma irradiation pretreatment on SSB is capable of improving *in vitro* rumen fermentation. The dose of 100 kGy was the best gamma irradiation dose to improve SSB quality. Further study is needed to determine *in vivo* degradability and economically benefits of this processing. The treatment of reducing CH_4 productions is also needed, when gamma irradiated SSB would be used as fiber source in ruminants diets.

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