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Low Irradiation Dose for Sorghum Seed Sterilization: Hydroponic Fodder System and *In Vitro* Study

Teguh Wahyono*, Shintia Nugrahini Wahyu Hardani, and Irawan Sugoro

Agricultural Division, Center for Application of Isotope and Radiation, Indonesia National Nuclear Energy Agency, South Jakarta, 12040, Indonesia

ABSTRACT

The purpose of this study was to evaluate the influence of low gamma irradiation dose on growth performance, *in vitro* gas production and rumen fermentation product of sorghum hydroponic fodder (SHF) to utilize them in ruminant diets. Three polyethylene packages of Samurai 2 sorghum seeds were exposed at 25°C gamma irradiation in gamma cell (Co-60) at doses of 100, 200 and 300 Gy in the presence of air. Samples were then refrigerated (< 5°C) before planting. All seeds were planted in nutrient film technique hydroponic system. This study used Completely Randomized Design with four replications. The observed parameters were total fresh yield, plant height and conversion ratio from seeds to SHF. *In vitro* gas test evaluation was done to compare all treatments with sorghum straw. The observed parameters were total gas production, kinetics gas, CH₄ concentration, CO₂ concentration and rumen fermentation products. Results showed that lower irradiation dose for seeds sterilization decreased plant height and total fresh yield on SHF production. *In vitro* total gas production of all SHF treatment was higher ($p < 0.05$) than sorghum straw (control) at 2-48 h time of incubation. Gamma irradiation dose of 200 and 300 Gy on SHF seeds sterilization decreased ($p < 0.05$) *in vitro* CH₄ concentration for 19.51 and 15.43% respectively compared to SHF control (hypochlorite sterilization). In the same dose, seeds sterilization with gamma irradiation increased ($p < 0.05$) CO₂:CH₄ ratio by 23.46 and 20.73% respectively compared to SHF control. The treatment of 100 Gy gamma irradiation for seed sterilization also increased ($p < 0.05$) TVFA by 30.63% compared to sorghum straw. It was concluded that lower irradiation dose for seeds sterilization decreased growth performance of SHF. However, 100 Gy gamma irradiation increased *in vitro* total gas production.

Keywords: *In vitro*, Low irradiation dose, Sorghum hydroponic fodder, Total gas production

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* Corresponding author:

Telp. +62 878 0926 8855

E-mail: teguhwahyono@batan.go.id

Introduction

Traditional farmers in Indonesia are a major component to fight against various constraints in the good nutrients supply for livestock. The problems are the limited land for forage and conversion of agricultural land to other purpose. Limited supply of forage land could decrease the supply of green forage. El-Morsy *et al.* (2013) also reported that the availability of fodder is decreasing due to climate change, higher competition for land and water resources between fodder and cereal crops. According to vulnerability in agriculture system, especially in green fodder production, strategies are needed in order to improved water and land efficiency. Hydroponic fodder is the right solution for the provision of forage in a limited land.

Hydroponic green fodder is produced from forage grains that are germinated and grown in

short period time inside controlled growing rooms with appropriate growing conditions (Gebremedhin, 2015). Hydroponic technique can be used for green fodder production in a hygienic environment free of chemical residue (insecticides, herbicides, fungicides and growth promoters). Hydroponic fodder makes seed germinated and sprouted into high quality, highly nutritious and disease free fodder. This green fodder is also extremely high in protein and metabolisable energy (El Morsy *et al.*, 2013; Gebremedhin, 2015). This planting system has enabled the production of green fodder forage from oats, barley, wheat and other grains (Fazaeli *et al.*, 2012). Sorghum is potential cereal crops to be developed in Indonesia as hydroponic fodder. National Nuclear Energy Agency (BATAN) have produced sorghum variety which has the advantage of high biomass production and great nutrient composition (Samurai 2 variety). This

varieties have not been optimally utilized as a source for forage, so that it is need to be developed as Sorghum Hydroponic Fodder (SHF). In many case, cereal grains may become contaminated with a range of microorganism especially fungi during their development.

Surface sterilization should be done to eliminate and decontaminated of seed surface affected with fungi. Sodium hypochlorite (NaOCl), mercury chloride (HgCl₂) and Methyl bromide are conventional used for surface sterilization and fumigation of seeds (Younesikelaki *et al.*, 2016). Alternative method is gamma irradiation that useful for both sterilization and for preservation of food and seed in nutrition and agriculture. Gamma irradiation also used for long-storage seeds and fumigation (range 1-6 kGy) on *Oryza sativaseeds* (Maity *et al.*, 2009). Aynehband and Afsharinafar (2012) studied the effect of gamma irradiation (100-250 Gy) on germination characters of amaranth seeds. Surface sterilization effect with gamma irradiation depended on plant species and the dose of irradiation. From the above information, there was no information yet on low gamma irradiation effect for sterilization seed, in growth and degradability of sorghum hydroponic fodder. Therefore the objective of this study was to evaluate the influence of low gamma irradiation dose on growth performance, *in vitro* gas production and rumen fermentation product of sorghum hydroponic fodder to utilize them in ruminant diets.

Materials and Methods

Source of seed

Seeds of Samurai 2 sorghum (*Sorghum bicolor* (L.) Moench) variety were obtained from Agricultural Division, Center of Isotope and Radiation Application, National Nuclear Energy Agency, Indonesia. Samurai 2 variety was sorghum variety from mutation radiation technique breeding.

Gamma Irradiation

Gamma irradiation was carried out in a cobalt-60 irradiator in Center of Isotopes and Radiation Application. Three polyethylene packages of seeds were exposed at 25°C gamma irradiation in gamma cell (Co-60) at doses of 100, 200 and 300 Gy in the presence of air. Samples were then refrigerated at < 5°C before planting.

Hydroponic system

A growing plan was conducted using a steel hydroponic rack in controlled sterilized room equipped with automatic irrigation system with capacity of 10 polyethylene trays sized 60x18 cm² each. The hydroponic system was nutrient film technique system (Lee and Lee, 2015). Temperature inside room controlled to get a range of 20-22°C and the relative humidity adjusted about 60-70% controlled by air circulation. Clean seeds of sorghum were soaked in tap water for 24 h then were spread in the trays. The density

obtained was 0.38 g/cm². The irrigation system automated by using digital timer to control water pumping. Trays were irrigated with hydroponic nutrition solution on 1st day and then tap water on the day of 2-8. Trays were irrigated each two hour periods during daily hours for two minutes each time.

Sample preparation

Sorghum hydroponic fodder was harvested at 8 d after planting, where the fodder biomass was ready for harvest (Figure 1). The following data were recorded per tray: total fresh yield, plant height and conversion ratio from seeds to SHF. Representative fresh plant samples from every tray were taken and oven-dried at 60°C for 48 h. Samples ground to pass a fine particle size. Organic material (OM), crude protein (CP) and ether extract (EE) (AOAC, 2010) were analyzed. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also analyzed using Goering and Van Soest (1970) procedures.

Rumen fluid for *in vitro* gas study obtained from fistulated male buffalo fed a 30:70 concentrate:field grass. Buffalo was fed twice daily at 8.00 am and 4.00 pm. Rumen fluid was collected from middle part of the rumen.

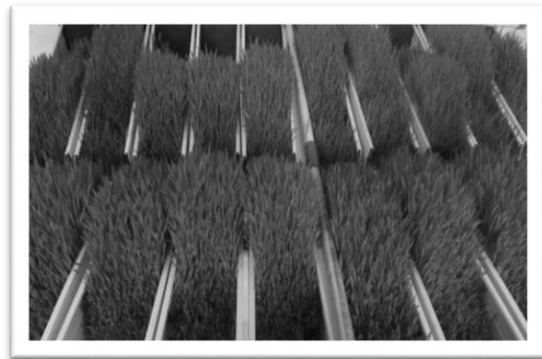


Figure 1. Sorghum hydroponic fodder ready to harvest.

Treatments

Treatments described as follows: SHF growing study treatments were: control (10% hypochlorite seeds sterilization); 100 (dose of 100 Gy seeds sterilization); 200 (dose of 200 Gy seeds sterilization) and 300 (dose of 300 Gy seeds sterilization). *In vitro* gas study treatments were: sorghum straw; SHF control; SHF 100; SHF 200 and SHF 300.

In vitro gas study

Approximately of 380 mg dry matter (DM) samples were added with 40 ml of rumen fluid-buffer in calibrated 100 ml syringe (Fortuna model, Germany) by following the method of Menke *et al.* (1979) as modified by Blümmel *et al.* (1997). The glassware was infused with CO₂ and kept at approximately 39°C before use the rumen strained and filtered through nylon net. The incubation was carried out in waterbath at 39°C for 48 h. All of

measurements were repeated four times. Variables observed were total gas production, kinetics gas, CH₄ concentration, CO₂ concentration, CH₄:CO₂ ratio, pH, ammonia (NH₃), total volatile fatty acid (TVFA) and protozoa population.

Measurement of rumen fermentation product variables

Gas production was recorded on 2, 4, 6, 8, 10, 12, 24 and 48 h. Gas production reading was quickly conducted to minimize temperature change. Kinetics gas was measured by exponential equation of Ørskov and McDonald (1979) 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/380 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/380 mg DM) and t is the incubation time (h).

The CH₄ and CO₂ concentrations, CH₄:CO₂ ratio, pH, NH₃, TVFA and protozoa population were measured after 48 h incubation. The CH₄ and CO₂ concentrations were measured using MRU gas Analyzer®. Readed value on MRU gas Analyzer® was per centation of CH₄ or CO₂ stored in the syringe. The pH value was measured by pH meter Hanna instrument. Measurement of total VFA using AOAC (2010). The NH₃ concentration was analyzed using Conway microdiffusion methods (Conway, 1950). Protozoa population was measured according to the method of Ogimoto and Imai (1981).

Experimental design and statistical analysis

This study used completely randomized design with four replications from four tray unit with following model: $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$; where: Y_{ij} is the i^{th} treatment observation and j^{th} replication, μ is general mean, α_i is effect of i^{th} treatment and ϵ_{ij} is random affect of i^{th} factor and j^{th} replication.

Data were statistically analyzed using SPSS 20.00. Differences among treatments were compared using Duncan's multiple range test (Steel and Torrie, 1980).

Result and Discussion

Growth performance

Growth parameters included plant height, total fresh yield and ratio of SHF: seeds conversion are presented in Table 1. All parameters were significantly decreased after gamma irradiation treatments for seed sterilization ($p < 0.05$). Plant height decreased by 16.39, 19.15 and 22.42% after 100, 200 and 300 Gy seeds sterilization treatment, respectively. Total fresh yield also decreased by 18.40, 22.23 and 24.09% respectively. SHF: seeds conversion calculated from comparison of total fresh yield after harvested and seeds yield before planting. SHF:seeds conversion decreased by 15.33, 16.75

and 20.28% after 100, 200 and 300 Gy seeds sterilization treatment respectively.

The higher dose of gamma ray (800 Gy) had negative effect on the morphological characteristics of corn (Emrani *et al.*, 2013). By contrast, Ayneband and Afsharinafar (2012) reported that gamma irradiation were effective in improving germination index. In present study, dose of 100, 200 and 300 Gy for sorghum seed sterilization were not effective in improving plant growth. Gamma irradiation could decrease seed germination (Harding *et al.*, 2012; Aparna *et al.*, 2013). Lower growth performance on treatments of gamma irradiation for sterilization might be caused by DNA structural changes in sorghum seeds. Franco *et al.* (2015) reported that there is change in lettuce DNA bands after gamma irradiation treatment. These effect might be due to the structural rearrangements in DNA caused by different types of DNA damages. One of proper consideration on gamma irradiation for sterilization is mechanism DNA damage after irradiation process. Maity *et al.* (2009) also reported that the carbohydrate content and total protein are decrease due to higher metabolic activities and hydrolyzing enzyme activity in germinating seeds (range 3-4 kGy). Change of enzym activity due to induction by stress effect after irradiation process.

The range of SHF:seeds conversion ratio were 3.38 - 4.24 (Table 1). Green fodder:seed ratio depended on several factors such as type and quality grain, amount and frequency irrigation, nutritious solution, humidity, lights position and harvested time (Fazaeli *et al.*, 2012). From study Al-Karaki and Al-Hashimi (2012) have shown that sorghum produce lower conversion ratio (fodder:seeds) than cowpea, barley and alfalfa. However, sorghum had conversion ratio higher than wheat. Higher amount of barley green fodder to seed ratio was reported by Fazaeli *et al.* (2012) who obtained a ratio of 7.21 after harvested at 8 d.

In vitro gas study

Total gas production after *in vitro* gas study are presented in Table 3. CH₄ concentration, CO₂ concentration and CO₂:CH₄ ratio from *in vitro* total gas measurements are presented in Figure 2. *In vitro* total gas production of all SHF treatments were higher than sorghum straw (control) at 2-48 h incubation time ($p < 0.05$). However, potential gas production ($a+b$) in sorghum straw was higher than all SHF treatments ($p < 0.05$). Gamma irradiation dose of 200 and 300 Gy on SHF seeds sterilization decrease *in vitro* CH₄ concentration for 19.51 and 15.43% respectively lower than SHF control (hypochlorite sterilization) ($p < 0.05$). In the same dose, seeds sterilization with gamma irradiation increased CO₂:CH₄ ratio by 23.46 and 20.73% respectively compared to SHF control ($p < 0.05$).

Total gas production of SHF at 2-48 h incubation was significantly different ($p < 0.05$) when compared to sorghum straw treatment. The increasing of *in vitro* total gas production on SHF

Table 1. Growth parameters of SHF after harvested at 8th day after planting

	Treatments				SEM
	SHF control	SHF 100	SHF 200	SHF 300	
Plant height (cm)	15.61 ^a	13.05 ^b	12.62 ^{bc}	12.11 ^c	0.317
Total fresh yield (kg)	0.913 ^a	0.745 ^b	0.710 ^b	0.693 ^b	0.021
Conversion ratio (SHF:seeds)	4.24 ^a	3.59 ^b	3.53 ^{bc}	3.38 ^c	0.084

SHF (sorghum hydroponic fodder); SHF control (SHF after 10% hypochlorite seeds sterilization); SHF 100 (SHF after dose of 100 Gy seeds sterilization); SHF 200 (SHF after dose of 200 Gy seeds sterilization); SHF 300 (SHF after dose of 300 Gy seeds sterilization).

^{a,b,c} Different superscripts at the same column indicate significant differences ($p < 0.05$).

Each value is a mean of four samples.

SEM: Standard error of the Means.

Table 2. Nutrient composition of sorghum straw and SHF

	Sorghum Straw	Treatments				SEM
		SHF control	SHF 100	SHF 200	SHF 300	
Organic matter (% DM)	88.21 ^b	96.12 ^a	96.43 ^a	96.72 ^a	96.89 ^a	0.897
Crude protein (% DM)	8.65 ^b	10.17 ^a	10.13 ^a	10.03 ^a	10.00 ^a	0.198
Ether extract (%DM)	3.50 ^b	6.13 ^a	6.21 ^a	5.85 ^a	5.96 ^a	0.289
Neutral detergent fiber (% DM)	78.99 ^a	56.49 ^b	57.26 ^b	56.01 ^b	56.50 ^b	2.408
Acid detergent fiber (% DM)	50.34 ^a	32.48 ^b	33.81 ^b	31.99 ^b	33.18 ^b	1.896

DM (dry matter); SHF (sorghum hydroponic fodder); SHF control (SHF after 10% hypochlorite seeds sterilization); SHF 100 (SHF after dose of 100 Gy seeds sterilization); SHF 200 (SHF after dose of 200 Gy seeds sterilization); SHF 300 (SHF after dose of 300 Gy seeds sterilization).

^{a,b,c} Different superscripts at the same column indicate significant differences ($p < 0.05$).

Each value is a mean of four samples.

SEM: Standard error of the Means.

could be as a result of the higher of soluble carbohydrates, minerals and vitamin of SHF than sorghum straw. Sorghum straw has highest content of NDF and ADF (Table 2). High fibre in sorghum straw might cause decrease on rumen microbial activity on less than 24 h of diet incubation, however gas production rate from fermentation by microbe was higher on 48 hours of incubation. It could be seen on potential gas production (a+b) after kinetics gas calculation. This was caused by rumen microbial started to digest substrate after 24 h incubation. Total gas production from *in vitro* fermentation represent the extent of feed digestibility and fermentation (Krizsan *et al.*, 2013). Fazaeli *et al.* (2012) reported that variations in gas production are the result of variations in chemical composition. The increase of total gas production not yet represented the efficiency of feed substrate utility. The feed efficiency should be observed from variable of CH₄ and CO₂ concentration.

The increase of total gas production was followed by CH₄ production. Higher total gas production caused high CH₄ production. CH₄

variable was used to measure CH₄ emission decrease level (Wahyono, 2015). SHF 100 treatment had highest total gas production but it had high CH₄ concentration. Bodas *et al.* (2012) reported that CH₄ gas production represent energy losses in form of gas reflecting low feed efficiency. The increase of microbial activity followed by increasing in protozoa population (Table 4). Protozoa population could increase due to more easily digested carbohydrate from SHF. Bhatta *et al.* (2015) reported that CH₄ production was related to the protozoa population. CH₄ was generated by methanogens bacteria that consumed hydrogen from carbohydrate fermentation.

Rumen fermentation product

In vitro rumen fermentation products of SHF vs sorghum straw were presented in Table 4. pH value in all SHF treatments were higher than sorghum straw ($p < 0.05$). TVFA results showed that SHF control and SHF 100 had higher value than sorghum straw ($p < 0.05$). However, SHF 200 and SHF 300 treatments had lower TVFA than

Table 3. Total gas production and gas kinetics of sorghum straw and SHF

Treatments	Incubation periods (h)								Gas kinetics	
	2	4	6	8	10	12	24	48	a+b	c
Sorghum straw	0.59 ^c	2.72 ^d	4.71 ^e	6.84 ^c	9.36 ^c	12.16 ^c	34.88 ^c	58.01 ^c	147.71 ^a	0.012 ^c
SHF control	6.19 ^b	12.97 ^c	19.06 ^d	25.29 ^b	36.81 ^b	48.74 ^b	92.23 ^b	111.46 ^b	130.85 ^b	0.046 ^b
SHF 100	6.97 ^{ab}	14.26 ^b	20.87 ^c	29.64 ^a	43.14 ^{ab}	57.85 ^a	97.92 ^a	116.99 ^a	132.51 ^b	0.054 ^b
SHF 200	7.48 ^a	15.4 ^{ab}	22.69 ^b	31.97 ^a	45.29 ^a	57.54 ^a	96.55 ^a	114.45 ^{ab}	127.79 ^b	0.055 ^{ab}
SHF 300	7.82 ^a	16.51 ^a	24.26 ^a	32.67 ^a	49.65 ^a	61.41 ^a	96.15 ^{ab}	111.78 ^b	121.36 ^b	0.063 ^a
SEM	0.572	1.033	1.471	1.981	3.081	3.902	5.147	4.640	3.126	0.004

SHF (sorghum hydroponic fodder); SHF control (SHF after 10% hypochlorite seeds sterilization); SHF 100 (SHF after dose of 100 Gy seeds sterilization); SHF 200 (SHF after dose of 200 Gy seeds sterilization); SHF 300 (SHF after dose of 300 Gy seeds sterilization).

a+b (potential gas production); c (gas production rate)

^{a,b,c} Different superscripts at the same row indicate significant differences ($p < 0.05$).

Each value is a mean of four samples.

SEM: Standard error of the Means.

Table 4. Rumen fermentation products of sorghum straw and SHF after 48 h incubation

	Treatments					SEM
	Sorghum straw	SHF control	SHF 100	SHF 200	SHF 300	
pH	6.84 ^a	6.54 ^b	6.58 ^b	6.60 ^b	6.62 ^b	0.022
NH ₃ (mg/100 ml)	36.37	41.67	39.19	36.02	38.84	0.773
TVFA (mM)	98.28 ^b	118.18 ^a	128.38 ^a	72.15 ^c	72.15 ^c	6.185
Protozoa population (log 10/ml)	3.93 ^b	4.15 ^{ab}	4.21 ^a	4.13 ^{ab}	3.93 ^b	0.037

SHF (sorghum hydroponic fodder); SHF control (SHF after 10% hypochlorite seeds sterilization); SHF 100 (SHF after dose of 100 Gy seeds sterilization); SHF 200 (SHF after dose of 200 Gy seeds sterilization); SHF 300 (SHF after dose of 300 Gy seeds sterilization).

NH₃ (ammonia); TVFA (total volatile fatty acids).

^{a,b,c} Different superscripts at the same column indicate significant differences ($p < 0.05$).

Each value is a mean of four samples.

SEM: Standard error of the Means.

sorghum straw ($p < 0.05$). CH₄ concentration and protozoa population also increased on 100 Gy irradiation for seeds sterilization treatment higher than sorghum straw ($p < 0.05$). There was no significant difference between all treatments in NH₃ parameter.

The range of pH from this study at the level > 6.2 . The results indicated that all treatments had no negative effect for the growth of cellulolytic bacteria. pH level > 6.2 could inhibit the growth of bacteria. The optimum and suboptimal pH values in the rumen for growth of bacteria were 6.4 and 5.5, respectively (Cardozo *et al.*, 2000). pH balance rumen might effect on cellulose digestibility due to carbohydrate type of forage have a reciprocal relationship with pH value (Wahyono *et al.*, 2014). The lower pH of all SHF treatments due to a higher content of soluble carbohydrate than sorghum straw. Wahyono (2015) reported that the decrease of pH value

might be due to higher content of soluble carbohydrate from the substrate. The NH₃ value in present study ranged from 36.02 to 41.67 mg/100 ml. These values were higher than normal range value for *in vitro* culture (5 mg/100 ml) (Wanapat *et al.*, 2013). This is due to incubation technique was carried out in closed system so there was NH₃ product accumulated (Wahyono *et al.*, 2014). The higher TVFA value in SHF control and SHF 100 were reflection of the higher total gas production (Table 3). Wang *et al.* (2016) reported that fermentation rate was correlated with TVFA value. TVFA is the final product of carbohydrate fermentation. It is used for rumen microbial growth (Roza *et al.*, 2013). TVFA also plays a role as a major energy source of ruminant and as the building block for milk synthesis in dairy animals (Jayanegara *et al.*, 2016). High protozoa population is also a reflection of the high concentration of CH₄ (Figure 2).

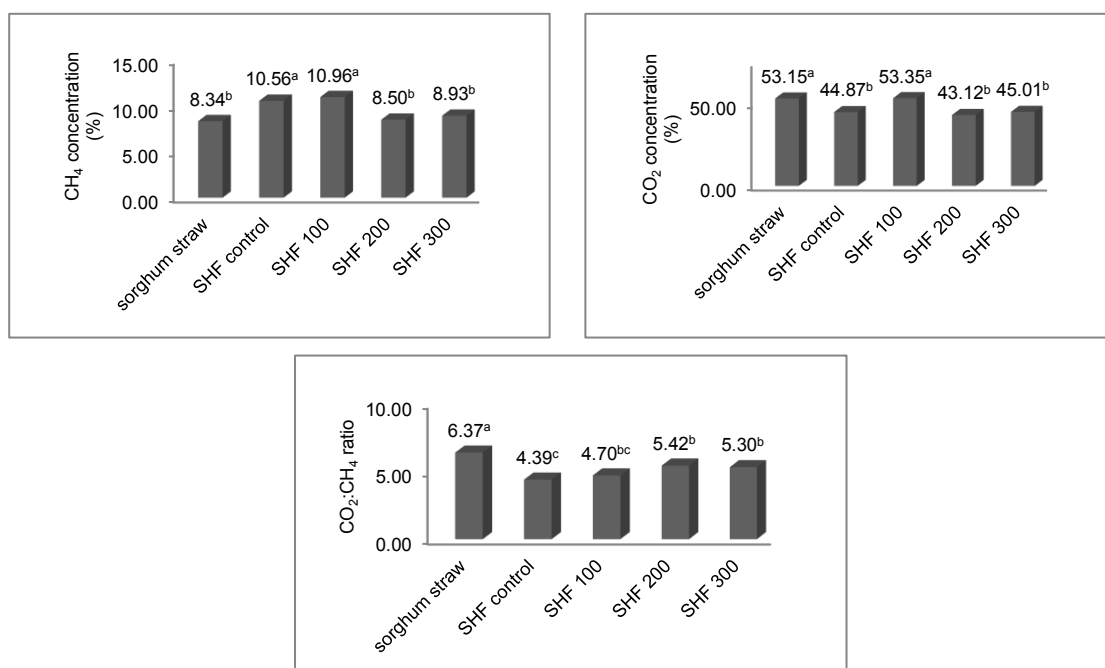


Figure 2. CH₄ concentration (a), CO₂ concentration (b) and CO₂:CH₄ ratio (c) of sorghum straw and SHF after 48 h incubation. SHF (sorghum hydroponic fodder); SHF control (SHF after 10% hypochlorite seeds sterilization); SHF 100 (SHF after dose of 100 Gy seeds sterilization); SHF 200 (SHF after dose of 200 Gy seeds sterilization); SHF 300 (SHF after dose of 300 Gy seeds sterilization). Means with different superscripts are differ ($p < 0.05$). Each value is a mean of four samples.

Conclusions

Lower irradiation dose for seeds sterilization decreased growth performance of SHF. Nevertheless, this study showed that SHF was a potential solution for the provision of forage in a limited land. Dose of 100 Gy gamma irradiation could increase *in vitro* total gas production better than SHF control and sorghum straw. CH₄ concentration and protozoa population are increased on 100 Gy irradiation for seeds sterilization treatment, so there is still need more appropriate strategy to overcome this problem.

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