Research Article

Nutrient Profile and *In vitro* Degradability of New Promising Mutant Lines Sorghum as Forage in Indonesia

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Abstract | G5 and G8 are two sorghum mutant lines projected as special sorghum varieties for forage in Indonesia. The objective of this research was to investigate the nutrient profile and *in vitro* gas production characteristics from two sorghum mutant lines as forage for ruminants. The two mutant lines were compared with Numbu and Pahat variety. Nutrients and *in vitro* digestibility studies were also investigated at different generative phase. This research was arranged into a randomized block design with two factors. The first factor was the variety/mutant lines of sorghum. The second factor was the generative phase (flowering, soft dough and hard dough). Variables measured were nutrients profile, *in vitro* gas production and rumen fermentation products. Results showed that G5 at hard dough phase produced the highest stem sugar content ($P<0.01$). The highest potential *in vitro* gas production (a+b) was found at G5 mutant lines at hard dough stage ($P<0.01$). Pahat and G5 at hard dough phase had the highest *in vitro* true digestibility (IVTD) with 54.48 and 54.61% repectively. The highest total volatile fatty acids (TVFA) was found at G5 and G8 mutant lines at hard dough stage ($P<0.01$). Meanwhile, the best CO_2:CH_4 ratio was produced by Pahat, G5 and G8 ($P<0.01$). Based on those findings, it can be concluded that G5 mutant lines had higher quality of nutrients profile, potential *in vitro* gas production and IVTD. Increasing generative phase also could increase *in vitro* digestibility for all varieties/ mutant lines.

Keywords | Generative phase, *In vitro* gas production, Mutant lines, Nutrient profile, Sorghum

Received | December 10, 2018; **Accepted** | March 09, 2019; **Published** | August 28, 2019
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Citation | Wahyono T, Sugoro I, Jayanegara A, Wiryawan KG, Astuti DA (2019). Nutrient profile and *in vitro* degradability of new promising mutant lines sorghum as forage in indonesia. Adv. Anim. Vet. Sci. 7(9): 810-818.
DOI | http://dx.doi.org/10.17582/journal.aavs/2019/7.9.810.818
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

The lack of fertile land and low production in dry season arethe typicalproblems in the supply of forage worldwide, including in Indonesia (Sriagtula et al., 2017). Sorghum (*Sorghum bicolor* (L.) Moench) forage is apparently suitable to be developed in Indonesia due to the characterization of drought tolerant and adaptive in the tropics (Sajimin et al., 2017). Sorghum has a 27% lower evapotranspiration ability than that of corn (Howell et al., 2008), and could grow in semi-arid and high salinity environments (Su-jiang et al., 2016). Sorghum is an important source for forage in developing countries (Singh et al., 2017). The National Nuclear Energy Agency of Indonesia (BATAN) has produced three sorghum varieties, namely Pahat, Samurai 1 and Samurai 2 (Wahyono, 2015). Recently, BATAN has also developed two mutant lines projected as special sorghum varieties for forage, namely G5 and G8. As forage, the difference in sorghum varieties will affect the nutrient characteristics and digestibility (Singh et al., 2017).

Based on the characteristics of leaf midrib color, sorghum could be categorized into three types: 1) white midrib (WMR) with the characteristics of white midrib and dry stem pith; 2) green midrib (GMR) with the characteristics of green midrib and juicy stem pith; 3) brown midrib (BMR) with the characteristics of reddish brown midrib and juicy stem pith (Li et al., 2015). The agronomic characteristics of Numbu and Pahat are within the WMR sorghum type, whereas the G5 and G8 mutant linesare within the BMR and GMR types, respectively. In addition to differences on variety/mutant line of sorghum, differences in generative phase also influence the nutrient quality and digestibility of sorghum. Forage quantity and quality are directly related with harvest times (Sriagtula et al., 2017). The digestibility of forage sorghum depends on age/phase of harvesting (Harper et al., 2017).

Several studies related to forage sorghum in Indonesia revolve around the topics of biomass production (Sriagtula, 2016), nutrient content (Sajimin et al., 2017; Sriagtula et al., 2017) and digestibility (Sugoro et al., 2015; Wahyono, 2015). These previous studies had not been associated with differences in sorghum nutrient and digestibility characteristics based on WMR, BMR and GMR types. So far, there is no reports describing nutrient profiles and degradability characteristics that compare two sorghum mutant lines (G5 and G8) from Indonesia. The objective of this research was therefore to investigate the nutrients profile and *in vitro* gas production characteristics from two sorghum mutant lines as forage for ruminants. The two mutant lines were compared with Numbu as national sorghum variety in Indonesia and Pahat as main variety in plant breeding from BATAN. Nutrient profiles and *in vitro* digestibility studies were also investigated at different generative phases to determine the best harvesting time.

MATERIALS AND METHODS

PLANTING AND SAMPLE PREPARATION

Field trials were conducted duringFebruary to June 2018 at laboratory field station in Center for Application of Isotope and Radiation, Indonesia National Nuclear Energy Agency (BATAN) (6°17'38.9" S; 106°46'28.8" E, elevation 38 m). This region is characterized as tropic near normal to semi-arid with mean low to medium annual precipitation of 100-300 mm (85-150% in February-June 2018) and average temperature of 28.7°C (BMKG, 2018).

Two sorghum mutant lines (G5 and G8), Pahat sorghum as mutant variety and Numbu sorghum as Indonesia national variety were used in this research. Seeds were sown in 20 × 60 cm planting area at 5-6 cm depth. At 7 days post planting, fertilizers (urea, tri sodium phosphate and potassium chloride) were applied in a ratio of 2:3:2 (g/g/g) at 210 kg/ha. Second fertilizer application was urea in an amount of 140 kg/ha, performed at 30 days post-planting. Harvesting was done after the plant entered the flowering (70 days after sowing/das), soft dough (95 das) and hard dough (115 das) phases. All edible parts (stems, leaves and panicles) were placed into individual paper bags and dried at 60°C for 48 h. Samples were then grinded at 1 mm and prepared for nutrient and degradability analyses.

NUTRIENT PROFILE DETERMINATION

The sugar content of the sorghum stem is measured using refractometer. Organic matter (OM), crude protein (CP) and ether extract (EE) were analyzed according to Associaton of Official Analytical Chemists method (AOAC,2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by following Van Soest et al. (1991) procedures. Non-fiber carbohydrate (NFC) was calculated as OM – CP – NDF–EE (Kondo et al., 2015). Determination of relative feed values (RFV) was calculated as follow: RFV (%) = (DMD x DMI)/1.29,dry matter digestibility (DMD) (%) = 88.9 – (%ADF x 0.779) and dry matter intake (DMI) (% live weight) = 120/%NDF (Rohweder et al., 1978; Kilic and Gulecyuz, 2017). According to the Quality Grading Standard by the Hay Marketing Task Force of the American Forage and Grassland Council, the RFV is classified as follow: reject (5) (score<75), poor (4) (75-86), fair (3) (87-102), good (2) (103-124), premium (1) (125-151) and prime (>151) (Kilic and Gulecyuz, 2017).

IN VITRO GAS PRODUCTION PROCEDURE

The *in vitro* gas production was conducted byusing Hohenheim gas test technique of Menke et al. (1979). Briefly, 200 ± 10 mg samples were weighted into 100 ml Fortuna® Optima glass syringe (Poulten & Graf, Germany). Three sheep as rumen fluid inoculum donor were fed with native grass and concentrate at the ratio of 70:30 (DM basis) for two weeks prior to experiment. Rumen fluid was obtained via oral stomach tube (OST) of Ramos-Morales et al. (2017). Rumen fluid (433.33 ml) was mixed with 827.85 ml McDougal buffer/artificial saliva. McDougal buffer consists of 413.33 ml aquadest, 206.67 buffer, 206.67 macromineral, 0.11 micromineral and 1.07 resazurin (Menke et al., 1979). Incubation was conducted in a waterbath at 39°C. Thirty ml media liquor was put into each syringe. Initial volume before sample incubated was recorded.

Gas production was recorded at 0, 2, 4, 6, 8, 10, 24, 48, 72 and 96 h. Gas production kinetics was measured using exponential equation of Ørskov and Mcdonald (1970) as follows: $p = a + b(1-e^{-ct})$, where p is the gas production at t time, a is the gas production from soluble fraction (ml/200 mg DM), b is the gas production from insoluble fraction (ml/200 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). The CH_4, CO_2 production and CO_2:CH_4 gas ratio were measured after 96 h incubation by an infrared VARIO luxx syngasanalyzer (Messgerätefür Rauchgase und Umweltschutz/MRU®gas analyzer, Germany) (An amount of 10 ml of *in vitro* fermentation medium was collected after 96 h incubation to determine NH_3 concentration (Conway, 1951), total volatile fatty acids (TVFA) production (AOAC, 2005) and pH. Metabolisable Energy (ME) was calculated by equation of Menke et al. (1979): ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP. GP was the total gas production (ml/200 mg DM) and CP was crude protein (% DM). Specifically for *in vitro* true digestibility (IVTD) measurement, after 48 h incubation, the residue of each substrate was treated by neutral detergent soluble (NDS) and dried at 105°C for 3 h.

DATA ANALYSIS

The data was analyzed using randomized block design with two factors. The first factor was sorghum variety or mutant line (Numbu, Pahat variety, G5 and G8 mutant line) and the second factor was generative stages (flowering, soft dough and hard dough phases). Data were analyzed using analysis of variance (ANOVA) by SPSS version 16.0. differences among treatments were separated using Duncan Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

NUTRIENT PROFILE

There wasa significant interaction on the stem sugar content between variety/mutant line and generative phase ($P<0.01$, Table 1). The G5 and G8 sorghum mutant lines produced higher stem sugar contents (11.83% and 10.41% brix, respectively) as compared to Numbu (9.9% brix) and Pahat (8.96% brix). The result showed that stem sugar content was influenced by generative phase ($P<0.01$). The interactions between sorghum variety/mutant lines and generative phase were significant for OM, CP, NFC, NDF and ADF contents ($P<0.01$; Table 2). The CP content decreased with the increasing generative phase ($P<0.01$). The NDF and ADF contents also decreased with advancing harvest time. According to RFV index, it was found that the forage quality increased with the increasing maturity stage.

The BMR sorghum has attractedmuch attention by the scientists in Indonesia due to its lower lignin content and higher digestibility as forage (Puteri et al., 2015; Sriagtula et al., 2017). Scully et al. (2016) reported that mutations in BMR6 gene were associated with reductions in lignin and changes in lignin subunit composition, which improved saccharification and sugar fermentation efficiency. Our result also showed that BMR type had higher sugar stem content than WMR (Numbu and Pahat) and GMR (G8) type. This may be a form of association between high sugar stem content due to the influence of lower lignin content. Lower lignin in BMR mutant line could affect sugar content of the stem (Sriagtula et al., 2017). A similar statement was also expressed by Li et al. (2015) that BMR populations had significantly ($P<0.05$) lower acid detergent lignin (ADL) content than WMR or GMR populations. Generally, sugar stem in all variety/mutant line increased with the increased maturity (hard dough) stage. This is due to increased sugar stem production to follow the physiological development of panicle. Teixeira et al. (2017) reported that sucrose, glucose and fructose are participate in total sugar concentration in the stem during the phenological stages.

In previous studies, there were many different results about expression of CP content difference between BMR and non-BMR sorghum types. Godin et al. (2016) Indicated CP content of BMR type was 7.31% higher than wild sorghum type. BMR sorghum produced higher CP content than non-BMR sorghum (Puteri et al., 2015; Sriagtula et al., 2017) However, Bean et al. (2013) reported that no significant differences was observed between BMR and non-BMR forage sorghum type. Our result showed that G5 as BMR type had higher CP content than Numbu (WMR type), nevertheless, G8 (GMR type) produced highest CP content in this study. It is still not clear about the explanation for the causes of differences in protein content in some types of sorghum. Some explanations are still limited speculation. Li et al. (2015) speculated that mutation process probably induced the change of their protein-synthesis pathway of BMR type. In this study, CP content was decrease with the increased generative phase. This is related to the inhibition of protein synthesis during maturation (Baloyi et al., 2013).

Kondo et al. (2015) reported that the NFC fraction included rapidly fermentable carbohydrates, including soluble sugars and starch. Our result was indicated that G5 as BMR sorghum produced higher NFC than non-BMR at hard dough phase ($P<0.01$) even though no difference in mean for all generative phase with Pahat variety. The reason for causing this response was likely to be connected with lower lignin content, as mentioned in previous statement. Mutation process in BMR affect reduced activity of two enzymes activity that connected with lignin biosynthetic activity. Both of these enzymes are cinnamyl alcohol dehydrogenase (CAD) in bmr-6 mutants and caffeic acid O-methyltransferase (COMT) in bmr-12 mutants (Li et al., 2015). High mean value in NFC content at hard dough phase is due to the increasing content of starch in grains. In soft dough to hard dough phase, Carbohydrate proportion will be trans-located from stems to grain and at the same time sugar accumulates in the stems (Qu et al., 2014; Sriagtula et al., 2017). The NFC fraction can be underlie specific consideration, especially to provide soluble carbohydrate in ruminant ration, which are usually provided by grains.

As feed, NDF content represents structural carbohydrate content in plants. On the other hand, structural carbohydrates also function as an energy source/adenosine triphosphate (ATP) but have a slow rate value of degradation (Kondo et al., 2015). NDF content of BMR was decreased significantly (P<0.05) as compared with WMR and GMR populations. The lower NDF content could be associated with lowering lignin content in BMR type. The highest NDF content was produced by WMR type (Li et al., 2015). Our results showed that G5 as BMR type had lower NDF content than Numbu as WMR type (P<0.01). even though, Pahat as WMR type produced the lowest NDF content at all generative phase. There are two things that could be explained: 1) The reason was probably influenced by the character of Pahat that has short height and affect to high proportion of panicle/grains. With the result that, Pahat has lower NDF content than other tall sorghum type; 2) the possibility of Pahat sorghum has BMR-12 gene due to these variety was the parent breed of G5 mutant line. However, this speculation needs to be further investigated. The content of NDF in the four variety/mutant line has decreased with the increase of generative phase. This due to requirement of plants to produce grains at the dough phase (Li et al., 2015). Our results indicated that NDF content decreased in whole plant while the stem sugar content were higher at the same phases (Table 1).

Variation of mean ADF among variety/mutant line was similar to variation of mean NDF. G5 and G8 mutant lines had lower ADF content than Numbu at all generative phase (P<0.01). Even though, Pahat produced the lowest ADF content (P<0.01). The explanation is in line with the previous speculation in NDF content. A low ADF content is an indicator of digestibility in feed (Kilic & Gulecyuz, 2017). BMR populations consistently higher nutritive value than non-BMR since joint stage (130 cm heigh of plant) (Li et al., 2015). Sriagtula (2016) reported that BMR sorghum had lower ADF content than non-BMR types. Lower ADF content associated with lower lignin content. G8 as GMR/stay green type of sorghum had lower ADF content than Numbu (P<0.01). This result is in accordancewith the results reported by Sugg et al. (2017) that stay green sorghum obtained numerical lower ADF content than traditional sorghum (25.66% vs 26.34%). Low ADF content in stay green sorghum contributed to greater yield of nutritive components than other line/variety (Vietor et al., 2010).

There was no significant difference in EE content between all variety/mutant line. In this study, the EE content was found between 1.83%-2.38%. Puteri et al. (2015) was analyzed the EE content of BMR and sweet sorghum variety and registered between 0.34%-1.84%. The EE content of sorghum forage also previously reported between 1.16%-2.27% (Sriagtula, 2016). We can presume that this difference, probably due to: 1) The differences in the wax content in leaves due to the differences in water loss stress in each planting condition. As we know, the wax is soluble in ether and included into ether extract fraction; and 2) The differences in the starch content between all variety.

High RFV values in Pahat, G5 and G8 at hard dough phase were associated with low levels of NDF and ADF. The values of RFV reflect digestibility levels (from %ADF) and potential feed intake (Basaran et al., 2017). RFV values are associated with nutritional value and quality of forage. The differences in nutritional value will associated with different fermentation and gas production characteristics. Forages with higher RFV are more digestible and palatable (Jahansouz et al., 2014). The RFV value was increased with the increased harvesting age. This due to the decrease of NDF and ADF value linearly with increase of generative phase. The RFV value is important to determine the feed quality. However, the results of calculations needs to be further observed to determine the true digestibility.

In Vitro Gas Production

There were significant interaction in total gas production, potential gas production (a+b) and gas production rate (c) between variety/mutant line and generative phase (P<0.01). High dynamics of total gas production showed in all varieties/mutant lines (P<0.01, Table 3). At 2-10 h incubation time, The lowest gas production was produced by Numbu and G5 at flowering phase (P<0.01). At the same incubation time, G8 at hard dough phase produced highest gas production of around 10.14-26.67 ml. 200 mg DM (P<0.01). At 24-96 h incubation time, the highest gas production was produced by G5 and G8 at hard dough phase (P<0.01). There was a tendency that hard dough phase was produced higher total gas and optimum gas production than flowering and soft dough phase.

Total gas production was a reflection of nutrient profiles presented in Table 1 and 2. The difference in ADF content will affect the difference of *in vitro* total gas production (Jayanegara & Sofyan, 2008). Furthermore, gas production has negative correlation with ADF content (Jayanegara et al., 2009). In present study, the high total gas production was obtained by G5, G8 and Pahat at hard dough phase. The highest total gas production was produced by G5 and G8 at hard dough phase due to a low ADF content (Table 2). G5 and G8 mutant lines also contain higher soluble carbohydrate represented by NFC content (Table 2). Zhong et al. (2016) reported that gas production in the rumen is generated associated with carbohydrate fermentation.

Table 1: Sugar content of sorghumstem at different generative phase (% brix)

Generative phase	Variety/mutant line				Mean
	Numbu	**Pahat**	**G5**	**G8**	
Flowering	8.31 ± 0.50^{b}	7.68 ± 0.75^{a}	9.39 ± 0.91^{c}	9.56 ± 0.64^{c}	8.73 ± 1.04^{a}
Soft dough	10.73 ± 0.86^{d}	9.80 ± 0.60^{c}	12.44 ± 0.59^{f}	9.86 ± 0.49^{c}	10.71 ± 1.25^{b}
Hard dough	10.67 ± 0.62^{d}	9.40 ± 0.40^{c}	13.66 ± 0.57^{g}	11.83 ± 0.58^{e}	11.39 ± 1.67^{c}
Mean	9.90 ± 1.32^{b}	8.96 ± 1.10^{a}	11.83 ± 1.95^{d}	10.41 ± 1.17^{c}	

Means with different superscripts within row or column are different (P<0.01).

Table 2: Nutrient content of sorghum and RFV value at different generative phase (% DM).

Parameter	Variety/mutant line	Generative phase			Mean
		Flowering	**Soft Dough**	**Hard Dough**	
OM	Numbu	90.52 ± 0.58^{b}	93.51 ± 0.62^{d}	94.70 ± 0.53^{f}	92.91 ± 1.88^{c}
	Pahat	92.23 ± 0.29^{c}	91.66 ± 0.51^{c}	89.66 ± 0.62^{a}	91.18 ± 1.22^{a}
	G5	89.56 ± 0.50^{a}	93.79 ± 0.91^{de}	94.42 ± 0.44^{ef}	92.59 ± 2.28^{c}
	G8	90.01 ± 0.77^{ab}	92.02 ± 0.76^{c}	93.80 ± 0.95^{de}	91.95 ± 1.77^{b}
	Mean	90.58 ± 1.16^{a}	92.74 ± 1.16^{b}	93.15 ± 2.16^{c}	
CP	Numbu	7.98 ± 0.33^{ab}	7.91 ± 0.17^{a}	7.89 ± 0.17^{a}	7.93 ± 0.23^{a}
	Pahat	10.95 ± 1.15^{f}	8.77 ± 0.39^{cd}	8.32 ± 0.30^{abc}	9.35 ± 1.36^{b}
	G5	9.95 ± 0.42^{e}	8.85 ± 0.26^{cd}	8.48 ± 0.26^{bc}	9.09 ± 0.71^{b}
	G8	10.52 ± 0.93^{f}	10.52 ± 0.56^{f}	9.19 ± 0.49^{d}	10.08 ± 0.92^{c}
	Mean	9.85 ± 1.38^{c}	9.01 ± 1.02^{b}	8.47 ± 0.57^{a}	
NFC	Numbu	12.19 ± 1.77^{a}	29.06 ± 2.16^{d}	31.56 ± 0.93^{e}	24.27 ± 8.92^{a}
	Pahat	20.30 ± 1.16^{c}	33.57 ± 2.49^{ef}	34.54 ± 1.91^{fg}	29.47 ± 6.88^{c}
	G5	14.25 ± 1.50^{ab}	33.31 ± 2.80^{ef}	38.79 ± 1.92^{h}	28.78 ± 10.92^{c}
	G8	15.84 ± 1.66^{b}	27.05 ± 1.11^{d}	36.65 ± 2.18^{gh}	26.51 ± 8.82^{b}
	Mean	15.64 ± 3.36^{a}	30.75 ± 3.54^{b}	35.39 ± 3.21^{c}	
NDF	Numbu	68.52 ± 1.36^{i}	54.44 ± 2.01^{e}	52.78 ± 0.67^{d}	58.58 ± 7.33^{c}
	Pahat	58.77 ± 1.30^{f}	47.08 ± 2.32^{b}	44.70 ± 1.53^{a}	50.18 ± 6.49^{a}
	G5	63.31 ± 1.19^{h}	49.25 ± 2.21^{c}	45.11 ± 1.84^{a}	52.56 ± 8.12^{b}
	G8	61.72 ± 1.17^{g}	52.16 ± 1.26^{d}	45.86 ± 1.37^{ab}	53.25 ± 6.75^{b}
	Mean	63.08 ± 3.78^{c}	50.73 ± 3.42^{b}	47.11 ± 3.61^{a}	
ADF	Numbu	44.24 ± 1.06^{i}	33.59 ± 0.67^{f}	31.93 ± 0.51^{e}	36.59 ± 5.61^{d}
	Pahat	32.61 ± 0.62^{e}	24.69 ± 1.27^{a}	24.14 ± 0.91^{a}	27.14 ± 4.05^{a}
	G5	37.14 ± 0.63^{h}	28.30 ± 0.52^{c}	26.14 ± 0.46^{b}	30.53 ± 4.88^{b}
	G8	35.75 ± 0.51^{g}	30.44 ± 0.76^{d}	27.97 ± 0.93^{c}	31.39 ± 3.38^{c}
	Mean	37.43 ± 4.37^{c}	29.25 ± 3.38^{b}	27.55 ± 2.99^{a}	
EE	Numbu	1.83 ± 0.16^{a}	2.09 ± 0.26^{bcd}	2.46 ± 0.38^{f}	2.13±0.38
	Pahat	2.21 ± 0.23^{cde}	2.24 ± 0.23^{cdef}	2.11 ± 0.10^{bcd}	2.19±0.19
	G5	2.05 ± 0.17^{abcd}	2.38 ± 0.37^{ef}	2.03 ± 0.12^{abc}	2.16±0.29
	G8	1.94 ± 0.09^{ab}	2.29 ± 0.22^{def}	2.10 ± 0.15^{bcd}	2.11±0.21
	Mean	2.01 ± 0.21^{a}	2.25 ± 0.29^{b}	2.17 ± 0.27^{b}	
RFV	Numbu	73.96 (5-reject)	107.49 (2-good)	112.44 (2-good)	
	Pahat	99.81 (3-fair)	138.14 (1-premium)	144.65 (1-premium)	
	G5	88.01 (3-fair)	127.98 (1-premium)	141.17 (1-premium)	
	G8	91.38 (3-fair)	115 (2-good)	137.47 (1-premium)	

Means with different superscripts within row or column in same parameters are different (P<0.01). Dry matter (DM); organic matter (OM); crude protein (CP); non-fiber carbohydrate (NFC); neutral detergent fiber (NDF); acid detergent fiber (ADF); ether extract (EE); relative feed value (RFV).

The sum of a and b fractions (a+b) can be interpreted as the optimum gas production, while c fraction is the rate of degradation (Orskov and Mcdonald, 1970; Kisworo et al., 2017). Jayanegara et al. (2009) reported the evaluation of gas production kinetics needs to be observed at 72-96 h, especially in fibrous samples/ingredients. The statement based on to find out the accurate of kinetics coefficient and estimation of optimum gas production. The optimum gas production (a+b) of G5 at hard dough stage was relatively high at 96 h incubation time. G5 mutant line has a higher digestibility value than non-BMR sorghum. In previous studies, BMR sorghum type had higher digestibility than non-BMR type due to the lower lignin content (Bean et al., 2013; Puteri et al., 2015; Sriagtula et al., 2017; Sriagtula, 2016). All variety/mutant line sorghum at hard dough phase had higher total and optimum (a+b) gas production than flowering phase. This due to the decrease of fiber fraction linear with increase of maturity stage until hard dough phase (Table 2). Kisworo et al. (2017) stated that the c fraction will be lower in feed containing high fiber content.

Rumen Fermentation Characteristics

In vitro true digestibility and rumen fermentation characteristics were showed in Table 4. There were significant interaction in all parameters (except NH_3) between variety/mutant line and generative phase (P<0.01). Within the same variety/mutant line, generative phase showed significant linear effects on IVTD (P<0.01). Numbu at the flowering phase had the lowest of IVTD and TVFA (P<0.01). Pahat and G5 at hard dough phase produced highest IVTD value (P<0.01). The pH value ranges of all treatments were from 6.62-6.79. The highest ME was also produced by G5 and G8 at hard dough phase by 2373.90 and 2356.96 kcal/kg DM (P<0.01). The best CO_2:CH_4 ratio was produced by Pahat, G5 and G8 at hard dough phase by 6.72, 6.90 and 6.86 respectively (P<0.01).

The different fermentation characteristics due to the differences in nutritional value (Zhong et al., 2016). It was reported that IVTD have the same pattern with total gas production, because the gas production in the rumen is representation of substrate degradability. Pahat and G5 sorghum at hard dough phase had highest IVTD value due to the lower ADF value compare to Numbu and G8 (Table 2). Furthermore, G5 as BMR type contains low lignin content, that associated with highly degradability of fiber fraction (Bernard and Tao, 2015). G8 had higher IVTD than Numbu at all generative phase due to higher CP content (Table 2). This also due to possibility of lower lignin content in BMR mutant lines. Samples with a low fiber content will be easy to be digested and requires a shorter time per unit weight (Kisworo et al., 2017). Bean et al. (2013) showed that forage digestibility was best with the BMR clases followed by the grain classes. Present study obtained that IVTD increased linearly with increasing harvesting time. Our result related with Sriagtula et al. (2017) that reported IVTD values also increase with increasing generative stages of plants. Decreasing fiber fraction after the soft dough phase will increase feed digestibility (Qu et al., 2014).

The value of pH, NH_3 and VFA characteristics are mainly indicate the degradation patterns of substrate by microbes. Each sample is a source of fiber content, therefore the pH ranges were neutral. Wahyono (2015) reported that pH values were influenced by the concentration of fiber in feed and the interaction with saliva and other fermentation products. Generally, the concentration of NH_3 was not significantly different between treatments, even though there were differences in CP content (Table 2). This due to accumulation of NH_3 from buffer/artificial saliva and there is no absorption in closed culture fermenter system (Firsoni et al., 2010). Kisworo et al. (2017) also stated that there was NH_3 accumulate in *in vitro* fermentation system because NH_3can not be recycled as in the actual rumen conditions. This will affects the less representative of NH_3 concentration. Furthermore, ruminal NH_3 concentration is not only influenced by CP content of the substrate incubated. Difference in the nature and amount of various protein fractions in the substrate, i.e., soluble protein, fastly degraded, moderately degraded, slowly degraded and undegradable protein also affect the extent and rate of NH_3 production in the rumen (Jayanegara, et al., 2016). Presence of plant secondary compounds that interact with protein such as tannins may cause alteration on ruminal NH_3 concentration as well (Kondo et al., 2014).

Ruminal VFA productions indicate the degradation pattern of carbohydrates (Zhong et al., 2016). The high value of TFVA in G5 and G8 at hard dough phase could be caused by the low ADF content (Table 2). TVFA productions are representations of fermentation rate, digestibility and gas production (Sugoro et al., 2015; Wahyono et al., 2018). TVFA production also has linearly pattern with total gas production (Table 3). Kondo et al. (2015) stated that TVFA production in tropical grass highly correlates with *in vitro* gas production (r=0.96) and NFC content (r=0.84).

The production of CH_4 tends to be high in the flowering phase and decreased at the hard dough phase. This due to different NDF content characteristics in each treatments. Jayanegara et al. (2009) reported that CH_4 production will increase associated with the high NDF and hemicellulose content. Our results showed that G5 and G8 mutant lines produce low CH_4 emissions. Pahat variety, G5 and G8 at hard dough phase also produce high ratio of CO2:CH_4 and associated with low NDF content (Table 2). Ratio of CO_2:CH_4 represents the efficiency of fermentation in the rumen. The availability of soluble carbohydrate in BMR

Table 3: *In vitro* total gas production and gas kinetics of sorghum at different generative phase

Treatment		Time of incubation (h) (ml/200 mg DM)									Gas kinetics	
		2	4	6	8	10	24	48	72	96	a+b	c
Numbu	flowering	1.47^{a}	2.09^{a}	2.88^{a}	4.19^{a}	5.29^{a}	10.43^{a}	21.33^{a}	25.06^{a}	30.25^{a}	38.14^{a}	0.015^{a}
	soft dough	5.26^{c}	8.58^{e}	11.58^{f}	13.32^{e}	15.27^{e}	27.54^{f}	36.08^{d}	38.56^{d}	42.14^{b}	41.95^{b}	0.041^{f}
	hard dough	5.01^{c}	8.92^{e}	11.89^{f}	13.50^{e}	15.38^{e}	25.96^{e}	35.03^{d}	37.85^{d}	41.55^{b}	41.59^{b}	0.038^{de}
Pahat	flowering	2.76^{b}	5.16^{c}	7.66^{d}	8.96^{c}	10.95^{c}	22.73^{d}	35.40^{d}	38.68^{d}	42.75^{b}	45.22^{c}	0.030^{c}
	soft dough	3.16^{b}	6.26^{d}	9.16^{e}	10.92^{d}	13.76^{d}	29.60^{g}	40.15^{e}	42.17^{e}	45.48^{c}	45.83^{c}	0.042^{f}
	hard dough	2.72^{b}	5.63^{cd}	8.64^{e}	10.62^{cd}	12.85^{d}	30.26^{g}	43.55^{f}	45.58^{f}	50.25^{e}	51.15^{e}	0.037^{de}
G5	flowering	1.80^{a}	2.73^{a}	3.91^{b}	4.89^{a}	6.18^{a}	15.55^{b}	30.51^{b}	35.12^{b}	44.28^{c}	51.41^{e}	0.018^{a}
	soft dough	6.78^{d}	12.21^{f}	14.67^{g}	16.13^{f}	17.59^{f}	31.63^{h}	42.65^{f}	45.36^{f}	50.32^{e}	50.72^{e}	0.036^{d}
	hard dough	8.63^{e}	14.34^{g}	17.93^{h}	20.27^{g}	23.44^{g}	32.29^{hi}	45.44^{g}	47.32^{g}	53.36^{f}	53.03^{f}	0.036^{d}
G8	flowering	2.67^{b}	3.81^{b}	5.49^{c}	6.53^{b}	7.73^{b}	19.70^{c}	32.13^{c}	36.47^{c}	42.00^{b}	47.82^{d}	0.022^{b}
	soft dough	5.25^{c}	9.31^{e}	12.01^{f}	13.93^{e}	16.53^{e}	31.56^{h}	42.11^{f}	44.29^{f}	48.19^{d}	48.22^{d}	0.041^{f}
	hard dough	10.14^{f}	15.91^{h}	21.52^{i}	23.19^{h}	26.67^{h}	33.01^{i}	45.07^{g}	48.60^{g}	52.55^{f}	51.95^{ef}	0.039^{ef}
SEM		0.261	0.422	0.521	0.546	0.606	0.685	0.678	0.620	0.603	0.460	0.001

Means with different superscripts within column are different ($P<0.01$).
Standard error mean (SEM); potentialgas production (a+b); gas production rate (c).

Table 4: *In vitro* true digestibility and rumen fermentation characteristics of sorghum at different generative phase

Treatment		IVTD (%)	pH	NH_3 (mM)	TVFA (mM)	ME (kkal/kg DM)	CH_4 Production (ml/100 mg IVOMD)	CO_2 Production (ml/100 mg IVOMD)	CO_2:CH_4 Ratio
Numbu	Flowering	18.98^{a}	6.77^{de}	6.71	89.62^{a}	1616.39^{a}	5.12^{f}	26.17^{c}	5.11^{b}
	Soft dough	36.54^{cd}	6.79^{de}	6.46	100.20^{bc}	2001.61^{bc}	5.09^{f}	31.49^{h}	6.19^{de}
	Hard dough	44.71^{e}	6.75^{cd}	6.65	91.29^{a}	1982.10^{b}	4.44^{c}	27.61^{de}	6.21^{de}
Pahat	Flowering	35.10^{c}	6.81^{e}	7.05	94.08^{ab}	2062.72^{de}	4.69^{e}	27.65^{de}	5.90^{c}
	Soft dough	50.08^{g}	6.74^{cd}	6.59	90.74^{a}	2121.85^{f}	4.74^{e}	29.44^{f}	6.21^{de}
	Hard dough	54.58^{gh}	6.62^{a}	6.53	99.64^{bc}	2270.73^{gh}	4.55^{d}	30.59^{g}	6.72^{f}
G5	Flowering	31.52^{b}	6.75^{cde}	7.02	115.23^{d}	2098.95^{ef}	4.75^{e}	23.32^{a}	4.91^{a}
	Soft dough	44.57^{e}	6.77^{de}	6.73	119.13^{de}	2280.01^{h}	4.53^{cd}	28.39^{e}	6.27^{e}
	Hard dough	54.61^{gh}	6.77^{de}	6.80	125.81^{e}	2373.90^{i}	3.96^{b}	27.34^{d}	6.90^{f}
G8	Flowering	37.94^{d}	6.76^{cde}	6.74	106.88^{c}	2032.68^{cd}	4.56^{d}	27.77^{de}	6.10^{de}
	Soft dough	43.47^{e}	6.69^{b}	6.95	116.34^{d}	2233.68^{g}	4.02^{b}	24.36^{b}	6.06^{cd}
	Hard dough	47.25^{f}	6.71^{bc}	6.95	121.35^{de}	2356.96^{i}	3.59^{a}	24.59^{b}	6.86^{f}
SEM		0.967	0.007	0.280	1.409	19.785	0.043	0.242	0.059

Means with different superscripts within column are different ($P<0.01$).
In vitro true digestibility (IVTD); ammonia (NH_3); total volatile fatty acids (TVFA); metabolisable energy (ME); dry matter (DM); in vitro organic matter degradability (IVOMD); standard error mean (SEM).

type will increase the efficiency of rumen fermentation that represent by lower CH_4 production (Su-jiang et al., 2016). These studies concluded that G5 promising mutant lines produced lower NDF and ADF than Numbu varieties. Lower structural fiber fractions could affect the higher optimal gas production (a+b) and IVTD. G8 promising mutant lines also produced high ME and CO_2:CH_4 ratio. It could be attributed to G8 produced a nutrient profile that approaches G5 and Pahat. The best harvesting time to produced best nutrient profile and digestibility is hard dough generative phase. Further studies are needed to evaluate G5 and G8 promising mutant lines as ingredient in ruminant feed rations.

ACKNOWLEDGEMENTS

The author wish to thank Prof. Soeranto Human, Mr. Sihono, Mr. Dedi Ansori and Shintia Nugrahini Wahyu Hardani, A.Md and others who helped in the field during this project. This study was conducted under the support of Agricultural Division, Center of Isotope and Radiation Application (CIRA) BATAN project and Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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