Maturity Stages Effects of Brown Midrib Sorghum Mutant Lines on Nutrients, Fiber Fraction, and In Vitro Fiber Fraction Digestibility

By Riesi Sriagtula

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ABSTRACT

Brown midrib sorghum mutant line has lower lignin content than conventional sorghum and can replace maize as a forage fodder. The objectives of this research are to investigate the influence of plant maturity stages at harvest times on nutrient, fiber fraction and tannin content, VFA production and in vitro fiber fraction digestibility of BMR sorghum mutant lines. This research was arranged into Randomized Complete Block Design with Factorial in three replicated. The first-factor was non-BMR Patir 3.1 (control), BMR Patir 3.2, and BMR Patir 3.7. The second factor was the generative stages (flowering, soft dough, and hard dough stage). The observed parameters were nutrient, fiber fraction and tannin content, VFA production and in vitro fiber fraction digestibility. Data were analyzed by Anova and DMRT. The sorghum mutant line factor was affected ($P<0.01$) of crude protein, crude fiber, and TDN and maturity stages were on crude fiber, ash, and crude fat. Meanwhile, the fiber fraction and tannin generally affected by maturity stages. No effect of the maturity stage on acetate and propionate production was found. However, plant maturity stage and sorghum lines impact butyrate ($P<0.05$). BMR sorghum mutant lines (Patir 3.2 and Patir 3.7) produced higher ADF digestibility than non-BMR sorghum mutant lines (Patir 3.1). NDF digestibility is significantly influenced by both sorghum mutant lines and maturity stages ($P < 0.01$). This study concludes that BMR sorghum mutant lines and hard dough stage produced better nutrient and in vitro digestibility, but the butyrate acid is higher on non-BMR sorghum mutant.

Keywords: Acetat; ADF digestibility; BMR sorghum; NDF digestibility; Propionat ratio

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of the world's main important crops and ranks at the fifth world's widest spread after wheat, rice, maize, and barley (Dahir *et a*l. 2015). Sorghum is one of the cereal crops consisting of forage and grains which can be used for fodder replace maize (Zea mays) (Sriagtula *et al*. 2017). Sorghum has requirements low input to ability grow on marginal lands Mathur *et al*. (2017); Sriagtula *et al*. (2019). Sorghum has higher lignin content than maize, which limits its utilization by ruminants. This is due to conventional varieties of sorghum is a food crop and not design to be used as a feed (Sriagtula *et al*. 2017). Genetic modification through induced mutation using gamma rays irradiation has been developed in sorghum, produced brown midrib sorghum mutant lines (BMR) with lower lignin content. Some BMR sorghum mutant lines are produced in Indonesia (Supriyanto 2014), meanwhile Patir 3.2 and Patir 3.7 produce the highest biomass productivity of them (Sriagtula *et al*. 2016). Decreasing lignin content in BMR sorghum increases digestibility, energy conversion efficiency and nutrition content (Christensen and Rasmussen, 2019).

The nutrition content and fiber fraction of forage is directly related to staging maturity. Advance maturity causes low digestibility, it indicates the low quality of forages and influences livestock productivity (Beck *et al*. 2013). In vitro fiber fraction digestibility was much less is known than dry matter digestibility, although its more relevance to cell-wall utilization by ruminants. The observation of sorghum mutant lines and BMR sorghum variety in tropical countries, like Indonesia, is limited. Patir 3.2 and Patir 3.7 are the new generations of BMR sorghum mutant lines in Indonesia, their nutrient content and fiber fraction as forage need to be explored. Based on those ideas, the optimum harvest times of BMR sorghum mutant lines Patir 3.2 and Patir 3.7 should be investigated. Furthermore, the ruminal organic acids and in vitro fiber fraction digestibility in different maturity stages should also be evaluated.

MATERIALS AND METHODS

The research was conducted at SEAMEO BIOTROP Bogor, Indonesia, used Randomized Complete Block Design with Factorial in three replicated. The first factor was the sorghum mutant lines of Patir 3.1 (non-BMR/control), Patir 3.2, and Patir 3.7 (BMR lines). The second factor was generative stages (flowering, soft dough, and hard dough phase).

The culture technique and sample preparation: The technique culture of sorghum refers to Supriyanto (2010) and Sriagtula *et al*. (2016). Sorghum mutant seeds were sown in 20 x 60 cm planting area at 5 cm depth. At 14 days post-planting, urea, tri-sodium phosphate, and potassium chloride fertilizers were ap¬plied in a ratio of 4:3:2 (g/g/g) at 270 kg/ha. At 50 days post-planting, second fertilizer application with a ratio of 2:4:2 (g/g/g) at 200 kg/ha. At flowering (74 days after sowing/das), soft dough (90 das) and hard dough (110 das) phases the plant was harvested. The whole plant sorghum (leaves, stem, and panicle) were placed into individual paper bags and dried at 60°C for 48 h. Samples were then ground at a <1 mm mash for nutritional analysis.

Rainfall during the study was categorized as low at <100 mm to a high at 300-500 mm (Ishak *et al*., 2012). The the rainfall during the study is presented in Figure 1.

In Vitro Fiber Fraction Digestibility (IVFFD) Test: The in vitro digestibility test was refers to Tilley and Terry (1963) method. Each sample was 0.5 g weighed incubated for 48 h with 40 mL of McDougall buffer solution and 10 mL of rumen fluid addition with CO_2. At the end of the first fermentation, 2-3 drops of $HgCl_2$ was an addition to stopped microbial activity, follow the inoculation period of 48 h with the ad¬dition of 50 g pepsin HCl. This rumen liquid obtained from three rumen-fistulated adult Bali cattle that were fed ad libitum with the ratio 60:40 of roughage: concentrate respectively. The IVFFD for each ingredient was measured in duplicate.

Parameters: The observed parameters were nutrient and fiber fraction content, VFA production and in vitro fiber fraction digestibility of the whole plant of sorghum mutant lines. The tannin content observed in the panicle part.

Chemical analysis and calculation: The quality of the whole plant mutant sorghum was measured by proximate analysis referred to as the AOAC method (1980). Nitrogen Free Extract (NFE) was calculated from moisture, CP, CF, EE, and ash with formula NFE= 100- (moisture + ash + crude fat + Crude protein + crude fiber) referred to Tillman *et* al. (1998). The fiber fraction analysis referred to Van Soest (1994).

Statistical analysis: Data were analyzed by using analysis of variance (ANOVA) by the SPSS 16 software program. Duncan Multiple Range Test was conducted if a significant difference occurred (Steel and Torrie, 1997).

RESULTS

Nutrient content of whole plant sorghum mutant lines: The harvest time was effected the crude fiber, ash and crude fat significantly (P<0.01). The Crude fiber was decreased at flowering stage to a soft dough and hard dough stage from 27.47%, 18,16%, and 14.72% respectively, meanwhile decreasing ash content occurs on the hard dough stage. The crude fat (EE) content was increased on advance maturity, and at hard dough stage was produced the highest crude fat (2.02%).

Fiber content of whole plant sorghum mutant lines: The content of fiber fractions presented in Table 2. There was no interaction was found on sorghum mutant lines and harvest time to fiber fraction content. The ADF, NDF, lignin, and cellulose content were affected by harvest time significantly (P<0.01), and the other hands ADF and lignin content were affected by sorghum mutant lines (P<0.05), neither in NDF content. Meanwhile, no effect of sorghum mutant lines and harvest time in hemicellulose content in this study.

Calcium and Phospor content of whole plant sorghum mutant lines: The content of Ca and P were not affected by both of the sorghum mutant lines or harvest time and there was no interaction between them (P> 0.05). The Ca and P content range 0.24%-0.32% and 0.15%-0.20%, respectively.

Tannin content in panicle: In this study, the tannin content was analyzed in panicles (Table 4). There was a hight significant interaction (P <0.01) between the sorghum lines and harvest time for panicle tannins. The highest tannin content was produced in the combination treatment of the Patir 3.1 at the soft dough stage (1.04%), while the lowest was combination of Patir 3.1 at flowering stage (0.12%). The harvest time has a very significant effect on panicle tannin content (P <0.01). The highest content of tannin in the panicle was found at the soft dough stage and decreases in the hard dough stage.

Digestibility of Fiber Fraction In Vitro: Based on Table 5, the sorghum mutant line was affected in vitro ADF digestibility significantly (P<0.01) but neither harvest time (P>0.05), and no interaction between both. The ADF digestibility of Patir 3.1 was lower than Patir 3.2 and patir 3.7 (BMR line) from 48.68%, 55.08% 55.015, respectively. Meanwhile, the NDF digestibility affected both sorghum mutant lines and harvest time significantly (P<0.01), and no interaction between both. The NDF digestibility found was lower in Patir 3.1 and in Patir 3.2 and Patir 3.7 were higher from51.44%, 57.24%, and 53.60%, respectively. The hard dough phase produces the highest NDF digestibility was 57.74% in this study.

Rumen fluid characteristics and Ruminal Organic Acids Production in vitro: Rumen characteristics and proportions of VFA, acetate: propionate ratio in rumen liquid is presented in Table 6 and Table 7. The pH ranges were neutral in all treatments in this study. There was the interaction of sorghum mutant lines and maturity stages on VFA production in rumen fluid in vitro. The concentrations of acetate, propionate, and isobutyrate in rumen fluid were not affected by either sorghum mutant lines or the maturity stage (P>0.05). Although there was a decreased in NDF content in the BMR sorghum mutant lines (Patir 3.2 and Patir 3.7), it did not cause a significant decrease in acetic acid in the rumen fluid. The proportion of butyrate, isovalerate and valerate was influenced by sorghum mutant lines (P <0.05), but the harvest times were affected the proportion of both isovalerate (P <0.05) and valerate (P <0.01).

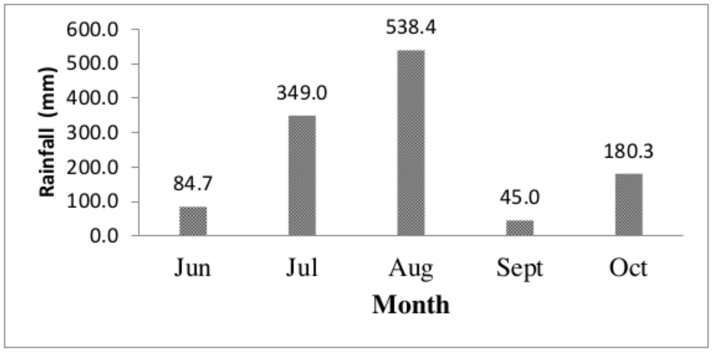

Figure 1. Rainfall (mm)

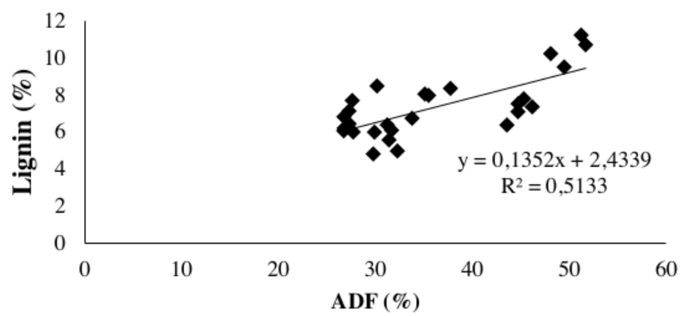

Figure 2. Correlation between lignin contents and ADF content of the the whole plant BMR sorghum mutant line

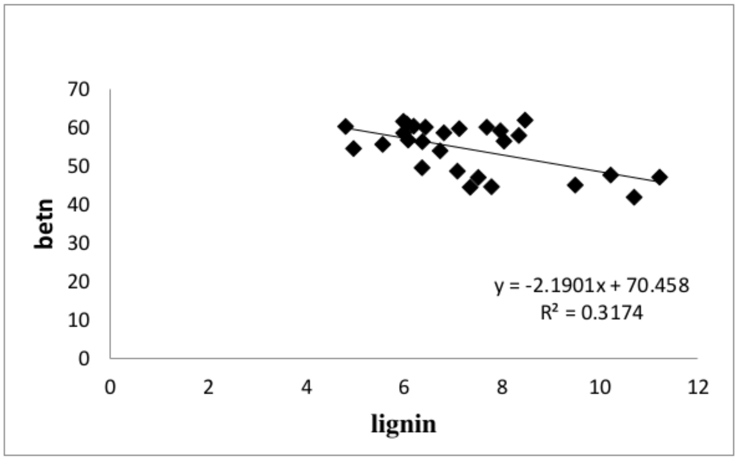

Figure 3. Correlation between BETN content and lignin contents of the the whole plant BMR sorghum mutant line

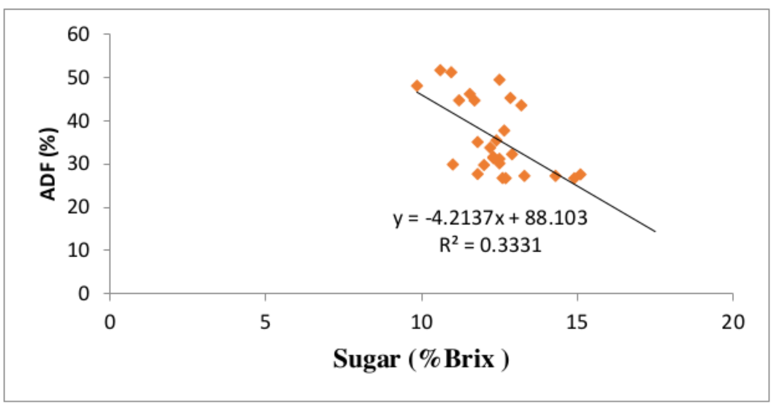

Figure 4. Correlation between ADF content and sugar (% Brix) of the the whole plant BMR sorghum mutant li

Table 1. Nutrien content of whole plant sorghum mutant lines (% DM basis)

Nutrients	Sorghum mutant lines	Harvest times			Mean
		Flowering	Soft dough	Hard dough	
Dry Matter	Patir 3.1	27.42±1.16	28.20±0.41	56.82±1.51	37.48±14.54
	Patir 3.2	26.77±0.46	28.70±0.91	56.31±4.05	37.26±14.46
	Patir 3.7	28.69±3.10	29.06±4.78	55.50±3.72	37.75±13.74
	Mean	27.63±1.88B	28.65±2.47B	56.21±2.91A	
Crude protein	Patir 3.1	9.20±0.37	8.54±0.58	7.89±0.26	8.54±0.41^{b}
	Patir 3.2	9.38±0.81	9.34±0.21	9.12±0.40	9.28±0.47^{a}
	Patir 3.7	8.79±0.31	9.04±0.56	9.36±0.46	9.06±0.45^{a}
	Mean	9.12±0.50	8.97±0.45	8.79±0.37	
Crude fiber	Patir 3.1	26.01±1.15	18.00±0.46	14.56±0.41	19.52±0.67^{b}
	Patir 3.2	28.51±1.57	19.66±0.93	15.52±1.50	21.23±1.33^{a}
	Patir 3.7	27.88±2.43	16.80±2.00	14.08±0.73	19.59±1.72^{b}
	Mean	27.47±1.72^{A}	18.16±1.13^{B}	14.72±0.88^{C}	
Ash	Patir 3.1	7.05±0.50	6.62±0.51	6.66±0.13	6.78±0.38
	Patir 3.2	6.96±0.56	6.37±0.32	6.02±0.48	6.66±0.45
	Patir 3.7	6.70±0.21	6.89±0.22	5.93±0.36	6.50±0.26
	Mean	6.90±0.42^{A}	6.63±0.35^{A}	6.20±0.32^{B}	
Crude fat	Patir 3.1	1.39±0.36	1.36±0.19	1.95±0.03	1.57±0.19
	Patir 3.2	1.16±0.27	1.86±0.34	1.83±0.34	1.61±0.32
	Patir 3.7	1.20±0.26	1.80±0.32	2.27±0.09	1.75±0.23
	Mean	1.25±0.30^{C}	1.67±0.28^{B}	2.02±0.15^{A}	
TDN	Patir 3.1	50.55± 0.11^{e}	54.73±0.15^{d}	57.73±0.50^{b}	54.34±0.25^{B}
	Patir 3.2	49.71±0.64^{e}	54.87±0.59^{d}	57.81±1.20^{b}	54.13±0.81^{B}
	Patir 3.7	49.83±0.84^{e}	56.18±1.00^{c}	59.98±0.82^{a}	55.33±0.89^{A}
	Mean	50.03±0.53	55.26±0.58	58.51±0.84	

Upper case with in a line and a column differ high significantly (P<0.01). Lower case with in a line and a column differ significantly (P<0.05). ns = non significant; Patir 3.1 = non BMR sorghum mutant line; Patir 3.2-Patir 3.7 = BMR sorghum mutant lines.

Table 2. Fiber fraction content of whole plant sorghum mutant lines (%)

Fiber Fraction	Sorghum mutant lines	Harvest times			Mean
		Flowering	*Soft dough*	*Hard dough*	
ADF	Patir 3.1	48.00±3.26	36.08±1.43	29.08±1.60	37.72±2.10[a]
	Patir 3.2	47.51±3.68	32.46±1.21	27.04±0.55	35.67±1.81[b]
	Patir 3.7	46.10±3.04	30.86±0.99	27.17±0.45	34.71±1.49[b]
	Mean	47.20±3.33[A]	33.13±1.21[B]	27.77±0.87[C]	
NDF	Patir 3.1	69.69±1.30	56.24±4.79	53.67±1.95	59.87±2.68
	Patir 3.2	69.25±3.65	56.34±1.85	50.75±1.78	58.78±2.43
	Patir 3.7	69.03±2.63	52.34±0.94	48.07±4.74	56.48±2.77
	Mean	69.32±2.53[A]	54.97±2.52[B]	50.83±2.82[C]	
Lignin	Patir 3.1	9.65±1.92	8.11±0.20	7.19±1.24	8.32±1.12[a]
	Patir 3.2	8.38±2.01	5.75±0.90	6.33±0.43	6.82±1.11[b]
	Patir 3.7	7.88±1.57	5.75±0.83	6.72±0.86	6.78±1.08[b]
	Mean	8.63±1.83[A]	6.54±0.64[B]	6.75±0.84[B]	
Selulosa	Patir 3.1	36.55±1.69	26.13±1.26	19.89±1.54	27.53±1.50
	Patir 3.2	37.38±1.07	24.68±0.39	17.36±0.50	26.47±0.65
	Patir 3.7	36.49±1.91	23.88±1.07	17.86±0.82	26.07±1.27
	Mean	36.81±1.56[A]	24.89±0.91[B]	18.37±0.95[C]	
Hemiselulosa	Patir 3.1	33.13±2.28	30.11±3.87	33.78±0.86	32.34±2.34
	Patir 3.2	31.87±3.24	31.66±2.09	33.38±2.17	32.30±2.50
	Patir 3.7	32.54±1.78	28.46±0.86	30.21±4.03	30.40±2.22
	Mean	32.52±2.43	30.08±2.28	32.46±2.35	

Upper case with in a line and a column differ high significantly (P<0.01). Lower case with in a line and a column differ significantly (P<0.05). ns = non significant; Patir 3.1 = non BMR sorghum mutant line; Patir 3.2-Patir 3.7 = BMR sorghum mutant lines.

Table 2. Fiber fraction content of whole plant sorghum mutant lines (%)

Fiber Fraction	Sorghum mutant lines	Harvest times			Mean
		Flowering	*Soft dough*	*Hard dough*	
ADF	Patir 3.1	48.00±3.26	36.08±1.43	29.08±1.60	37.72±2.10^a
	Patir 3.2	47.51±3.68	32.46±1.21	27.04±0.55	35.67±1.81^b
	Patir 3.7	46.10±3.04	30.86±0.99	27.17±0.45	34.71±1.49^b
	Mean	47.20±3.33^A	33.13±1.21^B	27.77±0.87^C	
NDF	Patir 3.1	69.69±1.30	56.24±4.79	53.67±1.95	59.87±2.68
	Patir 3.2	69.25±3.65	56.34±1.85	50.75±1.78	58.78±2.43
	Patir 3.7	69.03±2.63	52.34±0.94	48.07±4.74	56.48±2.77
	Mean	69.32±2.53^A	54.97±2.52^B	50.83±2.82^C	
Lignin	Patir 3.1	9.65±1.92	8.11±0.20	7.19±1.24	8.32±1.12^a
	Patir 3.2	8.38±2.01	5.75±0.90	6.33±0.43	6.82±1.11^b
	Patir 3.7	7.88±1.57	5.75±0.83	6.72±0.86	6.78±1.08^b
	Mean	8.63±1.83^A	6.54±0.64^B	6.75±0.84^B	
Selulosa	Patir 3.1	36.55±1.69	26.13±1.26	19.89±1.54	27.53±1.50
	Patir 3.2	37.38±1.07	24.68±0.39	17.36±0.50	26.47±0.65
	Patir 3.7	36.49±1.91	23.88±1.07	17.86±0.82	26.07±1.27
	Mean	36.81±1.56^A	24.89±0.91^B	18.37±0.95^C	
Hemiselulosa	Patir 3.1	33.13±2.28	30.11±3.87	33.78±0.86	32.34±2.34
	Patir 3.2	31.87±3.24	31.66±2.09	33.38±2.17	32.30±2.50
	Patir 3.7	32.54±1.78	28.46±0.86	30.21±4.03	30.40±2.22
	Mean	32.52±2.43	30.08±2.28	32.46±2.35	

Upper case with in a line and a column differ high significantly (P<0.01). Lower case with in a line and a column differ significantly (P<0.05). ns = non significant; Patir 3.1 = non BMR sorghum mutant line; Patir 3.2-Patir 3.7 = BMR sorghum mutant lines.

Table 3. Calcium and Phospor content of whole plant sorghum mutant lines (%)

Mineral	Sorghum mutant lines	Harvest times			Mean
		Flowering	*Soft dough*	*Hard dough*	
Ca	Patir 3.1	0.26±0.09	0.23±0.09	0.21±0.09	0.23±0.09
	Patir 3.2	0.23±0.05	0.25±0.08	0.21±0.02	0.23±0.05
	Patir 3.7	0.14±0.01	0.24±0.03	0.32±0.16	0.24±0.07
	Mean	0.21±0.05	0.24±0.07	0.25±0.09	
P	Patir 3.1	0.17±0.01	0.17±0.03	0.15±0.01	0.16±0.02
	Patir 3.2	0.19±0.02	0.19±0.00	0.17±0.01	0.18±0.01
	Patir 3.7	0.17±0.02	0.17±0.03	0.20±0.04	0.18±0.03
	Mean	0.17±0.02	0.18±0.02	0.17±0.02	

The treatment differ non significant effect (P>0.05)

Table 4. Panicle tannin content of sorghum mutant lines (%)

Sorghum mutant lines	Harvest times			Mean
	Flowering	Soft dough	Hard dough	
Patir 3.1	0.12 ± 0.08^{B}	1.04 ± 0.07^{A}	0.42 ± 0.03^{B}	0,53 ± 0,03
Patir 3.2	0.77 ± 0.12^{A}	0.59 ± 0.15^{AB}	0.38 ± 0.02^{B}	0,58 ± 0,07
Patir 3.7	0.61 ± 0.12^{A}	0.51 ± 0.04^{AB}	0.37 ± 0.06^{B}	0,50 ± 0,04
Mean	$0{,}50 \pm 0{,}10^{AB}$	$0{,}71 \pm 0{,}09^{A}$	$0{,}39 \pm 0{,}04^{B}$	

Upper case differ interaction high significantly (P<0.01). Patir 3.1 = non BMR sorghum mutant line; Patir 3.2-Patir 3.7 = BMR sorghum mutant lines.

Table 5. Fiber fraction digestibility of sorghum mutant lines (%)

Parameters	Maturity stages	Sorghum mutant lines			Mean
		Patir 3.1	Patir 3.2	Patir 3.7	
ADFD	Flowering	46.70±2.23	54.03±2.16	53.73±3.82	51.48±2.74^{ns}
	Soft Dough	49.21±3.61	55.11±3.01	55.83±2.99	53.38±3.20^{ns}
	Hard Dough	50.14±3.39	56.09±3.12	55.47±0.29	53.90±2.27^{ns}
	Mean	48.68±3.08^{B}	55.08±2.76^{A}	55.01±2.37^{A}	
NDFD	Flowering	49,93±2.73	51,62±2.26	52,57±3.91	51,37±2.97^{B}
	Soft Dough	48,79±3.71	58,22±3.79	52,49±4.40	53,17±3.97^{B}
	Hard Dough	55,59±3.49	61,89±2.42	55,75±1.16	57,74±2.36^{A}
	Mean	51,44±3.31^{B}	57,24±2.82^{A}	53,60±3.16^{B}	

Upper case with in a line and a column differ high significantly (P<0.01). Lower case with in a line and a column differ significantly (P<0.05). Patir 3.1 = non BMR sorghum mutant line; Patir 3.2-Patir 3.7 = BMR sorghum mutant lines; DMD = dry matter digestibility, OMD = organic matter digestibility; ADFD = acid detergent fiber digestibility; NDFD = neutral detergent fiber digestibility.

Table 6. pH, NH_3 and VFA rumen fluid *in vitro*

Parameters	Harvest times	Sorghum mutant lines			Mean
		Patir 3.1	Patir 3.2	Patir 3.7	
pH	Flowering	6.70 ± 0.10	6.70 ± 0.10	6.70 ± 0.00	6.70 ± 0.07
	Soft dough	6.70 ± 0.00	6.63 ± 0.00	6.67 ± 0.06	6.67 ± 0.04
	Hard dough	6.70 ± 0.10	6.70 ± 0.00	6.73 ± 0.06	6.71 ± 0.05
	Mean	6.70 ± 0.07	6.68 ± 0.05	6.70 ± 0.04	
NH3	Flowering	10.67 ± 0.14	10.12 ± 2.10	10.20 ± 2.77	10.33 ± 1.67^{A}
	Soft dough	7.89 ± 1.63	5.81 ± 0.77	5.11 ± 0.37	6.27 ± 0.92^{C}
	Hard dough	9.94 ± 1.21	9.04 ± 1.19	7.33 ± 0.75	8.77 ± 1.05^{B}
	Mean	9.50 ± 0.99^{A}	8.32 ± 0.99^{AB}	7.55 ± 1.30^{B}	
VFA	Flowering	132.60 ± 11.94^{A}	116.78 ± 11.00^{B}	117.51 ± 17.80^{B}	122.30 ± 13.58
	Soft dough	90.34 ± 9.56^{C}	75.48 ± 14.79^{D}	73.45 ± 3.04^{D}	79.76 ± 9.13
	Hard dough	127.52 ± 11.10^{AB}	101.12 ± 11.55^{C}	73.34 ± 7.14^{D}	100.66 ± 9.93
	Mean	116.82 ± 10.87	97.79 ± 10.17	88.10 ± 9.33	

Upper case with in a line and a column differ high significantly ($P<0.01$). Patir 3.1 = non BMR sorghum mutant line; Patir 3.2-Patir 3.7 = BMR sorghum mutant lines.

Table 7. VFA proportions and acetate:propionate ratio of sorghum mutant lines

Ruminal organic acids (mM)	Maturity stages	Sorghum mutant lines			Mean
		Patir 3.1	Patir 3.2	Patir 3.7	
Acetat	Flowering	32.92±2.45	30.45±4.46	29.21±2.11	30.86±3.01^{ns}
	Soft dough	30.20±5.80	30.12±5.01	29.05±2.23	29.79±4.35^{ns}
	Hard dough	29.94±3.60	28.93±3.42	28.79±0.66	29.22±2.56^{ns}
	Mean	31.02±3.95^{ns}	29.84±4.30^{ns}	29.02±1.67^{ns}	
Propionat	Flowering	9.56±0.92	10.44±2.71	9.92±3.26	9.97±2.30^{ns}
	Soft dough	9.39±0.72	11.94±1.64	10.92±3.03	10.75±1.79^{ns}
	Hard dough	10.37±2.27	11.36±3.29	11.07±1.26	10.93±2.27^{ns}
	Mean	9.77±1.30^{ns}	11.25±2.55^{ns}	10.64±2.51^{ns}	
Isobutirat	Flowering	1.84±0.43	1.49±0.39	1.11±0.39	1.48±0.40^{ns}
	Soft dough	1.26±0.41	1.23±0.41	0.97±0.40	1.15±0.41^{ns}
	Hard dough	1.20±0.69	1.15±0.08	0.88±0.36	1.08±0.38^{ns}
	Mean	1.43±0.51^{ns}	1.29±0.29^{ns}	0.99±0.38^{ns}	
Butirat	Flowering	4.37±0.57	3.37±0.13	2.67±1.28	3.47±0.66^{ns}
	Soft dough	3.73±1.03	2.75±0.55	1.58±0.69	2.69±0.76^{ns}
	Hard dough	2.34±1.40	2.58±0.75	2.44±1.13	2.45±1.09^{ns}
	Mean	3.48±1.00^{a}	2.90±0.48^{ab}	2.23±1.04^{b}	
Isovalerat	Flowering	1.55±0.28	1.43±0.03	0.83±0.40	1.27±0.24^{a}
	Soft dough	1.02±0.45	0.88±0.71	0.85±0.41	0.91±0.52^{b}
	Hard dough	1.17±0.60	1.01±0.16	0.73±0.33	0.97±0.36^{b}
	Mean	1.25±0.44^{a}	1.10±0.30^{a}	0.80±0.38^{b}	
Valerat	Flowering	1.35±0.18	1.30±0.16	0.57±0.36	1.07±0.23^{A}
	Soft dough	0.66±0.40	0.71±0.36	0.70±0.66	0.69±0.47^{B}
	Hard dough	0.69±0.38	0.75±0.29	0.51±0.35	0.65±0.34^{B}
	Mean	0.90±0.32^{ab}	0.92±0.27^{a}	0.59±0.46^{b}	
Asetat : Propionat	Flowering	3.59±0.13	3.02±0.65	3.10±0.70	3.24±0.49^{ns}
	Soft dough	3.20±0.37	2.59±0.75	2.82±0.92	2.87±0.68^{ns}
	Hard dough	3.02±0.93	2.70±0.80	2.63±0.36	2.78±0.70^{ns}
	Mean	3.27±0.48^{ns}	2.77±0.73^{ns}	2.85±0.66^{ns}	

Upper case with in a line and a column differ high significantly ($P<0.01$). Lower case with in a line and a column differ significantly ($P<0.05$). ns = non significant; Patir 3.1 = non BMR sorghum mutant line; Patir 3.2-Patir 3.7 = BMR sorghum mutant lines

DISCUSSION

The CP content was higher on BMR sorghum mutant lines compared to non-BMR sorghum, it was influenced by genetic factors. It is in line with Nohong and Islamiyati (2018) that the CP of BMR sorghum has been higher than Samurai-2 variety (non-BMR sorghum mutant). Refers to de Aguilar *et al*. (2014) that the BMR is present the higher protein content compared to the normal.

CF on late maturity was affected by grain filling. From soft dough and hard dough stage is the grain filling stage to produce kernel. Sorghum grain consists non-structural carbohydrates as starch, and the other hands, sugar accumulation in the stem was increased at the time. Sriagtula *et al*. (2017) mention the CF content was decreased in stems caused by less proportion of structural carbohydrate as sugar accumulates in stems and carbohydrate translocation for grain development.

The content of ash in sorghum whole plant was decreased with increasing maturity stage at harvest. In the hard dough stage, the ash content decreases significantly (P <0.01), it is in line with Koten (2014) founds. Refers to Sriagtula *et al*. (2017); Rosser (2013) that in the hard dough phase the proportion of panicles dominates from the total plants, panicles are rich in starch which will affect the percentage of ash.

The CF content increases with increasing maturity stage at harvest, this is due to starch content which also increases during the grain filling process. In the hard dough stages, the proportion of panicles (grain) was reached 60% (Sriagtula *et a*l. 2016) so that the starch content in this phase was the highest too, as well as the crude fat content. Refers to Wang *et al*. (2018), lipids are part of starch in the FFA (free fatty acids) form.

Assessment of digestible NDF and ADF is important to quantify the nutritional value of the forage, they were strongly negatively correlated with both DMD and OMD

(Lee, 2018). The low of ADF content in the BMR sorghum mutant lines was due to the low lignin content in the BMR lines because lignin is part of the ADF (Salama and Nawar, 2016). Low lignin content in BMR sorghum mutant lines influenced by genetic factors, BMR genes in plants cause low lignin content and increased digestibility. A possible explanation for this might be that low-level activity of CAD and COMT enzymes plays a role in lignin biosynthesis (Li *et al*., 2015).

The content of ADF, NDF, lignin, and cellulose was decreased with late harvest time, this was explained by the fact that an increase in BETN content both of in sugar on the stem and starch in the grain (panicle) in this phase (Sriagtula *et al*. 2017). Refers to Sriagtula *et al*. (2016b) that there was a carbohydrate competition for the synthesis of sugar and starch in stem and panicles compared to fiber synthesis in the advanced maturity stage so that the fiber fraction content decreases. The negative correlation between BETN vs lignin and ADF vs sugar present in Figures 3 and 4.

The content of Ca and P was the same as in all harvest times, it was caused by the dynamics of mineral nutrition in the part of sorghum plants. The Ca and P in the vegetative part (stem and leaf) will be translocated to the generative part (panicle) during the grain filling period at the soft dough and hard dough stages, so that Ca dan P content on both of part of plant decreased. It is opposite on malai, Ca dan P content were increased. This causes the content of Ca and P in the whole plant of sorghum mutant lines were not different. Refers to Gracia and Grusak (2015) that mostly in cereals with respect to micronutrient remobilization from flag leaves to developing grain.

Tanin is an antinutrient compound that can inhibit the digestion of protein and starch. Human (2012) states, in sorghum plants the content of tannins is abundant in seeds and dhurrin in leaves. The highest content of tannin in the panicle is found at the soft dough stage and decreases in the hard dough stage. This is in line with Omondi *et al*. (2012) that the tannin content of grain sorghum increases in the early stages of maturity and decreases in the late stages until the grain was rape. The results showed that the dynamics of panicle tannins were different between BMR sorghum mutant line and non-BMR sorghum mutant lines at harvest time. In the non-BMR sorghum mutant line (Patir 3.1), the lowest tannin content was produced in the flowering stage, then increases in the soft dough mutant and decreases in the hard dough mutant. In the BMR mutant line, the tannin content in Patir 3.2 and Patir 3.7were higher at the flowering stage and decrease to at the soft dough and hard dough stages.

The tannin content in the study ranged from 0.12% - 1.04%. Sorghum mutant lines are not a high tannins sorghum, according to Pan *et al*. (2016) that the sorghum was high in tannins if the content of tannin ranges from 1.11% - 1.51%. In this study many bird attacks occurred on panicles (grain), this indicates that the sorghum mutant lines have low tannin content. Wu *et al*. (2012) state that the higher tannin level in the panicle produced the low damage caused by bird attacks. In other hands, white grain sorghum was showed low tannin content. Cheng *et al*. (2009); Sedghi *et al*. (2012) state that the high tannin content was characterized by pigmentation on the seed coat (testa).

The BMR sorghum mutant lines produced the higher ADF digestibility than non-BMR sorghum mutant line, because of ADF content on non-BMR sorghum mutant line (Patir 3.1) were higher than BMR sorghum mutant lines (Patir 3.2 and patir 3.7) measuring 37%, 35%, and 34%, respectively (Table 2). ADF content was described as part of the undigestible matter on forage (Dasci and Çomakli, 2011).

Both sorghum mutant lines and maturity stages were affected in vitro NDF digestibility significantly (P<0.01), while no interaction on a both (P>0.05). In this study, the higher NDF digestibility was measuring on BMR Patir 3.2 (57.24%). The study before refers to Sriagtula *et al*. (2017), dry matter and organic matter digestibility on Patir 3.2 was 65% and 66%, it was higher than BMR Patir 3.7 and non-BMR Patir 3.1 was 63% and 63%; 60% and 60%, respectively. This result may be explained by Jančík *et al*. (2010) that in general NDF is the best parameter of dry matter degradation other for NDF represents the total matrix of insoluble fiber.

The NDF digestibility of sorghum mutant lines was affected by maturity stages. Advanced maturity in the hard dough stage produced the higher NDF digestibility (57%) than flowering and soft dough stages measuring 51% and 52%, respectively. This result may be explained by the fact that reducing of NDF content with increase maturity stages. At flowering stage (74 Days After Sowing/DAS) NDF content measuring 69.32%, then the late maturity at the soft dough stage (90 DAS) and hard dough stage (110 DAS) the NDF content was lower 54.97% and 50.83%, respectively (Table 2). Lignin content at hard dough stage was 6.75% lower than flowering stages 8.63% (Table 2), it was contributed to increased NDF digestibility at hard dough stages in this study. This agrees with Raffrenato *et al*. (2017) that lignin content was the negative correlation to NDF digestibility.

The proportion of VFA is an important factor for determining feed utilization by ruminants (Saunders 2015). Total VFA production was higher in non-BMR sorghum mutant line (Patir 3.1) compared to BMR sorghum mutant line (Patir 3.2 and Patir 3.7), this was due to higher butyrate production in non-BMR sorghum mutant line (Table 6), but acetate and propionate were not significantly different (P >0.05) between both lines.

In Patir 3.1 (non-BMR sorghum mutant line) at the flowering phase produced the highest VFA concentration, meanwhile, ADF and NDF content were highest so ADFD and NDFD were lowest in the same phase (Table 2 and 5), compared BMR sorghum mutant lines. These findings in line with Wahyono *et al*. (2019) that NH3 and VFA production in vitro system was not significantly different between treatments, even though there were differences in CP, ADF, and NDF content. This is contra with Chaugool *et a*l. (2013) that the ruminal fermentation characteristic sorghum cultivars were directly associated with rumen degradability.

In general, the concentration of NH3 and VFA was not affected by CP and fiber content (Table 1). This due to no absorption of rumen fermentation products in vitro system so that there was an accumulate of fermentation products, because they can not be recycled as in the actual rumen conditions (Firsoni *et al*., 2010; Kisworo *et al*. 2017). The reduction in the proportion of acetate, propionate, and isobutyrate in both sorghum mutant lines and maturity stages were not significant (P >0.05) despite a decrease in the content of ADF and lignin (Table 2). This result is contradictive with the report of Rahman *et al*. (2013) state that fermentation will produce a higher molar proportion acetate and butyrate and a lower proportion of propionate, on the other hand, feed with low fiber content would result in a greater proportion of VFA in the form of propionate, although acetate is still dominant, and a reduction in the A:P ratio during rumen fermentation.

In this study the proportions of acetate, propionate, and butyrate were 28.79 mM - 32.92 mM; 9.39 mM - 11.94 mM; 1.58 mM - 4.37 mM respectively. This outcome is lower to that of Saunders (2015) who found the proportion of VFA in maize ration based on corn silage BMR as acetate, propionate, isobutane, butyrate, valerate, and isovalerate were 60.5 mM, 21.8 mM,1.05 mM, 12.3 mM, 1.44 mM, and 1.44 mM respectively. The lower proportion of VFA in this study was caused by the material tested was single feed material was not in the rations.

The acetate: propionate ratio in the study was not affected by sorghum mutant lines and maturity stages (P> 0.05), although there was a decrease in the acetate: propionate ratio in the BMR sorghum mutant lines (Patir 3.2 and Patir 3.7) compared to non-BMR sorghum mutant (Patir 3.1), but statistically not significant effect (P<0.05). This was because the proportion of acetate and propionate from BMR and non-BMR sorghum mutant lines in this study also showed no significant difference (P>0.05). Ratio A: P on the BMR sorghum mutant line Patir3.2 and Patir 3.7 were 2.77 and 2.85, respectively. It was as the same as Saunders (2015) that the ratio A: P corn silage based BMR ration is 2.75.

CONCLUSION

BMR sorghum mutant lines (Patir 3.2 and Patir 3.7) produce higher CP, fiber fraction digestibility and lower ADF and lignin than non-BMR sorghum mutant lines (Patir 3.1). The proportion of rumen organic acids (acetate and propionate) and A: P ratio were no different on both sorghum mutant lines and harvest times, except butyrate. Harvesting at the hard dough stage was increased in vitro NDFD. The sorghum mutant lines and harvest time produce the same acetate and propionate production.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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