

Nutrient Changes and *in Vitro* Digestibility in Generative Stage of M10-BMR Sorghum Mutant Lines

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ABSTRACT

The objective of this research was to investigate the influences of generative stage on crude protein, crude fiber, ash, and crude fat contents as well as *in-vitro* dry matter and organic matter digestibilities of M-10 BMR sorghum mutant lines. This research was arranged into a randomized block design with 2 factors. The first factor was M-10 BMR sorghum mutant lines (Patir 3.1, Patir 3.2 and Patir 3.7) and the second factor was generative stages (flowering, soft dough and hard dough phase). The observed variables were proximate contents of stem, leaves and panicle of sorghum plant and *in-vitro* digestibility of whole plant. The results showed that leaves crude protein (CP) was more influenced by M-10 BMR sorghum mutant lines. Stems and panicles CP were influenced by the interaction between M-10 BMR sorghum mutant lines and generative stages. Further generative stage reduced stems CP but increased panicles CP. Crude fiber (CF), ash, and ether extract (EE) in leaves were not influenced by generative stages. Stems CF was influenced by M-10 BMR sorghum mutant lines and generative stages, while stems EE was more influenced by generative stages. Stems ash content was influenced by the interaction between M-10 BMR sorghum mutant lines and generative stages while panicles ash content was more influenced by generative stages. M-10 BMR sorghum mutant lines and hard dough phase increased *in-vitro* dry matter and organic matter digestibilities. Based on those findings, it can be concluded that the increased maturity reduces CP and CF contents so it increases *in-vitro* digestibilities.

Keywords: brown midrib sorghum, in vitro digestibility, generative stage, sorghum mutant lines

ABSTRAK

Penelitian ini bertujuan untuk menganalisis pengaruh fase generatif pada kandungan protein kasar, serat kasar, abu, dan lemak kasar, serta pencernaan bahan kering dan bahan organik galur sorgum mutan BMR secara *in vitro*. Penelitian ini menggunakan rancangan acak kelompok pola faktorial dengan 2 faktor. Faktor pertama adalah galur sorgum BMR (Patir 3.1, Patir 3.2, dan Patir 3.7) dan faktor kedua adalah fase generatif (berbunga, *soft dough*, dan *hard dough*). Peubah yang diamati adalah kandungan proksimat batang, daun dan malai tanaman sorgum, serta pencernaan bahan kering dan bahan organik tebon sorgum. Hasil penelitian menunjukkan bahwa kandungan protein kasar pada daun dipengaruhi oleh galur sorgum mutan BMR M-10 ($P < 0,01$). Kandungan protein kasar pada batang dan malai dipengaruhi oleh interaksi antara galur sorgum mutan M-10 dan fase generatif. Fase generatif lanjut menurunkan kandungan protein kasar pada batang, namun meningkatkan kandungan protein kasar pada malai. Kandungan serat kasar, abu dan lemak kasar pada daun tidak dipengaruhi oleh fase generatif, sedangkan kandungan serat kasar pada batang dipengaruhi oleh galur sorgum mutan BMR M-10 dan fase generatif. Kandungan lemak kasar pada batang dipengaruhi oleh fase generatif. Kandungan abu batang dipengaruhi oleh interaksi galur sorgum BMR M-10 dan fase generatif, sedangkan kandungan abu malai dipengaruhi oleh fase generatif. Galur sorgum BMR M-10 dan fase *hard dough* meningkatkan pencernaan bahan kering dan bahan organik secara *in vitro*. Kesimpulan penelitian adalah semakin tua umur tanaman maka semakin menurun kandungan PK dan SK, serta meningkatnya pencernaan secara *in vitro*.

Kata kunci: fase generatif, galur sorgum mutan, pencernaan, sorgum mutan

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INTRODUCTION

In general, forage is the main feed for ruminants with a daily consumption rate reaches 70% of the total ration. Besides of this huge amount, forage also plays a role as the main nutrition source for ruminants, therefore its availability is absolutely necessary. Forages should be maintained in ruminant diets because the production costs for forages are lower than for concentrate and more environmentally friendly, so it is more suitable for the development of sustainable livestock industry. Forage in diets helps maintain rumen function, reduces the risk of acidosis, and improves intake (Sari *et al.*, 2015).

The development of sustainable forage is a challenge in running livestock business. The classical problem in the supply of forage in Indonesia is the lack of fertile land and low production in the dry season. To overcome those conditions, forage with high biomass production and more adaptive to marginal land such as sorghum is needed to be developed. Sorghum (*Sorghum bicolor*) is one of important crops and ranks at the fifth world's widest spread after wheat, rice, maize, and barley (Dahir *et al.*, 2015). Sorghum is one of cereal crops consisting of forage and grains which potential to be used for fodder. Sorghum is also used to substitute maize (*Zea mays*) which is very potential to be cultivated in Indonesia. Sorghum has a good adaptation, tolerant to drought, and relatively produces higher biomass in less suitable climate and soil condition (Abdelhadi & Tricarico, 2009).

However, forage is limited in supplying energy demand of ruminant due to the low digestibility and high content of cell wall. The lignin content of the maize plant is less than that of forage sorghums, so it also limits the consumption of sorghum by ruminants (Dann *et al.*, 2008). This condition is caused by the reality that the sorghum used as forage is conventional varieties that are not designed to be used as a feed. Genetic modification of the source of forage is absolutely necessary to increase the availability of energy in the forage.

Recently, genetic modification through induced mutation using gamma rays irradiation has been developed in plant breeding technology. Mutation can expand plant genetic diversity to produce several sorghum mutant lines which designed for feed as brown midrib sorghum mutant lines (BMR). BMR usually has lower lignin content (Dann *et al.*, 2008; Astigarraga *et al.*, 2014). A structure modification in cell wall during mutation process decreases lignin content and increases cellulose content, fiber content in forage (cellulose and hemicellulose) as energy sources for livestock. The decrease in lignin content in BMR sorghum increases the efficiency of energy conversion and nutrition content (Gressel, 2008).

Forage quantity and quality are directly related with harvest times. Optimum harvest time correlates with optimum nutrition content and maximum yield. Maturity stages of forage define the quality of forage, further maturity stage will increase fiber content while crude protein content and digestibility are reduced. It

indicates that the low quality of forages will influence the productivity of livestock (Beck *et al.*, 2013). Based on those mentioned ideas, the best maturity stage of M-10 BMR sorghum mutant lines should be found. In addition, it is necessary to investigate the changes of nutrient content in stems, leaves and panicles and *in-vitro* dry matter and organic matter digestibilities in different generative stages of sorghum mutant lines.

MATERIALS AND METHODS

This research was arranged into a randomized block design with 2 factors. The first factor was M-10 BMR sorghum mutant lines (Patir 3.1, Patir 3.2, and Patir 3.7) and the second factor was generative stages (flowering, soft dough and hard dough phase). Data were analyzed by using analysis of variance (ANOVA) by using the SPSS 16 software program. Duncan Multiple Range Test was conducted if significant difference occurred (Steel & Torrie, 1997).

The culture technique and sample preparation of sorghum mutant line referred to Sriagtula *et al.* (2016a). Seeds were sown in 20 x 60 cm planting area at 5 cm depth. At 14 days post planting, fertilizers (urea, tri sodium phosphate, and potassium chloride) were applied in a ratio of 4:3:2 (g/g/g) at 270 kg/ha. Second fertilizer application was performed at 50 days post-planting with a ratio of 2:4:2 (g/g/g) at 200 kg/ha. Harvesting was done after the plant entered the flowering (74 days after sowing/das), soft dough (90 das) and hard dough (110 das) phases. To determine dry weight, 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 mash and prepared for nutritional analysis.

In vitro digestibility test was carried out by using Tilley & Terry (1963) method. 0.5 g of each sample was weighed and incubated for 48 h with a mixture of 40 mL of McDougall buffer solution and 10 mL of rumen fluid with CO₂. At the end of the first fermentation, microbial activity was stopped with 2-3 drops of HgCl₂. This was followed by the inoculation period of 48 h with the addition of 50 g pepsin HCl. This method was employed with rumen liquor obtained from three rumen-fistulated adult Bali cattle that were fed *ad libitum* with the ratio of roughage : concentrate was 60:40.

The observed parameters were crude protein (CP), crude fiber (CF), ash, and crude fat (EE) contents in leaves, stems, and panicles. Sugar Brix was measured on sorghum stem. The *in vitro* dry matter digestibility (DMD) and organic matter digestibility (OMD) of the whole plant of sorghum mutant lines were also measured.

The quality of the mutant sorghum plant was measured by proximate analysis with AOAC method (1980) to obtain the crude protein, crude fiber, ash, crude fat content and Brix sugar was measured using a refractometer. 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).

RESULTS

Crude Protein, Crude Fiber, Ash, and Crude Fat Contents of M-10 Sorghum Mutant Lines

The magnitude of CP contained in stems, leaves, and panicles at three different generative stages are shown in Table 1. The interaction between sorghum mutant lines and generative stages had significant effect ($P < 0.05$) on crude protein in stems. Among of three generative stages, the highest CP content of all sorghum mutant lines was produced at the flowering phase i.e., 4.58%-5.22%. At the soft dough phase, the stems CP content reduced to 2.10%-3.03% and continually reduced to 2.13%-2.31% at the hard dough phase. The leaves CP content was significantly influenced ($P < 0.05$) by sorghum mutant lines. BMR sorghum mutant lines Patir 3.2 and Patir 3.7 had higher leaves CP contents compared with non-BMR sorghum mutant lines Patir 3.1 i.e., 11.61%, 11.07%, and 10.49%, respectively. The leaves CP content was also significantly influenced ($P < 0.05$) by generative stages. The highest leaves CP content was produced at the flowering phase and continually reduced at the soft dough and hard dough phase i.e., 12.36%, 11.61%, and 9.20% respectively.

Crude fiber (CF) contents of leaves, stems, and panicles of sorghum mutant which observed at three different generative stages are shown in Table 1. Crude fiber content of stems was significantly influenced by sorghum mutant lines or generative stage ($P < 0.01$) but no interaction effect of both parameters. Non-BMR sorghum mutant lines (Patir 3.1) and BMR sorghum mutant lines (Patir 3.2) produced higher CF content compared with Patir 3.7 i.e., 26.33%, 25.40%, and 22.33%, respectively. In leaves, sorghum mutant lines or generative stage had no significant effect on CF content as well as the interaction between both parameters. The crude fiber content in stems ranged between 23.20%-25.95%. Meanwhile, the interaction between sorghum mutant lines and harvest times influenced the content of leaves CF ($P < 0.05$). In all sorghum mutant lines, the highest CF content was produced during the flowering phase and decrease during the soft dough phase. The lowest content was produced during the hard dough phase.

Ash contents in stems, leaves, and panicles are presented in Table 2. The result showed that an interaction between sorghum mutant lines and generative stages influenced the stems ash content ($P < 0.05$). The highest ash content was produced during hard dough phase (7.86%) by non-BMR sorghum mutant lines (Patir 3.1) and the

Table 1. Crude protein and crude fibre contents of M-10 sorghum mutant lines (based on 100% dry matter)

Plant organ	Generative phase	Sorghum mutant lines			Mean
		Patir 3.1	Patir 3.2	Patir 3.7	
Crude protein					
Stem	Flowering	5.21 ± 0.00 ^a	5.22 ± 0.52 ^a	4.58 ± 0.46 ^b	5.00 ± 0.33
	Soft dough	2.81 ± 0.6 ^{cd}	3.03 ± 0.78 ^c	2.10 ± 0.32 ^e	2.64 ± 0.59
	Hard dough	2.15 ± 0.33 ^e	2.13 ± 0.07 ^e	2.31 ± 0.22 ^{de}	2.19 ± 0.21
	Mean	3.39 ± 0.34	3.46 ± 0.45	2.99 ± 0.34	
Leave	Flowering	11.65 ± 0.85	13.07 ± 0.94	12.36 ± 0.61	12.36 ± 0.80 ^A
	Soft dough	10.96 ± 1.03	12.36 ± 0.69	11.50 ± 0.43	11.61 ± 0.72 ^B
	Hard dough	8.88 ± 0.39	9.39 ± 0.83	9.33 ± 0.41	9.20 ± 0.54 ^C
	Mean	10.49 ± 0.76 ^b	11.61 ± 0.82 ^a	11.07 ± 0.48 ^{ab}	
Panicle	Flowering	9.33 ± 0.29 ^C	8.94 ± 0.49 ^C	8.51 ± 0.43 ^B	8.93 ± 0.40
	Soft dough	10.19 ± 0.29 ^B	10.79 ± 0.34 ^{AB}	10.92 ± 0.69 ^{AB}	10.63 ± 0.44
	Hard dough	8.92 ± 0.47 ^C	10.80 ± 0.26 ^{AB}	11.06 ± 0.63 ^A	10.26 ± 0.46
	Mean	9.48 ± 0.35	10.18 ± 0.37	10.17 ± 0.58	
Crude fiber					
Stem	Flowering	27.97 ± 1.79	28.13 ± 1.68	25.57 ± 1.78	27.22 ± 1.75 ^A
	Soft dough	22.95 ± 1.43	23.00 ± 0.86	20.02 ± 1.14	21.99 ± 1.14 ^C
	Hard dough	28.08 ± 1.72	25.08 ± 2.52	21.38 ± 1.61	24.85 ± 1.95 ^B
	Mean	26.33 ± 1.65 ^A	25.40 ± 1.69 ^A	22.33 ± 1.51 ^B	
Leave	Flowering	24.40 ± 0.79	25.95 ± 2.75	25.77 ± 3.08	25.37 ± 2.20
	Soft dough	23.20 ± 0.35	24.91 ± 1.79	23.90 ± 1.45	24.00 ± 1.20
	Hard dough	25.53 ± 1.00	25.38 ± 1.70	25.33 ± 0.66	25.41 ± 1.12
	Mean	24.38 ± 0.71	25.41 ± 2.08	25.00 ± 1.73	
Panicle	Flowering	25.63 ± 1.26 ^b	29.97 ± 1.91 ^a	29.95 ± 2.30 ^a	28.52 ± 1.82
	Soft dough	11.87 ± 1.25 ^d	14.75 ± 1.72 ^c	10.70 ± 1.70 ^{de}	12.44 ± 1.56
	Hard dough	6.85 ± 0.79 ^f	8.87 ± 2.23 ^{ef}	7.61 ± 1.26 ^f	7.78 ± 1.43
	Mean	14.78 ± 1.10	17.86 ± 1.96	16.09 ± 1.75	

Note: Means with different superscripts in uppercase and lowercase differ significantly at $P < 0.01$ and $P < 0.05$, respectively. Patir 3.1= non-BMR sorghum mutant line; Patir 3.2-Patir 3.7= BMR sorghum mutant lines.

lowest ash content was produced during soft dough phase (4.67%) by BMR sorghum mutant lines (Patir 3.2). Sorghum mutant lines or generative stage had no significant effect on ash content in leaves. The ash content in panicles was influenced by generative stage ($P<0.01$) and it decreased with the increased generative stage.

The crude fat (EE) contents in stems, leaves, and panicles are shown in Table 2. The result showed that EE in stems was influenced by generative stage ($P<0.05$). The EE content in stems during hard dough phase was higher than flowering and soft dough phase i.e., 1.30%, 0.94%, and 0.92% respectively. The EE in panicles was also significantly influenced by generative stage ($P<0.01$). The hard dough and soft dough phase produced the higher EE content (2.10% and 1.74%) compared to flowering phase (0.94%).

Brix Sugar of Stems of M-10 Sorghum Mutant Lines

The stem sugar in the study is present in Table 3. Studies have shown that sorghum stem sugar was affected by the interaction between sorghum mutant lines and maturity stages ($P<0.01$). BMR sorghum mutant lines produce higher stem sugar content (13.37%-13.93% Brix) compared to non-BMR sorghum mutant

line (11.58% Brix). Sugar content in the sorghum stem increased with the increased maturity stage (hard dough stage) in this study. Stem sugar contents from flowering, soft dough, and hard dough phase were 12.01%, 13.68%, and 14.81% Brix, respectively.

In Vitro Digestibilities of Dry Matter and Organic Material of M-10 Sorghum Mutant Lines

The DMD and OMD are presented in Table 4. There is no interaction between sorghum mutant lines

Table 3. The mean of stems sugar contents of sorghum mutant lines (% brix)

Sorghum mutant lines	Generative stage			Mean
	Flowering	Soft dough	Hard dough	
Patir 3.1	10.67 ± 0.72 ^C	12.37 ± 0.49 ^B	11.63 ± 2.30 ^{BC}	11.56 ± 0.86 ^B
Patir 3.2	11.28 ± 0.60 ^{BC}	14.77 ± 0.42 ^A	15.80 ± 0.61 ^A	13.95 ± 0.54 ^A
Patir 3.7	12.27 ± 1.17 ^{BC}	12.60 ± 0.10 ^B	15.23 ± 1.44 ^A	13.37 ± 0.90 ^A
Mean	11.41 ± 0.83 ^C	13.24 ± 0.38 ^B	14.22 ± 1.14 ^A	

Note: Means with different superscripts in uppercase differ significantly ($P<0.01$). Patir 3.1= non-BMR sorghum mutant line; Patir 3.2-Patir 3.7= BMR sorghum mutant lines.

Table 2. Ash dan crude fat contents of sorghum mutant lines (based on 100% dry matter)

Plant organ	Generative phase	Sorghum mutant lines			Mean
		Patir 3.1	Patir 3.2	Patir 3.7	
Ash					
Stem	Flowering	5.49 ± 0.17 ^{bc}	6.31 ± 1.13 ^b	5.29 ± 0.61 ^{bc}	5.69 ± 0.64
	Soft dough	5.44 ± 0.56 ^{bc}	4.67 ± 0.87 ^c	4.98 ± 0.04 ^{bc}	5.03 ± 0.49
	Hard dough	7.86 ± 0.05 ^a	4.87 ± 1.18 ^{bc}	5.48 ± 0.39 ^{bc}	6.07 ± 0.54
	Mean	6.27 ± 0.26	5.28 ± 1.06	5.25 ± 0.34	
Leave	Flowering	10.72 ± 0.58	11.12 ± 0.79	11.31 ± 0.28	11.05 ± 0.55
	Soft dough	11.01 ± 0.43	11.04 ± 0.56	12.26 ± 0.47	11.44 ± 0.49
	Hard dough	13.24 ± 0.25	12.03 ± 0.45	12.50 ± 1.29	12.59 ± 0.66
	Mean	11.65 ± 0.42	11.39 ± 0.60	12.03 ± 0.68	
Panicle	Flowering	5.16 ± 0.13	4.97 ± 0.13	4.75 ± 0.18	4.96 ± 0.15 ^A
	Soft dough	4.44 ± 0.21	4.41 ± 0.41	4.43 ± 0.25	4.43 ± 0.29 ^B
	Hard dough	3.62 ± 0.30	3.79 ± 0.34	3.28 ± 0.25	3.56 ± 0.30 ^C
	Mean	4.41 ± 0.21	4.39 ± 0.30	4.15 ± 0.22	
Crude fat					
Stem	Flowering	1.16 ± 0.34	0.70 ± 0.23	0.96 ± 0.32	0.94 ± 0.29 ^B
	Soft dough	0.85 ± 0.14	0.98 ± 0.70	0.93 ± 0.14	0.92 ± 0.33 ^B
	Hard dough	1.19 ± 0.47	1.22 ± 0.10	1.48 ± 0.08	1.30 ± 0.22 ^A
	Mean	1.07 ± 0.31	0.97 ± 0.34	1.12 ± 0.18	
Leave	Flowering	2.21 ± 0.57	1.93 ± 0.66	1.94 ± 0.58	2.02 ± 0.61
	Soft dough	1.98 ± 0.43	2.03 ± 0.88	2.31 ± 0.47	2.10 ± 0.60
	Hard dough	2.43 ± 0.34	1.99 ± 0.46	2.30 ± 0.16	2.24 ± 0.32
	Mean	2.20 ± 0.45	1.98 ± 0.67	2.18 ± 0.40	
Panicle	Flowering	1.02 ± 0.28	0.89 ± 0.04	0.89 ± 0.26	0.94 ± 0.19 ^B
	Soft dough	1.24 ± 0.08	2.09 ± 0.80	1.91 ± 0.35	1.74 ± 0.41 ^A
	Hard dough	1.95 ± 0.25	1.91 ± 0.38	2.45 ± 0.14	2.10 ± 0.26 ^A
	Mean	1.40 ± 0.20	1.63 ± 0.41	1.75 ± 0.25	

Note: Means with different superscripts in uppercase and lowercase differ significantly at $P<0.01$ and $P<0.05$, respectively. Patir 3.1= non-BMR sorghum mutant line; Patir 3.2-Patir 3.7= BMR sorghum mutant lines.

Table 4. *In vitro* dry matter and organic matter digestibilities of sorghum mutant lines (%)

Parameters	Generative phase	Sorghum mutant lines			Mean
		Patir 3.1	Patir 3.2	Patir 3.7	
DMD	Flowering	50.90 ± 1.27	57.28 ± 1.62	54.51 ± 0.30	54.23 ± 1.06 ^C
	Soft dough	62.93 ± 1.60	68.82 ± 1.01	67.64 ± 1.09	66.47 ± 1.23 ^B
	Hard dough	66.46 ± 1.26	71.55 ± 1.53	68.21 ± 0.57	68.74 ± 1.12 ^A
	Mean	60.10 ± 1.37 ^C	65.88 ± 1.39 ^A	63.45 ± 0.65 ^B	
OMD	Flowering	50.63 ± 1.45	57.05 ± 1.75	54.21 ± 0.42	53.96 ± 1.21 ^C
	Soft dough	63.62 ± 1.18	69.77 ± 1.46	68.53 ± 0.95	67.31 ± 1.19 ^B
	Hard dough	67.52 ± 1.29	72.97 ± 1.43	69.42 ± 0.12	70.03 ± 0.95 ^A
	Mean	60.59 ± 1.30 ^C	66.59 ± 1.55 ^A	63.38 ± 0.50 ^B	

Note: Means with different superscripts in uppercase differ significantly ($P < 0.01$). 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

and generative stage on dry matter digestibility (DMD). However, DMD was influenced by sorghum mutant lines ($P < 0.01$). The highest digestibility (65.88%) was found in BMR sorghum mutant lines (Patir 3.2) and the lowest digestibility (60.10%) was found in non-BMR (Patir 3.1). Dry matter digestibility was also influenced by generative stage ($P < 0.01$). The highest dry matter digestibility was obtained at hard dough phase (68.74%) and the lowest was obtained at flowering phase (54.23%).

Organic matter digestibility was influenced by sorghum mutant lines ($P < 0.01$). The highest organic matter digestibility (66.59%) was produced by BMR sorghum mutant lines (Patir 3.2) while the lowest organic matter digestibility (60.59%) was produced by non-BMR (Patir 3.1). The OMD was also influenced by generative stage ($P < 0.01$). The highest OMD was obtained at hard dough phase (70.037%) and the lowest was obtained at flowering phase (53.69%).

DISCUSSION

The declining trend of stems and leaves CP contents during further generative stage (soft dough and hard dough phase) could be related to the inhibition of protein synthesis in the aging plant (Baloyi *et al.*, 2013). In contrast to the stems and leaves, panicles experienced an increasing trend of CP. This trend can be due to the movement of nutrient content from stems and leaves to grain which associates with the increased maturity of the plant (Atis *et al.*, 2012; Fernandes *et al.*, 2014). In this research it was found that CP content at BMR sorghum mutant lines was higher than non-BMR sorghum mutant line. This result is in agreement with the report of de Aquilar *et al.* (2014) who also stated that CP content of BMR lines was 0.6% higher than non-BMR which harvested at soft dough stage.

In this study, the stems CF content was decrease with the increased generative stage, because of Sorghum bicolor is sweet sorghum that has two sinks: stems and grains. Low CF content in stems is due to the less portion of structural carbohydrate as sugar accumulates in stems and for grain development. Grain filling occurs at the soft dough and hard dough phase, therefore, carbohydrate proportion will be trans-located from stems to

panicles and at the same time sugar accumulates in the stems (Carmi *et al.*, 2006; Qu *et al.*, 2014). Low CF content in panicles at the further generative stage is due to the increasing content of starch in grains. This is in agreement with the findings of Marsalis *et al.* (2009) who stated that the increased non-fiber carbohydrate and decreased fiber fractions during the increased generative stage are due to the increased starch content in grains. In this study, it was found that the CF decreased in stem and panicle at soft dough and hard dough phases while the stem sugar were higher at the same phases (Table 1 and Table 3).

Ash content represents mineral content. There is no different in ash contents of leaves related to the mobile characteristic of nutrient at leaves which will be translocated to other parts of the plant to fill the grain (Atis *et al.*, 2012; Fernandes *et al.*, 2014). The ash content in this study was decreased with the increased generative stage. The same trends were also found by Koten *et al.* (2014) that sorghum harvested at the stage of initiation of grain development had higher ash content compared with those harvested at further maturity (soft dough phase). This result was due to the increased starch content of grains during further generative stage (Marsalis *et al.*, 2009) and the increased organic content such as starch in grain and sugar in the stem as an accumulation result of photosynthesis so that the inorganic matter (ash) was measured lower (Rosser, 2013).

Sorghum leaves have high EE content due to the presence of a wax coating on leaves surfaces. This coating material may prevent water loss during transpiration. Wax material is insoluble in ether leading to a higher crude fat contained in forage material (Buschhaus & Jetter, 2012). The general result showed that EE increased with the increased generative stage. The hard dough and soft dough phases result in a higher crude fat contents of panicles than others, due to the high starch content in this phase. In seeds, lipids are positively correlated with starch and is an integral part of starch in the form of lysophospholipid (LPL) and free fatty Acids (FFA) as a starch-lipid complex (Tang & Copeland, 2007). Sorghum leaves have abusive fat content which is quite large because of the wax coating on the leaf surface. Layers of wax in plants inhibit water loss due to transpiration. The wax is soluble in ether and

included into the ether extract so that the crude fat value in forage fodder has a higher value (Tillman *et al.*, 1998). The EE content in leaves found in this research was 1.98%-2.20%, that was lower than the result reported by Koten *et al.* (2014) i.e., 3.99%-6.96%.

In general, sugar levels in the sorghum stem increased with the increased maturity stage (hard dough phase) that was a seed maturation period. The sugar content of sorghum stem was influenced by stem maturity of sorghum. Increase in stems maturity will increase sugar content. The maturity of the sorghum stem due to the maximum sugar content is achieved at physiological maturity of sorghum seed. This result is in accordance with the results reported by Almodares *et al.* (2007) that the highest sugar content of sorghum stem was found when the plant enters the physiological maturity of seed at 120 das. A similar statement was also expressed by Long *et al.* (2006) that the sugar contents of two sweet sorghum varieties were lower at early flowering phase then gradually increased from the milky phase to seed maturation. The low sugar content in the flowering phase was caused by the translocation of photosynthesis materials into the seeds, and a part of the sugar in sorghum stem was used as energy to support plant physiological process (Sucipto, 2010). Low lignin content in BMR mutant lines is caused by the sugar content of the stem was higher than a non-BMR mutant line (Scully *et al.*, 2016).

The DMD is in line with the OMD. At this study, the highest *in vitro* digestibility was found at BMR sorghum mutant lines (Patir 3.2 and Patir 3.7) compared with non-BMR sorghum mutant lines (Patir 3.1). This finding corresponds to Puteri *et al.* (2015) that *in vitro* DMD and OMD of sorghum mutant lines (Patir 3.5, Patir 3.6, and Patir 3.7) were higher than Samurai (M-17) (non-BMR sorghum mutant line). The increased digestibility was caused by the gene mutation in BMR which reduces lignin content (Ledgerwood, 2009; Tesso & Ejeta, 2011; Rao *et al.* 2012; Sattler, 2014). Our previous research (Sriagtula *et al.*, 2016a) reported the decrease in stem lignin in Patir 3.2 and Patir 3.7 BMR sorghum mutant lines that were 3.03% and 3.28%. Based on generative stage, the higher *in vitro* digestibility was obtained during hard dough compared to during the soft dough and flowering. This result is due to the decreased content of crude fiber, and the increased content of sugar in stem and NFE in sorghum at further maturity (Table 3 and Figure 1). Crude fiber was reflected as ADF, NDF, and lignin. Refer to Carmi *et al.* (2006); Sriagtula *et al.* (2016a) that decreasing of ADF, NDF, and lignin during hard dough stage and increasing the content of sugar in stems and grain proportion eventually affected *in vitro* digestibility. Refer to Sriagtula *et al.* (2016b) the highest panicle proportion produced by all sorghum mutant lines was at the hard dough phase. Qu *et al.* (2014) stated that the decrease in plant fiber content at soft dough phase would increase *in vitro* DMD. However, Rosser *et al.* (2013) stated that further maturity of forage crops did not reduce *in vitro* OMD.

In this research, *in vitro* DMD at the flowering phase was 54.23% and at soft dough phase was 66.47%. Those values were slightly different from the result

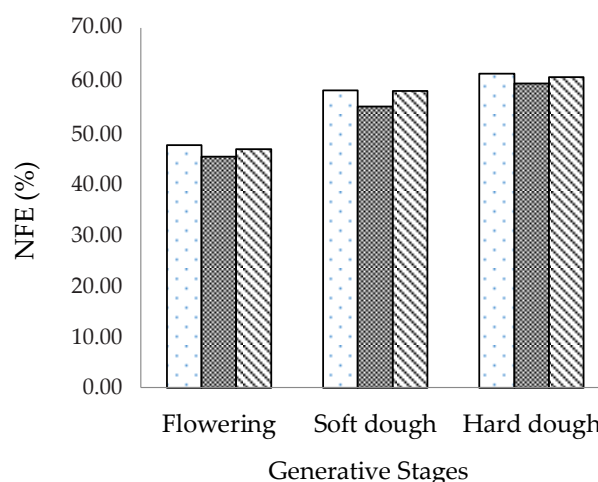


Figure 1. Nitrogen Free Extract of sorghum mutant lines, Patir 3.1 (□), Patir 3.2 (■), Patir 3.7 (▣).

found by Carmi *et al.* (2006) who stated that the ranges of *in vitro* DMD of sorghum at flowering phase was 61%-70% and soft dough phase was 61%-65%.

CONCLUSION

BMR sorghum mutant lines produced higher CP and lower CF compared with non-BMR sorghum mutant lines (Patir 3.1). The increasing generative stage decreased CP, CF, and ash contents meanwhile increased EE. BMR sorghum mutant lines produced higher digestibility compared to non-BMR sorghum mutant lines. The maturity stage at hard dough stage increased *in vitro* digestibility. The highest *in-vitro* DMD and OMD were produced at Patir 3.2 and hard dough phase.

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