White Midrib (WMR) vs Brown Midrib (BMR) sorghum: perspective of nutrient value for ruminant forage

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Abstract. Sorghum (Sorghum bicolor) cultivars can be classified as white midrib (WMR) and brown midrib (BMR). The BMR cultivar is the most potentially to be used as ruminant forage. However, the WMR, as conventional sorghum, has fairly good nutrient characteristics as roughage. The objective of our study was to evaluate the nutrient composition, fiber characteristics and nutrient value predictions of stem, leaf and panicle of Numbu variety (WMR type) and G5 mutant lines (BMR type). Five replication of two cultivars made up a randomized block design during 115 days cultivation in South Jakarta. Nutrient composition, fiber compounds and nutrient value predictions of stem, leaf and panicle were measured. There was no difference on crude protein (CP) content on stem, leaf and panicle between Numbu and G5. Numbu, as WMR type, has higher stem and leaf acid detergent fiber (ADF) and cellulose content (p<0.05) than G5. This study also proved that, G5, as BMR sorghum, had lower stem acid detergent lignin (ADL) level (p<0.05) than WMR. In panicle part, G5 had higher non fiber carbohydrate (NFC) (p<0.01) than Numbu. The stem relative feed value (RFV) represented by G5 is 136.37 and include in premium class. Conversely, Numbu included in good class, with a value of 109.83. Nutrient value characteristics of each part of sorghum is expected to be used as reference in preparing the ingredients for rations based on sorghum forage.

1. Introduction

Forage plants that grow optimally in dry environments is the main requirements in the livestock system in the future. Sorghum is an important dual-purpose crop for grain and forage yields in arid and semi-arid agro-ecosystem in the world [1]. As forage, the nutrient profiles of sorghum was not much different with napier grass and maize [2]. According to the color of leaf midrib, types of sorghum can further be divided into white midrib (WMR) and brown midrib (BMR) classes. WMR sorghum have white leaf midrib and dry stem pith, while the BMR type exhibit the characteristic reddish brown coloration in leaf midrib and juicy stem pith [3]. BMR sorghums are generally associated with low lignin content and high forage digestibility [4]. Generally, decreasing lignin content decreased neutral detergent fiber (NDF) and acid detergent fiber (ADF) while increasing digestibility [5].

The National Nuclear Energy Agency of Indonesia (BATAN) has developed G5 mutant lines as BMR forage sorghum [6]. In addition, Indonesia also has Numbu as national sorghum variety. Previous study reported that G5, as promising mutant lines had lower NDF and ADF than Numbu variety in whole parts of plant [7]. However, there are no information that represents the differences in nutrient content, fiber fractions and nutrient value prediction of the part of stem, leaves and panicle. Therefore, it is
necessary to evaluate the comparison of nutrient value between G5 and Numbu. The objective of this study was to evaluate the nutrient composition, fiber characteristics and nutrient value predictions of stem, leaves and panicle of Numbu variety (WMR type) and G5 mutant lines (BMR type).

2. Method

2.1. Cultivation and experimental design
Field trials were conducted in April–July 2018 at field laboratory of Agriculture Division, Center for Isotope and Radiation Application, National Nuclear Energy Agency of Indonesia, South Jakarta, Indonesia (6°17’38.9” S; 106°46’28.8” E, elevation 38 m). This region characterized as tropic near normal with mean annual precipitation of 100-300 mm 85–115% in April–July 2018 and average temperature of 28.7°C [8]. Soil type in this area is typically latosol, with pH during 4.7–5.6, low C-organic (1.1%), low N-total (0.16%), available P of 610 mg/kg and available K of 110 mg/kg. The previous crop was soybean.

G5 mutant lines as brown midrib and Numbu variety as white midrib sorghum were cultivated in this study. All cultivars were sown on April 2018 and planted at depth 5 cm. Completely randomized design (CRD) was arranged with five replications. Seeds were sown in 20×60 cm planting area. At 7 days post planting, a NPK fertilizer (2-2-2) was applied at rate 210 kg/ha with an additional 140 kg/ha urea applied 30 days after planting. Harvesting was done after the plant entered the hard dough (115 day after sowing) phases. The panicle, leaf and stem were cut into pieces and placed in an individual paper bag and dried 60°C for 48 h. samples were then grinded at < 1 mm and prepared for nutrient analyses.

2.2. Nutrient determination
The content of organic matter (OM), crude protein (CP) and ether extract (EE) were determined according to Association of Official Analytical Chemists (AOAC) method [9]. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) content were analyzed using an ANKOM 200 fiber analyzer according to Van Soest et al. [10]. Hemicellulose, cellulose and non-fiber carbohydrate (NFC) were determined using following equations:

\[
\text{Hemicellulose} \, (\%) = \text{NDF} - \text{ADF} \quad (1)
\]

\[
\text{Cellulose} \, (\%) = \text{ADF} - \text{ADL} \quad (2)
\]

\[
\text{NFC} \, (\%) = \text{OM} - \text{CP} - \text{NDF} - \text{EE} \quad (3)
\]

2.3. Nutrient value prediction
Dry matter intake (DMI), dry matter digestibility (DMD) and relative feed value (RFV) were predicted by formulas [11]:

\[
\text{DMI} \, (% \, \text{LW}) = \frac{120}{\% \, \text{NDF}} 
\]

\[
\text{DMD} \, (%) = 88.9 \times (\% \, \text{ADF} \times 0.779) 
\]

\[
\text{RFV} = \frac{(\text{DMD} \times \text{DMI})}{1.29} 
\]

According to The Hay Marketing Task Force of the American Forage and Grassland Council, the quality grading standard are: reject (<75), poor (86-75), fair (102-87), good (124-103), premium (151-125) and prime (>151).

The net energy lactation (NEL), estimated net energy (ENE) and total digestible nutrients (TDN) were predicted by formulas [12]:

1st formula (prediction equation from Pennsylvania State):

\[
\text{NEL} \, (\text{Mcal/lb}) = 1.0876 - (0.0127 \times \text{ADF} \, (\%)) 
\]

\[
\text{ENE} \, (\text{Mcal/lb}) = \text{NEL} \times 0.826 
\]

\[
\text{TDN} \, (\%) = 4.898 - (89.796 \times \text{NEL}) 
\]
The 2nd formula (prediction equation from New York State):

\[
\text{NEL (Mcal/lb)} = 1.085 - (0.0150 \times \text{ADF} \%) \quad (10)
\]

\[
\text{ENE (Mcal/lb)} = \text{NEL} \times 0.826 \quad (11)
\]

\[
\text{TDN (\%)} = 34.9 + (53.1 \times \text{NEL}) \quad (12)
\]

Mcal/lb was converted to Mcal/kg

For comparison, the calculation of TDN is also determined according to Jayanegara et al. [13]:

\[
\text{TDN} = (0.479 \times \%\text{NDF}) + (0.704 \times \%\text{NFC}) + (1.594 \times \%\text{EE}) + (0.714 \times \%\text{CP})
\]

2.4. Statistical analysis

Data were subjected to RBD design using SPSS 25.0 (IBM, Armonk, New York, USA). Differences among cultivar means with (P<0.05) were accepted as representing statistically significant.

3. Results and discussion

Data for nutrient content and fiber fractions of sorghum are given in table 1. Except for OM on stem, there was no significant difference between Numbu and G5 on OM and CP fractions for all sorghum parts. Sorghum leaves produced high CP content varied between 9.25–9.96 % dry matter (DM). Similar trend was also noted in the EE and NFC concentration of the stem and leaves. The ADF, ADL and cellulose content in stem were higher (P<0.05) in Numbu variety than G5 mutant lines. In leaves part, the NDF, ADF, hemicellulose and cellulose concentration of Numbu were higher (P<0.05) than G5. In panicle part, the EE, NDF, ADF, ADL, hemicellulose and cellulose of Numbu were highest (P<0.05) compared to G5. However, G5 had the higher NFC (P<0.05) than Numbu in panicle part.

Table 1. Nutrient and fiber fractions of stem, leaves and panicle of Numbu and G5 cultivars (n=5)

| % dry matter          | OM  | CP  | EE  | NDF | ADF | ADL | NFC | Hemicellulose | Cellulose |
|-----------------------|-----|-----|-----|-----|-----|-----|-----|---------------|-----------|
| Stem                  |     |     |     |     |     |     |     |               |           |
| Numbu                 | 96.40a | 2.80 | 1.98 | 52.74 | 35.17a | 5.36a | 38.89 | 17.57         | 29.82a    |
| G5                    | 95.94b | 2.57 | 1.98 | 46.00 | 27.56b | 2.46b | 45.62 | 18.45         | 25.09b    |
| SEM                   | 0.106 | 0.115 | 0.055 | 2.130 | 1.973 | 0.683 | 2.141 | 0.553         | 1.368     |
| Leaves                |     |     |     |     |     |     |     |               |           |
| Numbu                 | 90.23 | 9.25 | 2.59 | 57.22a | 32.11a | 5.13 | 21.34 | 25.10a         | 26.99a    |
| G5                    | 90.19 | 9.96 | 2.50 | 54.73b | 29.83b | 4.85 | 22.73 | 24.89b         | 24.98b    |
| SEM                   | 0.089 | 0.355 | 0.089 | 0.647 | 0.566 | 0.127 | 0.479 | 0.138         | 0.498     |
| Panicle               |     |     |     |     |     |     |     |               |           |
| Numbu                 | 94.88 | 8.11 | 2.63a | 60.42a | 34.99a | 4.09a | 23.91b | 25.43a         | 30.92a    |
| G5                    | 95.06 | 8.99 | 1.77b | 37.43b | 18.42b | 3.43b | 47.05b | 19.01b         | 14.99a    |
| SEM                   | 0.199 | 0.440 | 0.167 | 5.158 | 3.713 | 0.166 | 5.184 | 1.458         | 3.563     |

OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non fiber carbohydrate; SEM: standard error of mean; ‘a,b’ means with different superscripts in a column differ significantly (P<0.05).

There was no significant difference between BMR versus WMR on CP content in all parts of sorghum. It is apparent that BMR traits does not influence the CP proportion, despite G5 has numerically higher CP content on leaves and panicle. This possibly related to a same supply of N fertilizer at cultivating. In agreement with our study, OM and CP did not vary significantly between BMR and WMR.
genotypes of sorghum [13]. In contrary, several studies reported that BMR types produced higher CP content than WMR [3,14]. It is interesting to note that BMR sorghum produced lower ADF in the parts of stem, leaves and panicle. Despite NDF content of stem was similar, BMR is relatively had lower ADL value than WMR. Brown midrib trait were lower by five units in ADF, NDF and cellulose content than WMR genotypes [13]. The BMR genotype is associated with reduction in lignin content [15]. Lignin is an important component to support the uprightness of plants, thus a clear difference between BMR versus WMR was found in stem part.

Sattler et al (2010) also reported that stover from BMR sorghum had lower ADL and higher saccharification process [16]. There are two mechanisms suggested reducing lignin: 1) the activity of cinnamyl alcohol dehydrogenase (CAD) was reduced in BMR-6 mutants and 2) the activity of caffeic acid O-methyltransferase (COMT) was decreased in BMR-12 mutants [16]. Unfortunately, the BMR specific type was not reported in this study. Lignin had negative impacts for lignocellulosic conversion into monomeric sugar due to the blocking mechanism for the enzymatic liberation of sugar and inhibit microbes fermentation [15]. In present study, NDF and ADF contents in stem and leaves parts of G5 were lower than previous study. Vietor et al (2015) conducted that NDF fraction on stem and leaves were 48.0–49.8 and 61.0–63.4%, respectively, while ADF fractions were 28.6–28.9 and 32.5–36.5% [17]. This results were probably related to the variation of BMR varieties and agro-ecosystem environment.

The RFV is an important value to determine forage quality [7]. The estimation of DMI, DMD and TDN are reported in table 2. In stem part, no difference was noted in the DMI and RFV of the sorghum cultivars. Dry matter digestibility was higher (P<0.05) in G5 versus Numbu cultivars. The highest DMI, DMD and RFV on leaves and panicle parts were produced by G5 as BMR type. The prime RFV prediction was reported in the panicles of BMR type (RFV = 185.36). Furthermore, the RFV of stem on G5 also had premium quality (RFV = 136.37).

Table 2. Dry matter intake, digestibility and relative feed value of stem, leaves and panicle of Numbu and G5 cultivars

|          | DMI (% LW) | DMD (%) | RFV   |
|----------|------------|---------|-------|
| Stem     |            |         |       |
| Numbu    | 2.29       | 61.50   | 109.83|
| G5       | 2.61       | 67.43   | 136.37|
| SEM      | 0.099      | 1.537   | 7.641 |
| Leaves   |            |         |       |
| Numbu    | 2.09       | 63.88   | 103.91|
| G5       | 2.19       | 65.66   | 111.61|
| SEM      | 0.024      | 0.441   | 1.940 |
| Panicle  |            |         |       |
| Numbu    | 1.98       | 61.64   | 94.95 |
| G5       | 3.21       | 74.55   | 185.36|
| SEM      | 0.274      | 2.893   | 20.276|

DMI: dry matter intake; DMD: dry matter digestibility; RFV: relative feed value; SEM: standard error of mean; ab means with different superscripts in a column differ significantly (P<0.05).

The RVF index estimates DMI and DMD from NDF and ADF concentrations, respectively [18]. Low NDF and ADF contents on stem, leaves and panicle of G5 influence the higher value of DMI, DMD and RFV than Numbu, as WMR genotype. The results of present study were consistent with the research of Wahyono et al. [7]. In previous study, G5 produce higher RFV (141.17) than Numbu.
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The panicle part of G5 had highest RFV prediction because it has lowest NDF content (table 1). Apparently, the content of NDF and ADF should be measured carefully to improve RFV accuracy. It seems that some factors that affects fiber make-up and animal utilization could influence the accuracy of DMI and DMD prediction [19]. However, RFV calculations are still a practical option for rapid method utilization in the forage industry. Rohweder et al (1978) reported that this technique has immediate application in more effective grading for market hay [11].

The magnitude of energy values and TDN predictions contained in stem, leaves and panicles are shown in table 3. In all parts of sorghum, The energy (NEL and ENE) values and TDN predictions of G5 were higher (P<0.05) than Numbu variety. Despite there was no significant difference on TDN value (Jayanegara et al. [20] equation) in stem and leaves, G5 mutant line is relatively had higher value than Numbu. With regard to sorghum parts, Panicle had highest TDN values (55.72 – 81.55) compared with other parts.

**Table 3.** Energy values and total digestible nutrients prediction of stem, leaves and panicle of Numbu and G5 cultivars

|                      | Pennsylvania State prediction | New York State prediction | Jayanegara et al. [13] prediction |
|----------------------|-------------------------------|---------------------------|-----------------------------------|
|                      | NEL (Mcal/kg) | ENE (Mcal/100 kg) | TDN (%) | NEL (Mcal/kg) | ENE (Mcal/100 kg) | TDN (%) | TDN (%) |
| **Stem**             |                |                |          |                |                |          |         |
| Numbu                | 1.41ᵇ         | 116.61ᵇ        | 62.45ᵇ   | 1.23ᵇ         | 101.41ᵇ        | 64.49ᵇ   | 57.79ᵇ  |
| G5                   | 1.62ᵃ         | 134.19ᵃ        | 71.13ᵃ   | 1.48ᵃ         | 122.19ᵃ        | 70.56ᵃ   | 58.96ᵃ  |
| SEM                  | 0.055          | 4.558          | 2.249     | 0.065          | 5.384          | 1.571    | 0.438ᵇ  |
| **Leaves**           |                |                |          |                |                |          |         |
| Numbu                | 1.49ᵇ         | 123.67ᵇ        | 65.94ᵇ   | 1.32ᵇ         | 109.76ᵇ        | 66.93ᵇ   | 52.82ᵇ  |
| G5                   | 1.56ᵃ         | 128.95ᵃ        | 68.54ᵃ   | 1.40ᵃ         | 115.99ᵃ        | 68.75ᵃ   | 53.51ᵇ  |
| SEM                  | 0.016          | 1.307          | 0.645     | 0.019          | 1.544          | 0.451    | 0.207ᵇ  |
| **Panicle**          |                |                |          |                |                |          |         |
| Numbu                | 1.42ᵇ         | 117.02ᵇ        | 62.66ᵇ   | 1.23ᵇ         | 101.91ᵇ        | 64.64ᵇ   | 55.72ᵇ  |
| G5                   | 1.88ᵃ         | 155.31ᵃ        | 81.55ᵃ   | 1.78ᵃ         | 147.13ᵃ        | 77.84ᵃ   | 60.44ᵃ  |
| SEM                  | 0.104          | 8.580          | 4.234     | 0.123          | 10.134        | 2.958    | 1.073ᵃ  |

NEL: net energy for lactation; ENE: estimated net energy; TDN: total digestible nutrients; SEM: standard error of mean; ᵇᵃ means with different superscripts in a column differ significantly (P<0.05).

The most energy values are predicted from fiber fractions because fiber is negatively related to the animal’s ability to digest and utilize nutrients in the feed [12]. In the current study, we found that G5 produce higher energy values than WMR genotype, in all parts of plant. Low lignin content could improve cell wall digestibility, thus could lead to higher energy intake by cows [4]. Total tract digestibility of NDF for BMR sorghum was greater than conventional sorghum [21]. Previous in vivo studies reported that dairy cow rations based on BMR genotype produced higher milk yield, fat yield and protein yield than conventional sorghum (P<0.05) [4]. Moreover, dairy rations based on BMR sorghum silage have similar milk/DMI production (kg/kg) with rations based on corn or oat silage [22]. In the different kind of plant, the piper BMR had higher energy values than piper non-BMR, which represented in high OM and ADF digestibility [23].

4. Conclusion
Brown midrib and WMR sorghum genotypes have a similar CP content in all parts of plant. As BMR type, G5 had lower ADF, ADL and cellulose content than Numbu variety, especially on stem and panicle parts. Brown midrib sorghum also have greater RFV, energy value and TDN than WMR. The stem
relative feed value (RFV) represented by G5 is 136.37 and include in premium class. Conversely, Numbu included in good class, with a value of 109.83. Nutrient value characteristics of each part of sorghum is expected to be used as reference in preparing the ingredients for rations based on sorghum forage. Additional study is needed to compare the digestibility and nutritive value of BMR versus WMR genotypes by in vivo experiment.

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References
[1] Mahfouz H, Ali A M M, Megawer E A and Mahmoud A S 2015 Response of growth parameters, forage quality and yield of dual-purpose sorghum to re-growth and different levels of FYM and N fertilizers in new reclaimed soil Int. J. Curr. Microbiol. Appl. Sci. 4 762–82
[2] Sajimin S, Purwantari N D., S and . S 2018 Evaluation on performance of some Sorghum bicolor cultivars as forage resources in the dry land with dry climate J. Ilmu Ternak dan Vet. 22 135–43
[3] Li Y, Mao P, Zhang W, Wang X, You Y, Zhao H, Zhai L and Liu G 2015 Dynamic expression of the nutritive values in forage sorghum populations associated with white, green and brown midrib genotypes F. Crop. Res. 184 112–22
[4] Astigarraga L, Bianco A, Mello R and Montedónico D 2014 Comparison of brown midrib sorghum with conventional sorghum forage for grazing dairy cows Am. J. Plant Sci. 5 955–62
[5] Bean B W, Baumhardt R L, McCollum F T and McCuistion K C 2013 Comparison of sorghum classes for grain and forage yield and forage nutritive value F. Crop. Res. 142 20–6
[6] Wahyono T, Astuti D A, Wiryawan I K G, Sugoro I and Jayanegara A 2019 Fourier Transform Mid-Infrared (FTIR) spectroscopy to identify tannin compounds in the panicle of sorghum mutant lines IOP Conf. Ser. Mater. Sci. Eng. 546 42045
[7] Wahyono T, Sugoro I, Jayanegara A, Wiryawan K G and Astuti D A 2019 Nutrient profile and in vitro degradation of new promising mutant lines sorghum as forage in Indonesia Adv. Anim. Vet. Sci 7 810–8
[8] Meteorological C and G A 2020 Tropical climate characteristics https://www.bmkg.go.id/
[9] AOAC 2005 Official Method of Analysis (Maryland: Association of Official Analytical Chemists)
[10] Van Soest P J van, Robertson J B and Lewis B A 1991 Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition J. Dairy Sci. 74 3583–97
[11] Rohweder D, Barnes R F and Jorgensen N 1978 Proposed hay grading standards based on laboratory analyses for evaluating quality J. Anim. Sci. 47 747–59
[12] Undersander D, Mertens D R and Thiex N 1993 Determination of Acid Detergent Fiber by Refluxing (Nebraska: National Forage Testing Association)
[13] Jayanegara A, Ridla M, Lacoii E B and others 2019 Estimation and validation of total digestible nutrient values of forage and concentrate feedstuffs IOP Conf. Ser. Mater. Sci. Eng. 546 42016
[14] Singh S, Prasad S V and Katiyar D S 2003 Genetic variability in the fodder yield, chemical composition and disappearance of nutrients in brown midrib and white midrib sorghum genotypes Asian-austrialasian J. Anim. Sci. 16 1303–8
[15] Sriadutla R, Karti P, Abdullah L, Supriyanto S and Astuti D A 2017 Nutrient changes and in vitro digestibility in generative stage of M10-BMR sorghum mutant lines Media Peternak. 40 111–7
[16] Sattler S E, Funnell-Harris D L and Pedersen J F 2010 Brown midrib mutations and their importance to the utilization of maize, sorghum, and pearl millet lignocellulosic tissues Plant Sci. 178 229–38
[17] Sattler S E, Saballos A, Xin Z, Funnell-Harris D L and Vermerris W 2015 Characterization of Novel Sorghum brown midrib Mutants from an EMS-Mutagenized Population Agron. Hortic. - Fac. Publ. 898 2115–24
[18] Vietor D M, Rhodes G A and Rooney W L 2010 Relationship of phenotypic variation in sorghum to nutritive value of crop residues *F. Crop. Res.* **118** 243–50

[19] Jeranyama P and Garcia A D 2004 *Understanding Relative Feed Value (RFV) and Relative Forage Quality (RFQ)* (South Dakota)

[20] Sanson D W and Kercher C J 1996 Validation of equations used to estimate relative feed value of alfalfa hay *Prof. Anim. Sci.* **12** 162–6

[21] Oliver A L, Grant R J, Pedersen J F and O’Rear J 2004 Comparison of brown midrib-6 and-18 forage sorghum with conventional sorghum and corn silage in diets of lactating dairy cows *J. Dairy Sci.* **87** 637–44

[22] Harper M T, Oh J, Giallongo F, Lopes J C, Roth G W and Hristov A N 2017 Using brown midrib 6 dwarf forage sorghum silage and fall-grown oat silage in lactating dairy cow rations *J. Dairy Sci.* **100** 5250–65

[23] Ledgerwood D N, DePeters E J, Robinson P H, Taylor S J and Heguy J M 2009 Assessment of a brown midrib (BMR) mutant gene on the nutritive value of sudangrass using in vitro and in vivo techniques *Anim. Feed Sci. Technol.* **150** 207–22