

## ***In vitro* Digestibility and Gas Production Characteristics of Four *Brachiaria* Cultivars as Fresh Fodder**

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### **ABSTRACT**

*Brachiaria* is a grass that is potentially in the development of pastures, due to have a characteristic that is resistant on heavy grazing, trampling and drought and responsive to nitrogen fertilization. The potential of this grass is also survival in the dry season (drought resistant), other than that because it has very strong roots and quickly shut the soil so that it can be reduce soil erosion. However, there is a need to have comparative evaluation of these *Brachiaria* cultivars so that definite recommendations can be made in the choice and management of the respective cultivars. The experiment was conducted to evaluate the *in vitro* digestibility and gas production of four *Brachiaria* cultivars namelyg *Brachiaria brizantha*, *Brachiaria Mulato*, *Brachiaria ruzinensis*, and *Brachiaria decumbens*. Grass harvested at 40 days, and dried with air dried for 3 days, then grinded with a grinding machine. Sample collection was analyzed at the Laboratory of Biochemistry Nutrition, Faculty of Animal Science, Universitas Gadjah Mada. The parameters data consists of nutritional quality of grass and dry matter digestibility and organic matter. Data of the chemical composition, *in vitro* digestibility, *in vitro* gas production were analysed using one way annova in SPSS version 16. The model included the fixed effect of treatment. Data are presented as least squares means with standard error of the means. Significance was declared at  $p \leq 0.05$ , and tendencies were considered at  $0.05 < p \leq 0.10$ . Significant treatment effects were detected by Duncan multiple range test (DMRT). The results showed that the digestibility of dry organic matter by *in vitro* on *Brachiaria ruzizensis*, *Brachiaria mulato*, *Brachiaria decumbens*, and *Brachiaria brizantha* significantly different ( $P < 0.05$ ) in consecutive as much as 45.83%, 52.05%, 42.83%, 52.80% (Digestibility of dry matter) and 48.28%, 51.74%, 47.51%, and 54.90% (Digestibility of organic matter). Based on these results it can be concluded that dry and organic matter digestibility on the type of *Brachiaria* has the distinction of good digestibility of dry matter and organic matter.

**Keywords:** Nutritive quality, Digestibility of dry and organic matter, *Brachiaria* cultivars.

### **INTRODUCTION**

Ruminant livestock play key role as an integral part of farming and rural life in tropical countries by providing food, family income and employment (Peze and Davendra, 2002). Most developing countries are located in the tropical area, including Indonesia. Feeding of cattle in the tropics is often difficult because of seasonal decline in feed supply, in both quality and quantity (Wanapat and Davendra 1992). The main cattle feed is grass, either from natural or cultivated

pasture. The common problems that farmers face are cattle losing weight and lack of quality feed resources during dry season. One way to overcome this problem and to maintain the continuity of feed supply is to conserve surplus forage or crops as hay or silage for later use when feed is short supply.

*Brachiaria* is a tropical grass and commonly used in tropical countries as feedstuff for ruminants. These are local grass in Uganda, Africa. *Brachiaria* has been the grass most commonly sown on upland soils in developing countries because of the availability of relatively cheap seed (Hare *et al.*, 2005) despite poor dry season forage production (Hare *et al.*, 2009) *Brachiaria* including lawn grasses long-lived, can grow to form a dense overlay and is spreading rapidly through the stolon, furthermore it has heavy grazing grass resistant, resistant tread and pull as well as resistance to drought and responsive to nitrogen fertilization. Besides this grass is also rapidly growing and developing in ground cover, but not resistant to waterlogging. This grass is hay material Balk, because small trunk easily become dry. *Brachiaria* grass were planted well at altitude 0-1200 m (lowlands to highlands) with rainfall of 762 to 1500 mm/year, soil acidity (pH) 6 to 7 (Deutschman *et al.*, 2017). A *brachiaria* breeding program initiated in 1988 at CIAT (Centro Internacional de Agricultura Tropical) combined desirable attributes found in accessions of *Brachiaria brizantha* and *B. decumbens*. Three apomictic hybrids have been released (cvs. Mulato, Mulato II and Cayman). Mulato showed agronomic potential but seed yields were low. Trials in Central America demonstrated the superiority of Mulato II, a vigorous grass with very deep and branched roots, giving it excellent drought resistance in the Brazilian Cerrado and Mexico. Mulato II has excellent nutritional value. The development in Belgium of a tetraploidized sexual ruzigrass (*B. ruziziensis*) (Swenne *et al.* 1981). *Brachiaria* has already been used as animal feed for a long time (Tikam *et al.*, 2013). However, evaluation of *brachiaria* cultivars, grown under identical conditions at the same location, harvested at the same regrowth age and then utilised in fresh and preserved form has not yet been investigated.

*In vitro* gas production technique (IVGPT) was invented to evaluate the nutritional quality of feed by measuring the rate of production of fermentation gases that can be used to predict the rate of feed degradation, assuming that the amount of substrate degraded (Lopez *et al.*, 2000). Unlike this technique, an *in vivo* technique is time consuming, laborious, expensive, requires large quantities of feed and is unsuitable for large scale feed evaluation. Due to limitation in the number of animals and constraint of animal welfare, this technique has been practiced worldwide. In IVGPT, the feedstuff is incubated with buffered rumen fluid and the gas produced is measured as indirect indicator of fermentation kinetics. Fermentation of carbohydrate relatively produced 340 to 370 ml/g substrate (Cone and Gelder, 1999; Menke and Steingass, 1988). Comparative studies of dry matter productivity and nutritional content of *Brachiaria* cultivars had been conducted in number of places (Deutschman *et al.*, 2017; Tikam *et al.*, 2013; Evitayani *et al.*, 2004). Nevertheless, there were limited comparative studies conducted to assess digestibility of different *brachiaria* cultivars. Therefore, the objective in this study was to evaluate the *in vitro* digestibility and gas production characteristics of four *Brachiaria* (*Brachiaria brizantha*, *Brachiaria Mulato*, *Brachiaria ruzinensis*, and *Brachiaria decumbens*) cultivars as fresh fodder.

## MATERIAL AND METHODS

**Experimental site.** This study was conducted in Malang, Indonesia. The province is located in the tropical at altitude 456 m above sea level, 112 06 'south latitude, 7.06' east longitude, temperature: 22.7 to 25.1 max: 32,7°C, 79 to 85% humidity, Inceptisol soil type, as well as the average -rate rainfall of 1100 mm. There are two seasons during the year, dry season

from February to September and rainy seasons from November to March. The temperature is nearly constant, differing by only a few degrees among the dry and rainy season with daily temperature range from 18 to 34°C.

**Collection of forage samples.** The forages consisted of four species of grasses (*Brachiaria brizantha*, *Brachiaria Mulato*, *Brachiaria ruzinensis*, and *Brachiaria decumbens*). The grass sample was collected during the dry season (July, September). The grass samples were harvested from same location at the same regrowth age at cutting (45 days). Pasture were fertilized with N-P-K (15-15-15) compound fertilizer before the start of planting. Sprinkle irrigation for pasture was provided every 3 to 5 days during first the growing season. Wedding of pasture was removed 2 to 4 weeks after planting. Grasses were harvested at approximately 5 to 10 cm above the ground. The grass were dried at dry air for 3 days and coarsely milled to pass a 1 mm screen for further analysis. Chemical composition of the forages were analyzed by the standard method of the Association of Official Analytical Chemist (AOAC, 1984).

**Determination of in vitro digestibility and gas production.** In vitro digestibility of dry (IVDMD) and organic matter (IVOMD) of the forages were determined by the methods of Tilley and Terry (1963). The rumen fluid for measurement of in vitro digestibility and gas production was taken from healthy mature of Ongole crossbreed with permanent fistulated. One part of the rumen fluid was mixed with two parts of the medium consisting of buffer solution, macro and micro mineral solutions, resazurine and reduction solution. Two gram of each sample was incubated in the rumen fluid buffer medium mixture through water shaker bath at 39±0.1°C for 36 hour. After finishing the in vitro digestion trials, all the incubated materials were filtered and dried at 60°C for 72 hour to determine DMD. The residues were analysed for organic matter (OMD). In vitro gas production was measured with syringes according to the method described by Menke and Steingass, (1988). The produced gass was read at series of incubation times.i.e 3,6,12,24, and 36. The exponential equation proposed by Orskov and McDonald (1979) was used to determine characteristics of gas production using Neway-Exel computer program (Macaulay Institute, Aberdeen, UK). The equation was:  $p=a+b(1-e^{-ct})$ , where p: the volume of gas production; a=intercept of gas production curve; b=asymptote and c=the rate of gas production (ml/h). The value of (a+b) represented the potential extent of in vitro gas production.

**Statistical analysis.** Data of the chemical composition, *in vitro* digestibility, *in vitro* gas production were analysed using the Mixed procedure in SPSS version 16. The model included the fixed effect of treatment. Data are presented as least squares means with standard error of the means. Significance was declared at  $p\leq 0.05$ , and tendencies were considered at  $0.05 < p \leq 0.10$ . Significant treatment effects were detected by the global F-test, pairwise comparisons were performed employing Tukey test.

## RESULTS AND DISCUSSION

Chemical compositions. The chemical compositions of the forages are given in Table 1. Chemical characteristic of fresh *brachiaria* indicated a low forage quality when compared with various report Deutschman *et al.* (2017); Evitayani (2004); Santoso and Hariadi (2008); Imran *et al.* (2016), where crude protein (CP) measured in four different studies ranged from 8 to 12 % of dry matter. The CP content of grasses varied from 5.53% in *B. ruziziensis*, 5.69% in *B.mulato*, 6.79% in *B.decumbens*, 8.52% in *B.brizantha*. The crude protein content of the grass in the present study of *Brachiaria* cultivar in several country was relatively higher compared to the CP in Malang, Indonesia. As reported Maia (2014), showed that during rainy season, from the fifth

cut, there was an increase in CP, owing the beginning of the rainy season and the top dressing fertilization (nitrogen and potassium). Importantly, in periods of low rainfall, when the forage growth is impaired, it is required management strategy that result in higher percentages of green leaves in the pasture, to contribute and improve the nutritional value of forage during this period. Santos et al (2010) verified that a reduction in the period when the pasture remains deferred and accomplishment of fertilizations with nitrogen can contribute to the occurrence of larger mass of green foliage and lower masses of stem and dead material in the forage, being therefore actions recommended for, among other things, improving the nutritional value of deferred forage in some regions.

Comparative studies were developed by Euclides (2009), who evaluated the nutritional values of *B. brizantha cv marandu*, *Xaraes*, *Piata* and showed that regardless of the experimental year, The CP was higher during rainy season. Even presenting a decline in the CP content in the dry season, levels remained above the critical level quoted by Van Soest (1994) for the satisfactory development of ruminal cellulolytic bacteria, that is, The CP content must be equal to or higher than 7%. Similar content were also obtained by (Villela *et al.*, 2008), who found that the *Brachiaria decumbens* had CP content of 11.69%, 11.08%, 9.43% and 8.93% in spring, summer, autumn and winter, respectively, showing the reduction of CP content during the winter period due to the maturity of the plant. Moreover, In Indonesia, Similar on result were also obtained on several researcher (Imran *et al.*, 2016), who found that *B. mulato* had higher CP content as much as 8.5% and *Brachiaria decumbens* had CP content 12.8% (Evitayanti *et al.*, 2004). It is important to know the results of nutritional values in *Brachiaria* cultivar that has lower CP content in dry season. These features make *Brachiaria brizantha* excellent alternative for use integrated crop-livestock systems, with purpose of providing quality food in the dry season. By examining the contents of CP between *Brachiaria* species (Table 1) showed that *Brachiaria brizantha* has highest content of CP, differing from other species. This may be correlated with Maia *et al.* (2014) research who reported that *Brachiaria brizantha* had highest CP content differ other *Brachiaria* species until 6th cut in the dry seasons.

**Table 1.** The chemical composition of the *Brachiaria* cultivars as fresh fodder

Item	Nutrient compositions (%)				
	Dry matter (DM)	Organic matter (OM)	Crude protein (CP)	Crude fat (EE)	Crude Fiber (CF)
<i>B. ruziziensis</i>	89,61	81,11	5,53	3,5	29,84
<i>B. mulato</i>	91,30	87,26	5,69	5,06	32,44
<i>B. decumbens</i>	91,58	88,58	6,79	6,04	34,27
<i>B. brizantha</i>	89,32	79,4	8,52	2,66	32,55

Evaluating the IVDMD and IVOMD of *brachiaria* cultivars in the same area planting (Table 2), In the data showed that higher IVDMD and IVOMD values were obtained in *Brachiaria brizantha* and *Brachiaria mulato*, being different from other grasses, which showed similar IVDMD. These results can be related to higher content of CP and lower fiber fractions obtained for these grasses. Fernandes *et al.* (2002) described that increased digestibility is probably associated with shifts in the chemical composition with decreased content of hemicellulose and fiber, which certainly make available readily digestible carbohydrates for rumen microorganism. These results may possibly be explained by the most advanced



physiological maturity, due to seasonality of forage production, undermining the development of forages, once they were cut in the longer growth cycle (70 days), compared to other cuts which were performed in shorter cycles, and with this increases the cell wall component and reduces digestibility. Nevertheless, when comparing Maia *et al.* (2014) data research showed that the highest values of the IVDMD were detected in *Brachiaria brizantha*. Similar on data results showed that *Brachiaria brizantha* have the highest values of IVDMD and IVOMD with compared to other species.

For all forages, lowest IVDMD and IVOMD were obtained in dry season. These results may possibly be explained by the most advanced physiological maturity, due to seasonality of forage production, undermining the development of forages, once they were cut in the longer growth cycle (70 days), compared to other cuts which were performed in shorter cycles, and with this increases the cell wall component and reduces digestibility. Note that with the exception of the fourth cut, the digestibility is considered high for offseason; it shows once again the importance of crop-livestock integration, for supplying high quality food in the dry season, when low production and forage quality are obtained under normal conditions. Machado *et al.* (2011) investigated genotypes of *Brachiaria brizantha* succeeding soybeans in integrated crop-livestock system, and found a digestibility similar to that of the present study in the offseason with values in 2009 at 74.9%, 74.0%, 67.8%, 71.6%, 83.7% and 77.3% for *Marandu palisadegrass*, *MG-4 palisadegrass*, *Xaraes palisadegrass*, *arapoty* and B6, respectively.

**Table 2.** Digestibility quality of *brachiaria* cultivar by *in vitro*

Item	IVDMD (%)	IVOMD (%)
<i>B.ruziziensis</i>	45.83±1.21 <sup>a</sup>	48.28±2.71 <sup>a</sup>
<i>B.mulato</i>	52.05±6.81 <sup>ab</sup>	51.74±8.65 <sup>ab</sup>
<i>B. decumbens</i>	42.83±0.98 <sup>a</sup>	47.51±3.11 <sup>a</sup>
<i>B. brizantha</i>	52.80±2.37 <sup>ab</sup>	54.90±0.77 <sup>ab</sup>

The cumulative gas production rapidly increased with increasing incubation time from 2 to 36 h (Table 3). *Brachiaria mulato* and *Brachiaria brizantha* had the highest gas production within 36 h incubation. In comparasion the average digestibility of temperate grasses in Canada ranged between 89 to 92% *in vitro* true digestibility (Trovaldsson *et al.*, 2007). In Netherlands, a study conducted by Bruinenberg *et al.* (2002) showed that the digestibility of four temperate grasses ranged from 54 to 84% dry matter digestibility. Therefore, this information indicate that the digestibility of *Brachiaria* cultivars (50 to 60%) is within the range reported for several temperates grasses.

**Tabel 3.** *In vitro* gas production characteristic of *Brachiaria* cultivars at 36 h

Item	Gas production (ml/200 mg DM)						Characteristic			SE
	2	4	8	12	24	36	a	b	c	
<i>B.ruziziensis</i>	1.8	2.9	4.6	7.4	15.9	20.0	-0.64	32.81	0.026	0.99
<i>B.mulato</i>	3.4	7.0	11.1	18.6	36.5	43.7	-2.21	61.64	0.037	0.99
<i>B.decumbens</i>	4.2	8.0	12.6	12.4	34.9	32.9	-0.69	41.46	0.050	0.92
<i>B.brizantha</i>	4.0	8.7	13.9	20.3	35.1	41.4	-0.61	52.91	0.043	0.97

The asymptotic gas production (b) ranged from 61.64 and 52.91 for *Brachiaria mulato* and *Brachiaria brizantha*, respectively. Highest values of asymptotic gas production and potential gas production were observed in *Brachiaria mulato* and *Brachiaria brizantha* with 43.7

and 41.4 ml/200 mg DM, respectively. Low gas production in *Brachiaria ruziziensis* and *Brachiaria brizantha* (20 and 41.4 ml of potential gas production) was expected because of high fibrous fraction. Evitayani *et al.* (2004) reported that *Brachiaria decumbens* had potential gas production as much as 37.3 ml/200 mg. The present study indicated that *in vitro* gas production of the forages was closely related with their digestibility. It has been soon for *Brachiaria mulato* and *B. brizantha*. The results were also in agreement with the observation of Menke *et al.* (1979) that the amount of gas released when a feed is incubated *in vitro* with rumen fluid is closely related to the digestibility of the feed.

### CONCLUSIONS

Evaluation of nutritional quality and digestibility on *brachiaria* cultivars in the offseason, and the cultivars of *Brachiaria brizantha* and *Brachiaria mulato* show higher nutritional and digestibility values. There cultivars are the most suitable for presenting feed of better quality, compared with *Brachiaria decumbens* and *Brachiaria ruziziensis*.

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