

EVALUATION OF MORPHOLOGICAL TRAITS, FORAGE YIELD AND NUTRIENT QUALITY OF BRACHIARIA HYBRID MULATO II IN BHUTAN

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ABSTRACT: Dairying forms an integral part of livestock farming for smallholder farmers in Bhutan. Profitability of dairy enterprises continue to be constrained by non-availability of quality feed and fodder. There is currently limited choice of fodder species in Bhutan. The most popular grass species for subtropical belt is Ruzi grass (*Brachiaria ruziziensis*), used commonly for pasture development and forage production. Therefore, it is crucial to explore other *Brachiaria* cultivars with promising forage yield and nutrient quality. Accordingly, field experiments were conducted to compare the performance of *Brachiaria* hybrid grass Mulato II with *Brachiaria ruziziensis* on morphological traits and essential nutrients. The experiments were laid out in randomized complete block design with four replications in Lingmithang and three replications in Samrang. The data collected consisted of morphological characteristics such as DM yield, forage quality, plant height, number of tillers and leaves per plant and forage biomass. The chemical analysis was performed for crude protein, crude fat and total ash content. The *Brachiaria* hybrid cultivar Mulato II produced significantly ($p < 0.05$) higher forage and dry matter yield compared to Ruzi grass. Data revealed there is no significant difference ($p > 0.05$) in other morphological traits and essential nutrient content. Since Mulato II produced higher biomass and DM without compromising on other traits and nutrient content, the study concludes Mulato II presents good potential to promote it as an additional fodder species for the sub-tropical places.

Keywords: *Brachiaria* cultivars; crude protein; dry matter; forage yield and quality.

1. INTRODUCTION

Livestock production is an integral part of rural livelihood system and provide employment to majority of rural communities (Singh et al. 2018). However, despite its importance, the productivity of ruminant livestock is low due to deteriorating forage quality (Mustaring et al. 2014). According to FAO, (2016), the use of improved forage plants as a feed source is recommended to address the livestock feed shortage. Improvements in animal nutrition resulting in better performance of livestock can be achieved by utilizing improved forage varieties. One of the potential forage

grasses with promising results to address ruminant feed shortage is *Brachiaria* grass. These grasses are one of the most important tropical grasses widely available around the world. *Brachiaria* grass is drought resistant, perform well with low level of fertilizer inputs and control soil erosion (Wassie et al. 2018). It produces high herbage biomass with high nutritive value and palatability and has potential to reduce carbon foot print from livestock production through carbon sequestration (Djikeng et al. 2014).

In Bhutan, among the livestock ventures, dairy farming gained popularity as one main

economic activity with various opportunities and potentials (Ugyen 2020). The Department of Livestock under Ministry of Agriculture and Forest continues to initiate number of interventions and support programs in its effort to upscale livestock development in the country. With the adoption of improved breeds and good management practices, dairy farmers are gradually transforming from subsistence to commercial farming (Dendup and Dorji 2020). According to livestock statistics 2019, there are 92,449 milch cows including 11,000 milking yaks with annual milk production of 57,546.774 MT. The average annual milk yield per cow in the country is calculated around 622 kg which is one of the lowest in the South Asian region.

Recognizing the major limitations on livestock production owing to lack of year-round production of green fodder, the National Research and Development Center for Animal Nutrition (NRDCAN) Bumthang, initiated various interventions to enhance availability, utilization and quality of fodder resources. The center produced and supplied 196.24 MT of fodder seeds including winter fodder in the fiscal year 2019 - 2020 (DoL 2020).

At present, Bhutan has limited choice of fodder species and most popular grass species for subtropical belt is Ruzi grass (*Brachiaria ruziziensis*). Ruzi grass is commonly used for pasture development and forage production. The *ruziziensis* variety was released in the year 2002 by erstwhile RNR Research Center, Jakar. Thus, there is a need to identify and evaluate additional *Brachiaria* cultivars with promising forage yield and nutrient quality.

The *Brachiaria* hybrid grass Mulato II was introduced in Bhutan in 2018 from Department of Livestock Development Service, Thailand. The *Brachiaria* hybrid was the second hybrid introduced by Centro Internacional De Agricultura Tropical (CIAT) from crosses and selections of *Brachiaria ruziziensis*, *Brachiaria decumbens* and *Brachiaria brizantha* (Magalhaes et al. 2017). Among *Brachiaria* cultivars, Mulato II is the most preferred grass because of its possibility for year-round biomass production, drought tolerance, low fertilization inputs requirement and high palatability (Maass et al. 2015).

Therefore, this study was carried out with the aim of evaluating and comparing the performance

of *Brachiaria* hybrid Mulato II on morphological traits, herbage yield and quality over existing variety Ruzi grass (*Brachiaria ruziziensis*) under subtropical environmental condition in Bhutan.

2. MATERIALS AND METHODS

2.1 Experimental site

The field experiments were conducted from August 2018 to December 2019 at Regional Poultry and Piggery Breeding Farm, Lingmithang, Mongar and Bhutan Livestock Development Corporation Farm, Samrang, Samdrupjongkhar. The experimental site at Samrang is located at an altitude of 380m and Lingmithang at 600m above sea level. The average maximum and minimum temperature recorded in Lingmithang and Samrang were 29.7, 20.4, 32 and 20.3°C respectively. Both the locations fall under subtropical climate characterized by humid hot summer and cool dry winter.

2.2 Experimental design and treatment

The experiment was laid out in a randomized complete block design (RCBD) with two treatments and three replications at Samrang and same treatment with four replications at Lingmithang. The treatments were *Brachiaria* hybrid Mulato II, existing variety Ruzi grass (*Brachiaria ruziziensis*) and Ruzi grass was used as a control. The individual plot measured 3.75 m² (2.5 m x 1.5 m) at Lingmithang and 6m² (3m x 2m) at Samrang. The experimental plot size in respective locations were based on topographical condition of the land. In total, there were eight experimental plots at Lingmithang and six at Samrang with spacing of 50 cm between plots and 70 cm between replications in two locations.

2.3 Field management and sowing

The experimental field was properly ploughed and harrowed. The land was divided into blocks and each block comprised of required number of plots. The seed was sown at the rate of 8kg per acre. Fertilization was done with Single Super Phosphate (SSP), applied at the rate of 150 kg per acre during the establishment of trial and urea (Nitrogen fertilizer) was used as top dressing after first cut. No irrigation was done as it was sown as summer fodder crop when the rains were

frequent. The periodic weeding was carried out to maintain the field. Plants along the border of plots were cut and disposed off at the time of data collection and excluded from measurement.

2.4 Data collection

At Samrang, the grass was harvested at three months after sowing. The data for experimental site from Lingmithang was collected from 2nd year onward after establishment. The second cut was done at 60 days interval after first cut in two sites. The data recorded were; fresh and dry matter yield, number of tillers per plant, plant height, number of leaves per plant and leaf to stem ratio. Similar measurements were carried out on the second cut. To measure the fresh green biomass, the entire plot was harvested and fresh plant materials were weighed with weighing scale. Plant height was measured in centimeters from base to tip of the plant by randomly selecting five plants from each replicate. The tiller numbers per plant were counted from five plants randomly taken from the individual plot. Ten plants were selected at random and plant parts were segregated into leaf and stem and then weighed separately to calculate leaf to stem ratio. The leaf to stem ratio was obtained from dividing leaf sample weight by stem sample weight. The leaf numbers per plant were counted from five plant randomly selected from each plot.

2.5 Laboratory analysis

The plant materials were systematically mixed and a representative sample weighing 200 gm was collected from each plot after harvest from respective experimental sites. The samples were collected to estimate dry matter content and nutrients.

The laboratory test for dry matter and nutrient content was conducted at the Animal Nutrition Laboratory, NRDCAN, Bumthang. The fresh samples were dried in a hot air oven at 65°C for 48 hours and DM percent was obtained from dividing the dry mass by the wet mass, multiplied by 100. The proximate analysis was performed to determine crude protein, crude fat and total ash content.

2.6 Data analysis

The data for biomass and dry matter yield from first and second cuts were added and

converted to acreage to obtain total yield. For morphological traits, data was averaged from two cuts. The data was entered in Microsoft Excel and exported to IBM SPSS version 26 for statistical analysis. The data sets were checked for outliers followed by Shapiro Wilk's and Leven's tests for normality and homogeneity of variance respectively. Two sample independent t-test was performed to compare the statistical mean difference on various parameters between two *Brachiaria* grass varieties. Differences in means were considered significant when p-value was less than 0.05.

3. RESULTS AND DISCUSSIONS

3.1 Morphological traits

3.1.1 Plant height

The data for morphological traits (mean plant height, tiller and leaf number per plant and leaf to stem ratio) are presented in Table 1 and 2, respectively. The tallest plant recorded in the study for *Brachiaria* cultivar Ruzi was 192.66 cm followed by *Brachiaria* hybrid Mulato II with 174.33 cm in Samrang. Similarly, in Lingmithang, the plant height measured for *Brachiaria* hybrid Mulato II was 91.47 cm which was taller than *Brachiaria* cultivar Ruzi that had recorded height of 77.17cm. However, there was no significant difference ($p > 0.05$) in plant heights between two *Bracharia* cultivars. The observation in this study is in conformity with the findings of Demski et al. (2019) who reported similar result among different *Brachiaria* cultivars. Ansar et al. (2010) mentioned that plant height is a major factor contributing towards forage yield for different grasses. The variation in plant height is affected by genetic makeup of particular cultivar. In addition, availability of light also plays a major role in influencing plant growth and survival (Guenni et al. 2008). The mean plant height of the *Brachiaria* hybrid Mulato II in this study was found to be higher than result obtained by (Yberkew et al. 2020) for same grass species. The reason could be due to the variations in environmental conditions related to soil type, soil fertility, temperature, altitude and management practices applied on the plant. Harvesting stage of the plant also affects plant physiology in terms of plant height (Shoab et al. 2013). Asmare (2016)

mentioned that plant height increased with an advancement of harvest time of the plant. The increasing plant height in latter stage of the plant could be due to massive root development and efficient nutrient uptake (Melkei 2005).

Table 1: Morphological traits (M±SD) of two *Brachiaria* species at Samrang

Parameters	<i>Brachiaria</i> species		Sig.
	Mulato II	Ruzi	
Plant height (cm)	174.33±17.00	192.66±8.00	ns
Tiller number per plant	31.66±4.61	30.00±4.58	ns
Leaf number per plant	12.00±1.00	11.00±1.00	ns
Leaf to stem ratio	0.78±0.1500	0.56±0.07	ns

* $p \leq 0.05$; ns: nonsignificant

3.1.2 Tillers per plant

Tillering in forage grass is an important trait for the productivity and sustainability of grazing system (Silva 2015). As shown in the Table 1, tiller numbers counted for *Brachiaria* hybrid cultivar Mulato II was 31.33 and Ruzi grass was 30 in Samrang. In Lingmithang, the number of tillers recorded for cultivar Mulato II was 74.39 and Ruzi grass was 77.17 (Table 2). Although Mulato II cultivars produce higher number of tillers numerically, it is not statistically significant ($p > 0.05$). Rodrigues et al. (2014) reported the similar result regarding tiller density among *Brachiaria* cultivars. Saleem et al. (2015) mentioned that the number of tillers per plant is an important contributing factor for forage yield. In current study, both cultivars produced similar tiller numbers in respective locations and indicating the ability of two cultivars to recover faster after defoliation. The vegetative growth including tillering could be attributed to the morphological and physiological differences among the cultivars (Nguku et al. 2016a). Tillering response is affected by grazing management and nutrient supply and thus, identification of management strategies and tillering response associated with forage yield is important (Silva et al. 2020). Tiller number per plant increases with the increase in plant spacing. At wide spacing, where light can easily penetrate to the base of the plant, it further stimulates tiller development (Tilahun et al. 2017). In addition, competition for nutrients is also reduced and therefore, individual plant can support more tillers

under wider plant spacing (Yasin et al. 2003). However, in this study, seeds were sown using broadcast method. In current study, number of tillers per plant recorded for hybrid cultivar Mulato II in Lingmithang was higher than experiment site in Samrang. This was due to variation in harvest length between two locations. The grass cut in Lingmithang was done in second year after establishment due to late sowing. Tiller number of the plant also increases with the maturity of the grass (Zemene et al. 2020). Level of nitrogen content in the soil also influences the activation of dormant buds and increases the vegetation sward filling through the highest rate of tiller replacement (Mushtaque et al. 2010).

3.1.3 Leaf number per plant

The data on number of leaves per plant is shown in Table 1 and 2 respectively. Overall, the development and the growth of plant depends on total number of leaves (Wada et al. 2019) as it acts as the basic factory for food production in growing plant. The maximum number of leaves per plant was recorded for cultivar Mulato II than cultivar Ruzi in both locations. However, significant difference ($p > 0.05$) was not observed on the number of leaves per plant between two *Brachiaria* cultivars. Yield of the forage crops depend on increase or decrease in number of leaves per plant (Khan et al. 2014) and plant leaves play greater role in influencing forage biomass yield (Tessema and Getinet 2020). Numbers of leaves per plant are affected by soil fertility, stage and application of nitrogen fertilizer (Ali et al. 2017). The fertilizer was applied on initial stage during land preparation in this study. According to Tilahun et al. (2017) and Kezang et al. (2015), leaf number per plant also depends on harvesting stage which determines the photosynthetic capacity of the plant. Generally,

Table 2: Morphological traits (M±SD) of two *Brachiaria* species at Lingmithang

Parameters	<i>Brachiaria</i> species		Sig.
	Mulato II	Ruzi	
Plant height (cm)	91.47±15.95	77.17±12.87	ns
Tiller number per plant	74.39±10.24	65.01±10.59	ns
Leaf number per plant	8.00±1.00	7.00±1.00	ns
Leaf to stem ratio	1.24±0.26	0.70±0.12	ns*

* $p \leq 0.05$; ns: nonsignificant

the plant produces greater number of leaves when vegetative phase is longer due to formation of new tillers with increase in age at harvesting (Babale et al. 2020). In the current study, harvesting time for first cut in two locations varied but maintained same cutting interval of two months in subsequent cuts.

3.1.4 Leaf to stem ratio

The leaf to stem ratio was recorded numerically higher in cultivar Mulato II (0.78) than the Ruzi (0.56) grass in Samrang. But there was no significant difference ($p > 0.05$) between two *Bracharia* cultivars. This finding is in an agreement with similar result reported by Rodrigues et al. (2014) among different *Bracharia* cultivars. In Lingmithang, leaf to stem ratio for cultivar Mulato II (1.24) was significantly ($p < 0.05$) higher than Ruzi (0.70) grass in agreement to finding of Hare et al. (2015).

The leaf to stem ratio has important effect on the quality of the forage and materials preferred by grazing animals. It is also the main factor which determine the diet selection and forage intake in tropical grass (Smart et al. 1998). The variations in leaf to stem ratio in two locations may be attributed to soil fertility status and environmental conditions in different agro-ecological zones including the effect of temperature. Forage grasses grown in warm region are also associated with taller, low concentration of crude protein and higher concentration of fiber (Lee et al. 2017).

According to Yiberkew et al. (2020), type of fertilizer applied and plant spacing also affect the leaf to stem ratio and the highest leaf to stem ratio was observed at manure and wider plant spacing.

3.2 Yield parameter

3.2.1 Fresh biomass and dry matter yield

The green forage and dry matter yield of two *Bracharia* cultivars are shown in table 3 and 4 respectively. Fodder yield is the most important trait and ultimate product of fodder variety which reveals the total biomass attained by the plants during its life cycle.

The *Bracharia* hybrid cultivar Mulato II produced significantly ($p < 0.05$) higher fresh biomass and dry matter yield than Ruzi grass (*Bracharia ruzizensis*) in Samrang. This result is in an agreement with the finding of Esteban et al. (2013) who reported the similar result. However, in Lingmithang, although the yield recorded for Mulato II was numerically higher than Ruzi grass, there was no significant difference ($p > 0.05$) found between two cultivars. The current study demonstrated considerable variation in biomass and dry matter yield between two grass varieties in Samrang. Dry matter yield for Mulato II is mainly due to its large leaf size and thick stem. According to Ali et al. (2016), the variation in green fodder yield may be attributed to different genetic makeup and response to environmental condition. Lingmithang is located at higher elevation than Samrang and therefore, altitudinal effect must have been manifested in performance of these grass species. Hare et al. (2009) also mentioned that high production of lush, soft green leaves and low stem DM has always made Mulato II an attractive forage for livestock. In current study, DM yield per acre obtained from cultivar Mulato II in Samrang was 9.75 MT which is similar to that of DM yield (9.84 MT) obtained by Adnew et al. (2019). This is an indication that cultivar Mulato II performs better at lower elevation in Bhutan. Harvesting time is important to have better maturity level of forage to maximize DM yield and therefore, determination of appropriate time for harvesting forage is must (Mobashar et al. 2018). Variation in dry matter production among the different *Bracharia* cultivars could be attributed to differences in growth rate and growth habit which are mediated through genotypic and phenotypic differences (Gadisa et al. 2020). Application levels of nitrogen fertilizer also play a crucial role in determining forage yield and concentration of

Table 3: Total forage yield (M±SD) per acre of two *Bracharia* species at Samrang

Parameters	<i>Bracharia</i> species		
	Mulato II	Ruzi	Sig
Fresh biomass (MT)	46.90±6.48	32.60±1.03	*
Dry matter yield (MT)	9.75±1.38	6.41±0.81	*

* $p < 0.05$; ns: nonsignificant

nitrogen in tropical grasses (Worku et al. 2017). Polat et al. (2007) reported increased with increasing level of nitrogen fertilizer application. In this study, nitrogen fertilizer was applied as top dressing after subsequent harvest. In addition, Ansah et al. (2010) also mentioned that total herbage yield in Napier grass increased with upsurge in harvesting age and in which authors reasoned due to increase in tiller number, leaf elongation, leaf formation and stem development.

Table 4: Total forage yield (M±SD) per acre of two *Brachiaria* species at Lingmithang

Parameters	<i>Brachiaria</i> species		
	Mulato II	Ruzi	Sig
Fresh biomass (MT)	25.22±6.43	18.75±0.06	ns
Dry matter yield (MT)	6.33±1.37	4.77±0.58	ns

* $p < 0.05$; ns: nonsignificant

3.3 Fodder quality parameter

3.3.1 Crude protein

Crude protein is one of the vital factors affecting quality of fodder and represents important criteria for evaluating forage quality. Table 5 and 6 present the nutrient content, mainly crude protein, crude fat and total ash of two *Brachiaria* cultivars. This study found the *Brachiaria* hybrid cultivar Mulato II had the highest crude protein content (8.36%) than Ruzi grass (6.11%) in both the locations. However, there was no significant ($p > 0.05$) variation in crude protein content between two *Brachiaria* cultivars. Similar finding was reported by Mutimura and Everson (2012). However, crude protein content of cultivar Mulato II in present study was much lower than that obtained by Vendramini et al. (2014) who reported CP content of 12.29%. This variation may be due to nutrient inputs and climatic

Table 5: Forage quality parameters (M±SD) of two *Brachiaria* species at Samrang

Parameters	<i>Brachiaria</i> species		
	Mulato II	Ruzi	Sig
Crude protein (%)	8.36±1.40	6.11±2.57	ns
Crude fat (%)	1.16±0.28	0.83±0.57	ns
Total Ash (%)	10.75±2.18	9.64±1.85	ns

* $p \leq 0.05$, ns: nonsignificant

condition. In this study, cultivar Mulato II in Samrang surpassed minimum of 7% CP necessary from grass species for optimum rumen function as per the recommendation of Van Soest PJ (1994) as cited by Nguku et al. (2016b). The nutrient content in forage crop is also determined by stage of harvesting and CP content decreased with the stage of maturity (Kumar et al. 2018). The accumulation of CP content in plant is high at early stages of growth and as plants mature, CP content decreases due to increase in structural carbohydrates (Molla et al. 2018). Increasing cutting height also caused a decrease in crude protein in the forage grass due to plant aging which leads to decrease in cellular content and increase in cellular wall (Marques et al. 2017).

3.3.2 Crude fat

Though the result of this study found that crude fat content was higher in cultivar Mulato II (1.16%) in both the locations, there was no significant difference ($p > 0.05$) in crude fat content between two *Brachiaria* varieties. Mustaring et al. (2014) reported the similar result for crude fat content among different *Brachiaria* cultivars. Nutrient content of the plant depends on stage of maturity and as plants ages, increase in percentage of cellulose and hemicellulose reduce the digestible nutrients (Ribeiro et al. 2014). According to CSIRO (2007), crude fat levels of 2% in forage are considered low while levels of 5% or higher are considered high. In this study, crude fat levels obtained in cultivar Mulato II was below 5% in the two experimental sites. These variations could be due to soil fertility status and stage of defoliation. The ruminant diets high in fat increases energy intake and excessive fat in the diet is reported to inhibit rumen fermentation and effect volatile fatty acid (VFA) absorption (Palmquist 1994).

Table 6: Forage quality parameters (M±SD) of two *Brachiaria* species at Lingmithang

Parameters	<i>Brachiaria</i> species		
	Mulato II	Ruzi	Sig
Crude protein (%)	5.76±0.89	5.20±0.64	ns
Crude fat (%)	3.93±0.12	3.31±0.24	ns
Total Ash (%)	8.97±1.26	8.04±1.48	ns

* $p \leq 0.05$, ns: nonsignificant

3.3.3 Total ash content

Ash is the total mineral content of forage and accordingly, mineral composition in the grass is known through total ash content. Total ash content in cultivar Mulato II was higher than Ruzi grass in both locations. Statistically total ash content did not differ significantly ($p > 0.05$) between two *Brachiaria* varieties. Nguku et al. (2016) also reported the similar result among *Brachiaria* cultivars. The variation in concentration of minerals in forages can be influenced by various factors such as varieties, plant development stage, morphological fractions, climatic condition, soil characteristics and fertilization regime (Juknevičius and Sabiene 2007). In the current study, SSP fertilizer was applied during land preparation and sowing period with application of nitrogen fertilizer after each harvest. The forage varieties also varied in efficiency to absorb nutrient due to variable rooting depth and pattern (Ayub et al. 2011).

4. CONCLUSION

Brachiaria grass cultivars have promising potential to be promoted as the subtropical forage with desirable attributes such as palatability, nutritive value and climate resilience that will support sustainable dairy farming. In Bhutan, Ruzi grass (*Brachiaria ruziziensis*) had been widely adopted and promoted for pasture development and forage production in the subtropical region. The current study demonstrated that *Brachiaria* hybrid Mulato II has greater potential than Ruzi grass owing to higher palatability and biomass production, while their nutrient compositions are comparable. Therefore, it is concluded that new hybrid grass cultivar Mulato II can be an additional fodder species for pasture development and forage production under subtropical climatic condition in Bhutan. However, further study needs to be carried out for the production of seeds locally in the country.

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