

EFFECTS OF PLANT SPACING AND FERTILIZER LEVEL ON CHEMICAL COMPOSITION OF HYBRID *BRACHIARIA* CV. MULATO II GRASS DURING THE FIRST 150 DAYS OF GROWTH UNDER IRRIGATION SUPPLEMENTATION, IN CHAGNI RANCH, AWI ZONE, ETHIOPIA

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ABSTRACT

A study was conducted to evaluate the effects of plant spacing and N fertilizer application on chemical composition of *Brachiaria* hybrid cv. Mulato II grass for the first 150 days after planting. A factorial experiment with 3 urea fertilizer levels (0, 50 and 100 kg/ha) and 4 spacings between plants and rows (20 x 20, 30 x 40, 40 x 60 and 50 x 80 cm) with 3 replications was used. Chemical analyses were conducted for crude protein (CP), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Results indicated that DMY, CP%, CPY, NDF% and ADF% were significantly ($P < 0.05$) affected by plant spacing and fertilizer levels interactions. However, ash and ADL were significant ($P < 0.05$) affected by only main effects. The highest CP% was recorded for wider plant spacing (50 x 80 cm) with higher urea fertilizer level (100 kg/ha) (S4F3). Similar studies need to be conducted over much longer periods to determine to what extent these findings relate to performance over the life of a permanent pasture.

KEYWORDS: Urea; Spacing; Dry Matter Yield; Chemical Composition

Article History

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INTRODUCTION

Livestock are an important component of nearly all farming systems in Ethiopia, providing milk, meat, draught power, transport, manure, hides and skins and serve as a source of cash income (Funk et al. 2012). The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the Agricultural GDP. It also contributes 15% of export earnings and 30% of agricultural employment. The livestock subsector currently supports and sustains livelihoods for 80% of the total rural population (Samson and Frehiwot 2014). Despite the importance of livestock in the country, productivity is low (Sintayehu et al. 2010). One of the major constraints leading to such low productivity is shortage of feed in terms of both quantity and quality, especially during the dry season (Ahmed et al. 2010) combined with high feed prices (Sintayehu et al. 2010).

In order to solve the shortage of feed and increase livestock productivity, it is necessary to introduce and cultivate high-quality forages with high yielding ability and adaptation to the biotic and abiotic environmental stresses (Kahindi et al. 2007). Improved grasses, many of African origin, have greater palatability and productivity than other indigenous species and are therefore desirable additions to pastures and common grazing areas (Alemayehu 2002). Among the improved forage crops introduced into Ethiopia, Mulato II grass, which is the result of crosses of *Brachiaria ruziziensis*, *B. brizantha* and *B. decumbens*, is claimed to play an important role in providing a significant amount of quality forage (CIAT 2006).

The optimization of production and nutritive value of grass can be achieved by planting on fertile soils (ILRI 2010) and forage management tools such as plant spacing (Sumran et al. 2009). Nitrogen fertilizer application is a common practice since this nutrient is found to be one of the most limiting factors influencing yield and chemical composition of grass pasture including crude protein (CP) concentration and digestibility, which in turn improves livestock production (Marques et al. 2017). Nevertheless, information regarding the effects of fertilizer levels and plant spacing chemical composition of Mulato II grass is scarce in our country and specifically in the study area. We conducted the present study in order to generate information on chemical composition of Mulato II grass at different spacings with different rates of nitrogen fertilizer.

MATERIALS AND METHODS

Description of the Study Area

The experiment was conducted in Chagni Ranch, Guangua Woreda, Awi Zone, Amhara National Regional State, Ethiopia. Chagni (10°57'N, 36°30'E; elevation 1,583 masl), located at 528 km from Addis Ababa and 186 km West of Regional town, Bahir Dar, is the administrative center of Guangua District (Asnake 2009). The Ranch is located about 500 m south of the town. The area has average annual rainfall of 1,689 mm and mean minimum and maximum annual temperatures of 23 °C and 30 °C, respectively (Chagni ranch office).

Experimental Layout, Design and Treatments

The study was conducted using a 3 x 4 factorial arrangement in a randomized complete block design (RCBD) with 3 replications. The factors were 3 levels of urea fertilizer (0, 50 and 100 kg/ha) and 4 spacings (20 x 20, 30 x 40, 40 x 60 and 50 x 80 cm) between plants and rows, respectively, giving 12 treatment combinations (Table 1) and 36 experimental plots.

Table 1: Treatment Combinations

Fertilizer Level	Spacing			
	S1	S2	S3	S4
F1	F1 X S1 [T1]	F1 X S2 [T2]	F1 X S3 [T3]	F1 X S4 [T4]
F2	F2 X S1 [T5]	F2 X S2 [T6]	F2 X S3 [T7]	F2 X S4 [T8]
F3	F3 X S1 [T9]	F3 X S2 [T10]	F3 X S3 [T11]	F3 X S4 [T12]

S1 = 20 x 20 cm spacing; S2 = 30 x 40 cm spacing; S3 = 40 x 60 cm spacing; S4 = 50 x 80 cm spacing between plants and rows, respectively; T = treatments 1-12; F1= 0 kg urea/ha; F2 = 50 kg urea/ha; F3 = 100 kg urea/ha.

The size of each plot was 3 m long by 3.2 m wide with a gross plot size of 9.6 m² and the total experimental area was 12.6 m by 41.5 m (522.9 m²). The spacings between plots and replications were 0.5 and 1.5 m, respectively. Treatments were randomly assigned to plots within each replication.

Land Preparation, Experimental Management, Soil Sampling and Analysis

Land was oxen-ploughed, and harrowing and bed preparation were carried out before planting manually. Root splits of Mulato II grass were collected from Finota Selam grass nursery site at an age of 7 months re growth and planted at the experimental site on 6 September 2017. Urea was purchased from the local market and applied by split application at time of planting and again 30 days after planting with different levels based on treatment. The 25kg/ha for treatment taking 50kg/ha and 50kg/ha for treatment taking 100kg/ha of urea was applied at time of planting and again 30 days if planting. Weeding was done manually during the experimental period. The experiment was irrigated once a week when rain was limited, with precautions taken to avoid contamination of treatments by cross flooding. Soil samples were taken by auger from the center and corners of the experimental site prior to planting and from the individual plots immediately after harvesting to a depth of 15 cm. The collected samples were thoroughly mixed, dried, ground and preserved in plastic bags for chemical analysis to evaluate total nitrogen, available phosphorus, pH, organic matter and organic carbon. Total N was determined using the Kjeldahl procedure (Bermner and Mulvaney 1982) and available P using the Olsen method (Olsen et al. 1954). The totan organic carbon of soil was determined based on the Walkely-Black chromic acid wet oxidation method. Organic matter (OM) was calculated indirectly from organic carbon (OC) concentration by multiplying OC by 1.724 and the pH was determined using the method described by Van Reeuwijk (1993).

Chemical Analysis of Forage

The forage samples were thoroughly mixed and a 0.5 kg fresh subsample was taken from each plot for chemical analysis and dried in a forced-draft oven at a temperature of 105 °C for 24 hours. The dried material was ground to pass through a 1 mm sieve for chemical analysis and preserved in a plastic bag pending chemical analysis at Debre Birhan Agricultural Research Center Animal Nutrition Laboratory. Ash and nitrogen (N) were determined according to the procedures described by AOAC (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the procedures described by Van Soest and Robertson (1985).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS 2007). Differences among treatment means were determined using Duncan's Multiple Range Test (DMRT), at $P < 0.05$. The statistical model used was:

$$Y_{ijk} = \mu + B_i + F_j + S_k + (FS)_{jk} + e_{ijk},$$

Where:

Y_{ijk} = the response variable

μ = over all mean

B_i = i^{th} block effect

F_j = j^{th} main factor effect (Fertilizer level)

S_k = k^{th} main factor effect (Spacing)

$(FS)_{jk}$ = jk^{th} interaction effect (Fertilizer Level x Spacing)

e_{ijk} = random error.

RESULTS

Soil Analysis Report

Soil samples taken before planting of grass revealed the following: organic matter (OM) – 5.88%; organic carbon (OC) – 3.41%; total N – 0.30%; available P – 4 ppm; and pH – 5.6. After harvesting total N of the soil increased slightly with the highest level of urea as compared with the value before planting (Table 2), while pH declined. Available phosphorus (Av. P) declined on all treatments, while both OC and OM concentrations increased (Table 2).

Table 2: Soil Analysis Report After Forage Harvest for Various Urea Applications

Treatment	Soil Parameters				
	pH	Av. P (ppm)	OC (%)	OM (%)	Total N (%)
0 kg urea/ha	5.68	2	3.39	5.84	0.29
50 kg urea/ha	5.53	2	3.48	6.00	0.30
100 kg urea/ha	5.48	2	3.85	6.64	0.33
SSBP	5.6	4	3.41	5.88	0.30

SSBF = Soil Sample Before Planting; Av. P = Available Phosphorus; OC = Organic Carbon; and OM = Organic Matter.

Chemical Composition Brachiaria Hybrid (Mulato II) Grass

The significant effects of N fertilizer levels, spacing and their interactions on crude protein percentage (CP) and crude protein yield (CPY) is indicated in Table 3. CP concentration increased ($P<0.05$) with increase in row spacing at all fertilizer levels and urea application increased C at all plant spacings but differences were significant ($P<0.05$) at only the narrowest and widest spacings. Similarly, urea application increased CPY at all plant spacings but differences were significant ($P<0.05$) at only the narrowest spacing. Highest CP% (20.0%) was recorded where 100 kg urea/ha was applied at the widest plant spacing and the lowest (14.6%) for the Control treatment at the narrowest plant spacing. In contrast, highest CPY (1.45-1.57 t/ha) was recorded where urea was applied at the narrowest plant spacing.

Table 3: CP Concentration and Crude Protein Yield (CPY, t/ha) of Mulato II Grass as Affected by Urea Fertilizer Level, Plant Spacing and their Interactions

Spacing	CP (%)			CPY		
	Fertilizer Level			Fertilizer Level		
	F1	F2	F3	F1	F2	F3
S1	14.6h	16.2g	17.2defg	0.95cd	1.45ab	1.57a
S2	16.6fg	16.6fg	18.1bcdef	0.86cd	0.94cd	1.17bc
S3	17.7cdefg	18.3bcde	19.1abc	0.74de	0.83de	0.87cd
S4	18.5bcd	19.5ab	20.0a	0.53e	0.78de	0.74de

S1 = 20 x 20 cm; S2 = 30 x 40 cm; S3 = 40 x 60 cm; and S4 = 50 x 80 cm spacing between plants and rows; F1= 0 kg urea/ha; F2 = 50 kg urea/ha; and F3 = 100 kg urea/ha.

Means for different treatments with different letters are significantly different ($P<0.05$).

Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF)

The significant effects of N fertilizer levels, spacing and their interactions on neutral detergent fiber (NDF) and acid detergent fiber (ADF) are presented in Table 4. Both NDF and ADF concentrations declined as fertilizer level increased but differences were significant ($P<0.05$) only for the narrower two plant spacings. Similarly, NDF% declined as plant spacing increased but differences were significant ($P<0.05$) only in the Control treatment. ADF% also declined as plant spacing increased but there were no consistent significant differences. Overall trends were for highest values for both NDF and ADF concentrations to occur in the Control at the narrowest plant spacing and the lowest values in the higher urea level at the widest plant spacing.

Table 4: NDF and ADF Concentrations of Mulato II Grass as Affected by Urea Fertilizer Level, Plant Spacing and their Interactions

Plant Spacing	NDF (%)			ADF (%)		
	Fertilizer Level			Fertilizer Level		
	F1	F2	F3	F1	F2	F3
S1	52.8a	48.5abc	45.1cde	39.3a	36.9abc	33.7bcde
S2	51.0ab	47.8bcd	42.1e	38.2ab	36.3abcd	32.1cde
S3	48.3abc	43.6cde	46.6bcde	36.3bcd	30.8de	31.0de
S4	45.5bcde	46.2bcde	42.3de	34.4bcde	33.3bcde	30.1e

S1 = 20 x 20 cm; S2 = 30 x 40 cm; S3 = 40 x 60 cm; and S4 = 50 x 80 cm spacing between plants and rows; F1= 0 kg urea/ha; F2 = 50 kg urea/ha; and F3 = 100 kg urea/ha.

Means for different treatments with different letters are significantly different (P<0.05).

Ash and ADL content

As there was no significant interaction between fertilizer level and plant spacing for ash and ADL concentrations, main effect means are presented in Table 5. Ash concentration increased as fertilizer level increased (P<0.05) and as plant spacing increased (P<0.05), while the reverse was the case for ADL with ADL% decreased as fertilizer level increased and plant spacing increased (P<0.05).

Table5: Ash and ADL Concentrations of Mulato II Grass as Affected by Urea Fertilizer Level and Plant Spacing

Parameter	Fertilizer Level				Plant Spacing				
	F1	F2	F3	Mean	S1	S2	S3	S4	Mean
Ash (%)	9.86b	10.4ab	10.95a	10.4	9.43c	10.07bc	10.88ab	11.23a	10.4
ADL (%)	9.36a	8.56b	7.82b	8.58	9.55a	8.65ab	8.39ab	7.74b	8.58

S1 = 20 x 20 cm; S2 = 30 x 40 cm; S3 = 40 x 60 cm; and S4 = 50 x 80 cm spacing between plants and rows; F1= 0 kg urea/ha; F2 = 50 kg urea/ha; and F3 = 100 kg urea/ha.

Means for different treatments with different letters are significantly different (P<0.05).

DISCUSSION

Chemical Composition of *Brachiaria* Hybrid (*Mulato II*) Grass

Mulato II showed good response to fertilization, particularly to the application of nitrogen. Depending on the level of soil fertility, one or more maintenance applications may be required to maintain high yields of good quality forage. The percentage of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), invitro dry matter digestibility (IVDMD), depends on the soil fertility. Mulato II grass planted at wider spacing with higher N fertilizer level had excellent nutritional value, particularly CP concentration, limiting nutrient in forages. Even forage planted at all spacing with urea fertilizer application had CP concentration well above, the level of CP required for rumen function 7.5% (Van Soest 1982) and for lactation 15% (Norton 1982). This is a clear indication of the value of this particular grass to be used as valuable forage for livestock. The reason for the high CP percentage at higher fertilizer and wider spacing might be due to emergence of younger plants, enhancement of leafiness and leaf to stem ratio of grass which has high protein content than stem due nutrient and space availability. This result agree with finding Marques et al. 2017 who reported as Nitrogen is essential among nutrient to gate significant increase in forage and to have a linear increase in CP rate of the Mulato II grass

The crude protein yield (CPY) was increased with increasing of N fertilizer levels and reducing of spacing. Generally, the CPY was found to be higher for application of nitrogen fertilizer at narrower spacing (20x20 cm). The increment of CPY due to urea, in the current experiment was supported by Paulo et al. (2014) who recorded higher CPY from Rye grass fertilized by higher level of N. Despite the reduction of CP percentage with narrower spacing, CPY increased significantly as spacing become narrow. This might be due to, at closer space, DMY was higher with high dense plant population since the CPY is a product of CP percentage and DMY.

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations all declined as N fertilizer level and spacing increased. While increase steam percentage, maturity and increased lignifications would account for the narrower spacing without N fertilizer application. At lower fertilizer levels and narrower spacing's resource limitations is high which constrained the plants and make them to have higher structural components to cope with the stress full environmental condition. The narrower spacing's makes the plants to have higher structural materials and have higher NDF compared to the wider spacing which had less resources limitations and had higher leaf numbers and leaf to steam ratio which are assumed to be the factor for lower NDF and ADF content.

The current result is in line with Marques et al. (2017), who reported as Higher fertilizer dose led to higher protein, lower NDF and ADF levels of Mulato II grass, which could increase nutritive of value grasses and its intake (). Castagnara et al. (2011b) also detected the influence of N doses studied for NDF rates on three tropical grasses and four N doses (0, 40, 80, 160 kg/ha) with ureas as sources, and registered that the grasses had a quadratic behavior for the variable NDF. The lowest rate was obtained with higher N doses. Conversely, Hazary et al. (2015) on Jumbo grass did not find any significant effect on the NDF and ADF content with increasing level of N fertilizer. Berihun (2005) on Bana grass reported similar NDF and ADF increment trend with the current study at narrow spacings. However, Genet et al. (2017) on Desho grass reported higher NDF and ADL contents at wider spacings with the reasons being development of larger tillers in the wider-spaced plants.

The increase in cell wall contents (NDF) is a very important limiting factor of nutritive value of feeds (Van Soest and Robertson 1985). The NDF value greater than threshold level, 60% resulted in decreased voluntary feed intake, increased rumination time and decreased conversion efficiency of ME (Reed and Goe 1989). Roughage diets with NDF content of 45-65% and below 45% were generally considered as medium and high quality feeds, respectively (Singh and Oosting 1992). Additionally, roughages with less than 40% ADF content are categorized as high quality and those with greater than 40% as poor quality (Kellems and Church 1998). Mulato II grass established at different spacing and fertilizer level in the current study were bellow the critical value 60% NDF that decreased voluntary feed intake, feed conversion efficiency, longer rumination time (Reed and Goe 1989) and 40% ADF which considered as high quality grasses (Kellems and Church 1998).

The ash content in any feed is a positive indicator of the inorganic (minerals) content was significantly higher at higher N fertilizer level (F3). It was increased as the fertilizer levels increases, might be due to higher urea levels facilitate green leave production which uses more mineral for different physiological purposes and nitrogen fertilizer increased the mineral content in leaves. The observed high content of ash at higher levels of fertilizer was supported by the findings of Joorabi et al. (2014) who obtained higher mineral contents in Sorghum crops grown on higher fertilizer levels. Contrary to the current study, Abdi (2014) on *Cenchrus ciliaris* and *Panicum maximum* and Hazary et al. (2015) on Jumbo grass reported lower ash contents grasses with higher doses of nitrogen fertilizer levels. Total ash content was being reduced as

spacing become narrow. This might be due to less competition among plants for mineral resources uptake from soil and that facilitate emergence of more leaves. The current result supports the report of Genet et al. (2017) where increase in ash contents for Desho grasses grown on wider spacings.

The application of N fertilizer decreased the ADL content of the grasses, might be due to the continuous development of younger plants at N fertilizer application which could increase leafiness of grasses that in turn increases leaf to stem ratio. The urea fertilizer promotes the development of new leaves and shoots with low lignin content. When lignin is lowered digestibility plants increased since lignin is highly resistant to chemical and enzymatic degradation by the micro flora of the rumen. According to Abdi (2014), urea promotes the growth of new leaves and shoots resulting in low ADL contents of *Cenchrus ciliaris* and *Panicum maximum* grasses. The ADL content decreased as spacing increased, might be due to emergence of younger tillers from wider spacings which makes the plants to bear more leaves as a result of less competition for soil nutrient at wider spacing. Similarly, Berihun (2005) on Bana grass reported higher plant density per unit area increased the value of ADL.

The current result revealed that, quality of Mulato II grass can be improved by urea fertilizer application. More specifically the application of 100 kg/ha urea fertilizer is the most important to provides higher CP% and lower fiber content with better economic feasibility. On the other hand, CP% of grasses at wider spacings 50x80 cm were found to have high values. An important **limitations** of this study, that could be addressed in future works are:

- The trial needs to be conducted across a years to reach a complete recommend of optimum chemical composition more precisely.
- Further study on re-growth yield of Mulato II grass is useful to determine continuing productivity of grass in terms of chemical composition.
- The trial needs to be conducted for different types of soil to recommend optimum chemical composition of Mulato II grass.
- Further study on harvesting time and fertilizer type effects on Mulato II grass is useful to determine productivity of the grass in terms of chemical composition.
- The magnitude of improvement in the chemical composition of Mulato II grass needs to be studied in terms of animal performance.

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