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Title: Effect of Nitrogen Fertilization on Biomass Yield and Chemical Composition of German Grass (*Echinochloa polystachya*)

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ABSTRACT

The aim of the experiment was to evaluate the effect of N fertilizer in the form of urea on the biomass yield and nutritional value of German grass (*Echinochloa polystachya*). A randomized complete block design (RCBD) with three replications was used to design the experiment. Nitrogen fertilizer treatments were 130, 140, 150 and 160 kg ha^{-1}N with control. Doses of other fertilizers such as TSP, MoP, gypsum and zinc sulphate were kept constant for all treatments. The first and second cuttings were done at 60 and 90 days after planting (DAP), respectively. Data on selected parameters of biomass yield and nutrient composition were collected and analyzed on computer using SAS version 9.1. The results revealed that biomass yield of German grass increased with increasing N fertilizer dose at 60 DAP with highest at N dose of 160 kg ha-1 (52.78±2.82 t ha^{-1}) and lowest in control (32.11±1.64t ha^{-1}) ($p \leq 0.01$). Biomass yield increased numerically ($p \geq 0.05$) with increasing N fertilizer dose at 90 DAP. Crude protein (CP) percentage of German grass increased with increasing N fertilization and the highest value was observed with N dose of 160 t ha^{-1} for both cuttings at 60 DAP (10.52±0.04; $p \leq 0.001$) and 90 DAP (11.30±0.25; $p \leq 0.05$). Dry matter (DM), crude fiber (CF), ether extract (EE) and ash percentage of German grass did not vary significantly for both cuttings ($p > 0.05$). It can be concluded that biomass yield and CP content were significantly improved by application of 160 kg ha^{-1} N fertilizer.

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INTRODUCTION

Livestock is recognized as a pivotal sector for alleviating poverty, fostering income generation, and addressing nutritional deficiencies. According to the recent report prepared by Salim (2023) indicate that its substantial impact on the national Gross Domestic Product (GDP) amounted to be 1.85% in the fiscal year 2022-23. The development of livestock is mostly dependent on enhancing animal nutrition through better feeding and increased fodder supply. Roughage of low quality, namely rice straw and a tiny amount of green grass with minimal concentration, accounts for around 90% of the supply of cattle feed (Aquino et al., 2020). Protein, easily fermented carbohydrates, minerals, and vitamins are lacking in rice straw. Because of this, animals that only consume rice straw exhibit just 10% of their genetic potential in terms of milk and meat output. Therefore, it is frequently advised to add enough green grass to a diet based on straw in order to meet the needs of the animal (Jalal et al., 2016). One of the main obstacles to dairy production in Bangladesh is the lack of green fodder (Khan et al., 2009; Roy et al., 2012). The majority of the country's ruminants feed on fallows, naturally occurring trees, aquatic plants, native grasses from roadside verges, and crop leftovers and agro-industrial byproducts (Roy et al., 2012; Chowdhury et al., 2016). The area is not kept up to improve the output for animal feed; instead, they are utilized as pasture for ruminants of various kinds. These pastures' low productivity is determined by the season and climate (Tabassum et al., 2008). The majority of pastures are currently being converted to agricultural land due to rising pressure from the human population (Miah and Noman, 2003). Large farms are the only places where fodder is typically grown. Farmers who are poor and marginalized are nonetheless unfamiliar with the practice of growing fodder. This explains why there is insufficient feed available in the nation for ruminant animals. According to Khan et al. (2009), the majority of forages that livestock in Bangladesh feed are of relatively low quality.

One of the most important nutrients for plants is nitrogen, which is also the nutrient that is most commonly lacking in plants (Munir et al., 2004). A number of parameters, such as soil quality, plant density, fertilizer dosage, growth season, and maturation stage, have an impact on the yield and chemical composition of fodder. The amount and quality of fodder produced are directly impacted by the application of nitrogen, which is one of these important components. Increases in plant height, stem diameter, leaf area per plant, number of leaves per plant, and eventually fodder yield were the outcomes of applying nitrogen fertilizer (Mahmud et al., 2003). German grass has a reputation for producing large amounts of dry matter (DM), with average yields of 8–12 t ha^{-1} in South America and 10–20 t ha^{-1} in Australia (Cook et al., 2005). It has been reported that, in comparison to para grass, it generates noticeably more dry matter, crude protein, and green biomass in India (Hannan-Jones and Weber, 2008). In light of these findings, the current study was conducted to determine the effect of N fertilizer doses on the biomass yield and proximate composition of German grass.

MATERIALS AND METHODS

Site of the experiment

The experiment was conducted at Professor Dr. Purnendu Gain Field Laboratory of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh.

Experimental treatments and design

The experiment was laid out Randomized Complete Block Design (RCBD) with 15 plots, standardizing their size as 3 m × 5 m to create a homogeneous area for each plot. There were five treatment groups in the trial, designated as T_0, T_1, T_2, T_3, and T_4. Each treatment group corresponded to a different dosage of nitrogen and these were replicated thrice. While T_1, T_2, T_3, and T_4 represented N doses of 130 kg ha^{-1}, 140 kg ha^{-1}, 150 kg ha^{-1} and 160 kg ha^{-1}, respectively. T_0 considered as the control plot that did not receive N fertilizer.

Fertilizer management

In terms of fertilizer application, half the recommended dose of N in the form of urea was applied six days before planting, and the other half was applied 15 days after planting. TSP, MoP (muriate of potash), Gypsum and $ZnSO_4$ (zinc sulfate) application rates were done at 175 kg ha^{-1}, 20 kg ha^{-1}, 27.75 kg ha^{-1}, and 14.25 kg ha^{-1}, respectively. These are equivalent to the following doses: 35 kg ha^{-1} for phosphorus (P), 10 kg ha^{-1} for potassium (K), 5 kg ha^{-1} for sulfur (S), and 3 kg ha^{-1} for zinc (Zn), respectively.

Production of German grass

German grass cuttings were collected from the Buffalo Breeding and Development Farm, Fakirhat, Bagerhat, and planted on the next day of collection. There were four rows in every plot, and each row had eighteen cuttings, where cutting to cutting distance was 30 cm apart. Two weeks after planting the cuttings, the damaged cuttings were replaced with new cuttings. German grass was harvested for the first time at 60 DAP and again 90 DAP.

Data collection

Five equivalent fodder samples from each cutting were randomly selected from each plot; three of these samples were set aside for the purpose of separating the leaves and stems, while the other two were kept as whole plants for further examination. Using a knife, each leaf, stem, and entire plant sample was divided into pieces no larger than 1 cm. The samples that weren't used for the dry matter and ash tests were sun dried then pulverized using a blender machine and kept safe in sealed sample containers with the proper labelling. In total, 15 fodder samples underwent examination. Each sample was analyzed in triplicate. Dry matter, moisture, crude protein (CP), crude fiber (CF), ether extract (EE), and ash were among the quality characteristics that were evaluated following the AOAC (2003) standard procedure. After the forage cultivars were harvested, data was collected at 60 and 90 DAP.

Statistical method and analysis

SAS version 9.1 (SAS, 2009) was used to analyze the data using the General Linear Model (GLM) technique. Using DMRT, treatment means were compared with significance level set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Biomass yield

Data of the Table 1 shows the effect of nitrogen fertilizer on biomass output (t ha^{-1}) on German grass. Nitrogen (N) fertilizer had a highly significant impact on biomass yield at first cutting (60 DAP). At 60 DAP, the highest yield (52.78 t ha^{-1}) was obtained when 160 kg ha^{-1} of N was applied, and the lowest yield (32.11 t ha^{-1}) was obtained in control group. On the other hand, N did not significantly affect biomass yield during the second cutting (p>0.05) at 90 DAP. The application of 160 kg ha^{-1} of N produced the maximum yield (47.33 t ha^{-1}), while the absence of N at 90 DAP resulted in the lowest yield (32.67 t ha^{-1}). Notably, the total biomass yield from the current study was higher than previously reported findings. For example, Islam et al. (2017) reported 8.1 tons of biomass from a floating bed culture, however, it was marginally lower than 9.4 tons per acre as reported by Kanak et al. (2012) in conventional cultivation. The results revealed that the biomass yield of German grass increased with increasing N fertilizer dose indicating that the production of this grass is greatly influenced by N.

Table 1. Effects of N fertilizer on the biomass yield of German grass at first and second cuttings

N fertilizer dose (kg ha^{-1})	**Biomass yield** (t ha^{-1})	
	First cutting (60 DAP)	**Second cutting (90 DAP)**
0	32.11±1.64	32.67±6.68
130	36.45±2.12	34.67±3.71
140	43.22±4.70	40.44±4.96
150	49.78±3.86	45.56±5.20
160	52.78±2.82	47.33±5.00
p-value	0.0052	0.2623
Level of significance	**	NS

*** labelled as p≤0.01. NS = Non – significant, DAP = Days after plantation.*

Table 2. Effects of N fertilizer on the DM percentage of German grass at first and second cuttings

N fertilizer dose (kg ha^{-1})	**DM%**	
	First cutting (60 DAP)	**Second cutting (90 DAP)**

0	19.34±0.96	19.69±1.09
130	20.34±3.95	18.53±0.50
140	21.66±5.01	18.47±0.42
150	21.58±1.69	16.83±1.18
160	19.41±0.30	18.49±0.75
p-value	0.9624	0.2866
Level of significance	NS	NS

NS = Non – significant, DAP = Days after plantation.

Table 3. Effects of N fertilizer on the CP percentage of German grass at first and second cuttings

N fertilizer dose (kg ha^{-1})	**CP (%)**	
	First cutting (60 DAP)	**Second cutting (90 DAP)**
0	9.33±0.05	9.83±0.20
130	9.64±0.06	9.90±0.16
140	9.98±0.12	10.50±0.40
150	10.25±0.03	10.95±0.37
160	10.52±0.04	11.30±0.25
p-value	0.0001	0.0198
Level of significance	***	*

** Labelled as p≤0.05 and ***labelled as p≤0.001., DAP = Days after plantation.*

Proximate composition

Results from the current study illustrates (Table 2) that the application of N did not result in a statistically significant effect ($p \geq 0.05$) on dry matter (DM) during the initial cutting (60 DAP). The application of N at 140 kg ha^{-1} resulted in the highest DM content (21.66%) and the lowest DM content (19.34%) when no urea was applied at 60 DAP. On the other hand, N did not significantly affect dry matter content of German grass during the second cutting ($p \geq 0.05$). When no urea was administered, the highest DM level (19.69%) was seen, while the lowest DM content (16.83%) was recorded when 150 kg ha^{-1} of urea was applied at 90 DAP. In contrast to the current findings, Isaa et al. (2018) observed a lower DM content of German grass of 13.67% in their study.

Table 4. Effects of N fertilizer on the EE percentage of German grass at first and second cuttings

N fertilizer dose (kg ha^{-1})	**EE (%)**	
	First cutting (60 DAP)	**Second cutting (90 DAP)**
0	2.43±0.23	2.33±0.14
130	2.67±0.69	2.48±0.20
140	2.76±0.41	2.61±0.25
150	2.87±0.11	2.38±0.15
160	2.98±0.58	2.63±0.25
p-value	0.9254	0.7856
Level of significance	NS	NS

NS = Non – significant, DAP = Days after plantation.

Table 3 showed the effect of N fertilizer on German grass's on crude protein (CP) level. At first cutting, the impact of N had a highly significant effect ($p \leq 0.05$) on crude protein content of German grass. The application of 160 kg ha^{-1} N resulted in the highest CP level (10.52%), whereas control plot at 60 DAP resulted in the lowest CP content (9.33%). On the other hand, N had a substantial ($p<0.05$) effect on crude protein during the second cutting (90 DAP). The application of 160 kg ha^{-1} of N resulted in the highest CP percentage (11.30%), while the application of no N at 90 DAP resulted in the lowest CP content (9.83%). Notably, in contrast to the current results, Isaa et al. (2018) reported considerably lower CP concentrations of German grass (2.53%) in their investigation. Table 4 illustrates the effect of N fertilizer on German grass's ether extract (EE) concentration. Application of nitrogen showed no significant effect ($p>0.05$) on EE contents of German grass at both cuttings. At 160 kg ha^{-1} level of N application, the highest EE content (2.98%) was attained at 60 DAP, while the lowest EE content (2.43%) was recorded when no N was applied. When 160 kg ha^{-1} of N applied, the maximum EE content (2.63%) was seen at 90 DAP, and the lowest EE content (2.33%) was recorded when no N was applied. The results of this study are notable because they differ from those of a study by Isaa et al. (2018), which indicated a lower EE content (1.23%) in German grass. Table 5 summarizes the effect of N fertilizer on German grass's crude fiber (CF) content. It is clear that the nitrogen did not have a significant effect (p>0.05) on crude fiber. At 60 DAP, the CF concentration was at its lowest (37.97%) with a urea dose of 140 kg ha^{-1}, whereas the greatest CF level (40.84%) was obtained when no N was applied. On the other hand, nitrogen did not significantly affect crude fiber during the second cutting (p>0.05). With a N dose of 130 kg ha^{-1}, the highest CF content (38.47%) was found from the second cutting (90 DAP), however, the lowest CF content (36.00%) was recorded with 160 kg ha^{-1} of N. The results of the current study are in contrast to Isaa et al. (2018) who reported a lower CF content (21.16%) in German grass. The CP content of German grass was affected by N fertilizer and showed an increasing trend of this element in grass with increasing soil N application. These results also show that the higher the N content in the soil, the better its uptake by the grass.

Table 5. Effects of N fertilizer on the CF percentage of German grass at first and second cuttings

N fertilizer dose (kg ha^{-1})	CF (%)	
	First cutting (60 DAP)	**Second cutting (90 DAP)**
0	40.84±0.18	37.60±2.28
130	38.73±2.61	38.47±2.15
140	37.97±1.08	38.33±3.73
150	38.93±0.72	36.72±1.64
160	39.23±0.67	36.00±0.45
p-value	0.6546	0.9269
Level of significance	NS	NS

NS = Non - significant, DAP = Days after plantation.

Table 6. Effects of N fertilizer on the ash contents of German grass at first and second cuttings

N fertilizer dose (kg ha^{-1})	Total ash (%)	
	First cutting (60 DAP)	**Second cutting (90 DAP)**
0	18±1.04	16.79±2.26
130	12.87±3.35	17.62±2.14
140	13.27±1.40	14.07±0.94
150	13.47±2.48	15.06±0.44
160	17.83±1.14	14.21±0.96
p-value	0.2622	0.4200
Level of significance	NS	NS

NS = Non – significant, DAP = Days after plantation.

The influence of N fertilizer on the total ash content of German grass is presented in Table 6. It is evident that the application of N did not result in a significant effect (p>0.05) on ash content. The highest ash content (18%) was observed when no N was applied, while the lowest ash content (12.87%) was recorded with a N dose of 130 kg ha^{-1} at 60 DAP. Conversely, during the second cutting (90 DAP), the effect of N also did not yield a significant impact ($p \geq 0.05$) on ash content. The highest ash content (17.62%) was observed with a N dose of 130 kg ha^{-1}, while the lowest ash content (14.07%) was recorded with a N dose of 140 kg ha^{-1} at 90 DAP. In a study conducted by Isaa et al. (2018), the ash content of German grass was reported as 14.73%, which is aligned with the findings from the current study. Khan (2012) stated that the mineral content of grass leaves is higher than that of their stems.

CONCLUSION

It was found that nitrogen fertilizer had a significant effect on the levels of crude protein (CP) content ($p \leq 0.05$) and biomass production ($p \leq 0.01$) at first cutting (60 DAP). On the other hand, at 60 DAP, there were no discernible variations in the concentrations of ash, CF, EE, or DM (p>0.05). At 90 DAP, N fertilizer showed a significant impact on

CP content ($p \leq 0.05$). However, at this stage, variations in N fertilizer did not significantly affect yield, DM, CF, EE, or ash ($p > 0.05$). Markedly, at 60 DAP, biomass yield and CP recorded their greatest values, reaching 52.78 t ha^{-1} and 10.52%, respectively at the N dose of 160 kg ha^{-1} ($p \leq 0.01$). The study also showed that increasing the dose of N fertilizer raised biomass yield, DM, EE, and CP levels while lowering CF and ash contents. It could be concluded that the highest biomass yield and CP content of German grass were noted at 60 DAP with the application of N fertilizer dosage of 160 kg ha^{-1}.

Competing interest

The authors affirm that there isn't any conflict of interest with this article's publishing.

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