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Research Article

Influence of N fertilizing on Single-cut Sorgum × Sudangrass Hybrids' Forage Yield and Nutritive Profile

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ABSTRACT

The study aimed to observe the effects of two forage sorghum hybrids and different nitrogenous (N) fertilizer rates. The current study was set up in randomized complete block (RCB) as a split-plot design with triplicate at the Field Crops Department experimental area, University of Ankara in the 2022 summer season. This experiment included two treatments which had forage sorghum hybrids (Hay day and Super-graze) and nitrogen fertilization rates in the form of 18% ammonium sulfate [control group with no nitrogen (N_0), 120 kg ha^{-1} N (N_1), 180 kg ha^{-1} N (N_2)]. N fertilizing rates positively influenced in agronomic profiles, yield components and nutritive profiles. The plant height (197.24-221.72 cm), green herbage yield (GHY) (60.42-70.89 ton ha^{-1}), dry matter (DM) yield (17.77-23.98 ton ha^{-1}), crude protein (CP) yield (0.97-1.90 ton ha^{-1}), metabolic energy (ME) yield (33070.17-51840.85 Mcal ha^{-1}), net energy production (NE_p) yield (21164.91-33178.15 Mcal ha^{-1}) increased with applied N rates. The maximum of DM (33.83%), CP (7.90%), TDN (58.63%), ME (2.16 Mcal kg^{-1} DM), NE_p (1.80 Mcal kg^{-1} DM), and the minimum of ADF (33.09%), NDF (53.17%), ADL (5.14%) were obtained in N_2 rates. In light of the results; "Hay-day" had more GHY. "Sugar-graze" had more DM yield and it was determined more digestible forage. It was inferred that the best yield components and nutritive profiles were obtained in N_2 rate.

Keywords: *Fertilizing, Sorghum, Yield, Quality*

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INTRODUCTION

Forage sorghum (*Sorghum bicolor* (L.) Moench × *Sorghum sudanese* (Piper) Stapf) is a beneficial forage crop for livestock production dynamics in many regions around the World owing to the adaptation of different environments (Fonseca *et al.*, 2012; Amelework *et al.*, 2015). The capacity to forage from natural rangelands has declined drastically in recent years, on account of water shortage, salinity problems of soil, and degradation (Zhang *et al.*, 2014). As a consequence of natural grassland's poor quality in Turkey, the livestock ecosystem needs more quality feed. In this case, it is reflected a huge production cost for livestock producers. In arid and semiarid environments, forage sorghum is an important alternative plant that has high dry matter (DM) yield and morpho-physiological adaptations that could resist water shortage (Sankarapandian *et al.*, 2013; Ahmeda *et al.*, 2016). Also, it is suitable for silage production and it has high nutritive profiles, which has high soluble carbohydrates and low buffering capacity (Lema *et al.*, 2000; Sankarapandian *et al.*, 2013; Brocke *et al.*, 2014; Kumar *et al.*, 2015).

While agronomic specifications of forage sorghum are necessary, economic specifications must be considered too. Forage sorghum is known to be more economical than other cereal forages because of the fewer requirements in irrigating and fertilizing. It was demonstrated by Iqbal *et al.* (2015) that seed usage, fertilizing amount, irrigation, total expenditures and net earnings are cheaper than maize (Iqbal, 2015; Iqbal *et al.*, 2015). Some agronomic practices, especially fertilizing, have the potential to increase the green herbage yield and nutritive value of sorghum (Iqbal, 2015). Particularly, N fertilizer is essential for the plant's growth during the growing season. Sorghum utilizes nitrogen more efficiently than maize (Noori, 2020). Increasing N fertilizing positively affect to plant height, shoot elongation, palatability, yield components and nutritive profiles of forage crops (Ikanovic *et al.*, 2014). It also increases protein content, digestibility of dry matter, and decreases crude fibre (Sher *et al.*, 2016). Deficit of soil N leads to lower forage sorghum biomass in result of reductions on leaf area, chlorophyll index (Mahama *et al.*, 2014). Other than that, forage sorghum has an ability to regrow after cutting, especially when fertilization is applied (Afzal *et al.*, 2012). Forage sorghum hybrids is fertilized for optimal forage with application of 50 to 100 $kg \cdot N \cdot ha^{-1}$, applied in two equal rates, which is recommended by OMAF (2002).

The first objective of the current research was to study the effect of forage sorghum hybrids and N fertilizer rates on agronomic profiles, yield components, and nutritive profiles of the Central Anatolian region, Ankara. The second objective was to determine which parameters had a relationship with growing degree days (GDD).

MATERIALS and METHODS

Experimental Design and Agronomic Practices

The experimental site was established at Ankara University, Faculty of Agriculture, Field Crops Department's experimental area in summer season of 2022. Köppen-Geiger climate classification of Ankara province is Csa which has a temperate, dry, and hot summer climate (Rahimi *et al.*, 2020). The latitude of Ankara, Turkey is 39° 97' north, the longitude is 32° 86' east and the elevation is 891 m. Mean temperature and total accumulated precipitation were 23.20°C and 77.30 mm between planting time to harvest. Soil of the experimental area is clay-sandy (sand 23.12, clay 44.60) with 8.02 pH. Organic matter contents were low, particularly in the layers to below given depth. There was rich in potassium (582 ppm), medium phosphorus (13.28 ppm), and low nitrogen (0.074%).

Forage sorghum hybrids were planted 30 kg ha^{-1}; with row spacing of 35 cm; a mean plot size of 5.75 m^2 keeping a distance of 1m gap among 3 replicates in a randomized complete block design with split plots. In order to prevent the side effect in the study, one more row was added to the borders of the experimental area. Two forage sorghum hybrids, which are certified, Hay day and Super-graze (n=2) were established in the field. Forage sorghum hybrids were all planted on May 30, 2022, and harvested on September 16, 2022. Before planting; phosphorous (P) fertilizer (120 kg ha^{-1} in the form of 46% di-ammonium phosphate) was applied to the all plots. Control treatment (N_0) (0 kg ha^{-1}) and two different N rates [N_1 (120 kg ha^{-1} N); N_2 (180 kg ha^{-1} N)] (n=3), which were in the form of 18% ammonium sulfate, were applied to the soil. N was applied into two different times; half of it was applied during the planting and the second part was applied when the plants reached up to 20-30 cm in length. The mechanical weeding operation by hand was done at the vegetative growth stage of the plants, specifically when the plants reached a height range of 30 to 40 cm. Forage sorghum hybrids are harvested at the dough stage. Silking date (days), which is described as the beginning of the flowering time, and plant height (cm) were measured for obtaining an agronomic profile. Plant height was measured for twenty sorghum plants and then taken as an average per plot. All plants per plot were harvested, then weighed to determine GHY on a hectare basis (ton ha^{-1}). The dry matter (DM) yield (ton ha^{-1}) was calculated per plot by multiplying the dry matter (%) and GHY. Growing degree days (GDD) were calculated per all plots in accordance with seedling emergence date in the field to the harvest date (GDD = [(Tmax, °C + Tmin, °C)/2 - 5]) (McMaster and Wilhelm, 1997) (data now shown).

Sampling and Nutritive Profile Analysis

Twenty plants per plot were separated at the harvest stage. The collected sample (500 g) was retained and dried at 70°C for 48 h to determine the constant weight (Avci, 2000). Dried samples were ground to pass through a 1-mm screen in the mill. All the collected samples were analyzed for DM, CP, ADF, NDF, ADL, and TDN. DM was analyzed from the collected sample (10 g) (135°C for 2 hours) (AOAC, 2005 method 930.15). The traditional Kjeldahl acid digestion method was used for obtaining nitrogen compound, then it was converted to ammonia, which is distilled and titrated (AOAC, 2005 method 2001.11), and CP was calculated with N × 6.25 equation. Van Soest *et al.* (1991)'s a sequential procedure applied to determine the ADF and NDF with the ANKOM200 Fiber Analyzer (Ankom Technology Corp, Macedon, NY, USA) after pre-treatment with sodium sulfite and α-amylase and expressed inclusive of residual ash. ADL was analyzed with the direct sulphuric acid (72%) method using ADF residues by Robertson and Van Soest (1981). By Horrocks and Valentine (1999); TDN was calculated [(TDN = (-1.291 × ADF %) + 101.35)]. Metabolic energy (ME) and net energy production (NE_p) were calculated in accordance with NRC (1989). After identifying CP, and ME, NE_p values; these values were multiplied with dry matter yield, then converted on a hectare basis for obtaining yield values of these parameters (ton ha^{-1} and Mcal ha^{-1}).

Statistical Analysis

The data obtained from the current study were subjected to analysis of variance in accordance with the randomized completely block design (RCB) with split plots as triplicate (n=3) via JMP v.13 computer software (SAS, 2017). For each forage sorghum hybrid; agronomic profiles (the silking date, plant height) and yield components (GHY, DM yield, CP yield, ME yield, NEp yield) and nutritive profiles (DM, CP, ADF, NDF, ADL, TDN, ME, NE_p) were analyzed. DM, CP, ADF, NDF, ADL, and TDN were arcsine-transformed before the statistical analysis to stabilize variances and normalize proportional data. Probabilities equal to or less than 0.05 were considered significant (* P<0.05, ** P<0.01). If ANOVA indicated differences between treatment means, LSD test was performed to separate them. Correlations (r) between GDD to agronomic profiles, yield components, and nutritive profiles were determined. The dependent variable of this observation was GDD. Its relationship with agronomic profiles, yield components and nutritive profiles are presented in Table 2.

RESULTS AND DISCUSSION

Agronomic Profile and Yield Components

The effect of the forage sorghum hybrids on agronomic profiles; significant differences were not detected for all parameters (ns), excluding GHY (P<0.05). But, the effect of the N rates had significant differences in plant height, DM yield, CP yield, ME yield, and NE_p yield (P<0.01) in accordance with one-way ANOVA, excluding silking date (ns) and GHY (P<0.05). The interaction of forage sorghum hybrids × N fertilizer rates were found non-significant for all parameters. “Hay day” and “Sugar-graze” generally showed similar agronomic and yield values due to their non-differences in statistical data. An earliest silking date (66.89 days) was observed in “Hay day”. The longest plant height (212.25 cm), the maximum GHY (68.05 ton ha^{-1}), the maximum yield of DM (20.91 ton ha^{-1}), CP (1.44 ton ha^{-1}) were obtained in “Sugar-graze” (Figure 1, Figure 2). The minimum agronomic profiles and yield components were noted in “Hay day”, excluding ME (42567.55 Mcal ha^{-1}), and NE_p (27243.23 Mcal ha^{-1}). “Hay day” had the smallest plant height (210.37 cm), the minimum GHY (63.86 ton ha^{-1}), the minimum yield of DM (20.78 ton ha^{-1}), and CP yield (1.41 ton ha^{-1}) (Table 1, Figure 2).

The silking date (69.83-64.14 days), plant height (197.24-221.72 cm), GHY (60.42-70.89 ton ha^{-1}), DM yield (17.77-23.98 ton ha^{-1}), CP yield (0.97-1.90 ton ha^{-1}), ME yield (33070.17-51840.85 Mcal ha^{-1}), NE_p yield (21164.91-33178.15 Mcal ha^{-1}) were varied among N fertilizer rates (N_0 to N_2) (Table 1.). N_2 fertilizer gave more GHY (10.47 ton ha^{-1}), the yield of DM (6.21 ton ha^{-1}), and CP (0.93 ton ha^{-1}) over non-fertilizing (N_0) plots (Figure 2).

Table 1. *Mean values of agronomic profiles, yield component and nutrient values of Forage sorghum hybrids and N fertilizer rates.*

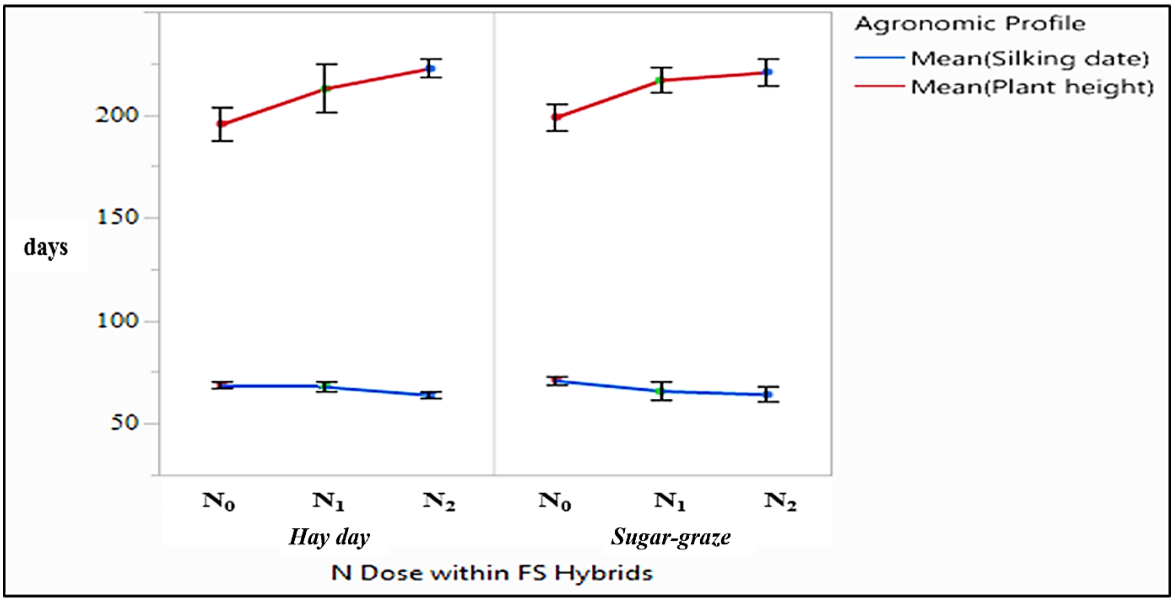
	Hybrids		Fertilizer				
Agronomic profile and yield components	Hay day	Sugar-graze	No (Control)	N_1 (120 kg ha^{-1})	N_2 (180 kg ha^{-1})	MEAN	± SD
Silking date, (day)	66.89	67.11	69.83	67.00	64.17	67.00	± 4.77
Plant height, (cm)	210.37	212.25	197.24b	214.97a	221.72a	211.31	± 15.45
GHY, ton ha^{-1}	63.86b	68.05a	60.42b	66.56ab	70.89a	65.96	± 6.38
DM yield, ton ha^{-1}	20.78	20.91	17.77b	20.79ab	23.98a	20.85	± 3.38
CP yield, ton ha^{-1}	1.41	1.44	0.97c	1.41b	1.90a	1.42	± 0.43
ME yield, Mcal ha^{-1}	42567.55	41778.95	33070.17c	41608.73b	51840.85a	42173.25	± 8991.78
NEp yield, Mcal ha^{-1}	27243.23	26738.53	21164.91c	26629.58b	33178.15a	26990.88	± 5754.75
Nutrient Profile							
DM, %	32.53	30.67	29.41b	31.23ab	33.83a	31.50	± 3.16
CP, % DM	6.66	6.77	5.48b	6.77a	7.90a	6.72	± 1.13
ADF, % DM	35.30b	36.14a	38.23a	35.83ab	33.09b	35.72	± 2.48
NDF, % DM	55.82	56.43	58.98a	56.21ab	53.17b	56.13	± 2.89
ADL, %DM	5.77	5.64	6.21a	5.75ab	5.14b	5.70	± 0.59
TDN, % DM	55.78a	54.69b	51.99b	55.09ab	58.63a	55.24	± 3.21
ME, Mcal kg^{-1} DM	2.03a	1.99b	1.86b	2.00ab	2.16a	2.01	± 0.14
NE_p, Mcal kg^{-1} DM	1.30a	1.27b	1.20b	1.28ab	1.38a	1.29	± 0.10

GHY: green herbage yield, **DM:** Dry matter, **CP:** Crude protein, **ME:** Metabolic energy, **Nep:** Net energy production, **SD:** Standart deviation.

An agronomic profile such as plant height reached the longest level in N_2 compared to non-fertilizing plots (Figure 1), for "Hay day" and "Sugar-graze" (210.37 and 212.25 cm). Silva *et al.* (2011) evaluated plant height in 25 forage sorghum hybrids and noted that was an average of 207 cm, which was below the forage sorghum hybrids in current study. The plant height findings of Chaudhary *et al.* (2018) (161.06-173.98 cm) were also lower, yet the findings of Atis *et al.* (2012) (257.70-278.00 cm) and Singh *et al.* (2016) (274.9-354.8 cm) were higher than the current study. "Hay Day", which was used in the current study, was stated the plant height as 284.60 cm by Keskin *et al.* (2018). Shivprasad and Singh (2017) also obtained the plant height as 234.00 cm with the same row spacing and N rate, which was similar to current study.

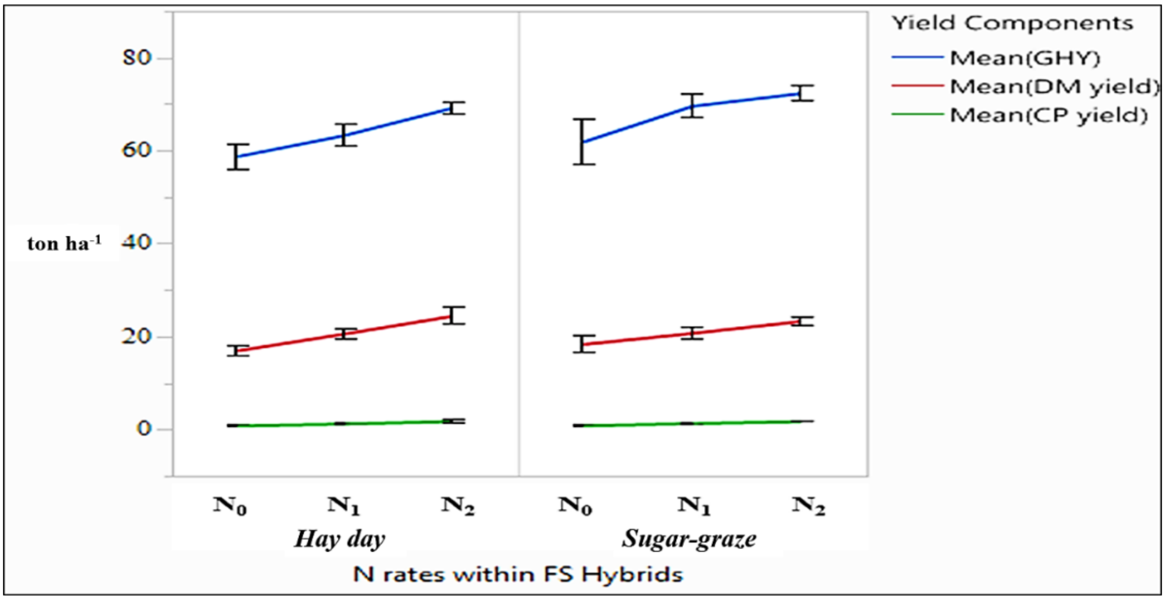
Monteiro *et al.* (2004) defined that plant height is an essential agronomic parameter for the GHY. It is also influenced positively by higher nitrogen rates (Cheema *et al.*, 2010). But a greater plant height does not always refer to a higher DM yield. "Sugar-graze" had a longer plant height (212.25 cm) and GHY (68.05 ton ha^{-1}) than the plant height (210.37 cm) and GHY (63.86 ton ha^{-1}) of "Hay day". But the DM yield of "Sugar-graze" (20.91 ton ha^{-1}) was very close to the DM yield of "Hay day" (20.78 ton ha^{-1}). The similarity of DM yield in forage sorghum hybrids emanated from the DM content of the "Sugar-graze" (30.67%) and "Hay day" (32.53%).

GHY, DM yield, CP yield, ME yield, and NE_p yield tended to increase in applied N fertilizer [N_0 to N_2 (0 to 180 kg ha^{-1})] (Figure 2). But individual hybrids' influence was less effective compared to different N rates on these yield components. With the parallel findings of Holman *et al.* (2019) and Almodares *et al.* (2009); GHY, and DM yield showed significant differences with the applied N rates. The GHY and DM yield results of Keskin *et al.* (2018) (83.37 ton ha^{-1} and 26.58 ton ha^{-1} for "Hay day") and Atis *et al.* (2012) (84.69 ton ha^{-1} and 21.0 ton ha^{-1}) showed higher yield potential from the current study. Similar agronomic practices to the current study by Shivprasad and Singh (2017), who conducted their study with row spacing of 30 cm and 120 kg ha^{-1} N rate, reported that GHY was as 36.90 ton ha^{-1}. CP yields of forage sorghum hybrids were very similar to each other, while N rates showed significant differences. The response of CP yield to the N rates is related to the increase in DM and CP content. When nutrient uptake is boosted in the plants with some agronomic practices like fertilizing, protein synthesis in the plants is disposed to increase which is resulted in higher protein content, CP yield per area also increases as a consequence of this (Księżak *et al.*, 2012; Sher *et al.*, 2017; Pal *et al.*, 2014) demonstrated that CP yield ranged 0.98-1.04 ton ha^{-1} and 1.02-1.76 ton ha^{-1}, which were below the CP yield of N_2 rate in the current study.



N_0: control treatment, N_1: 120 kg ha^{-1} N, N_2: 180 kg ha^{-1} N.

Figure 1. *Expression of agronomic profiles.*



N_0: control treatment, N_1: 120 kg ha^{-1} N, N_2: 180 kg ha^{-1} N, **GHY**: Green herbage yield, **DM**: Dry matter, **CP**: Crude protein.

Figure 2. *Expression of yield components.*

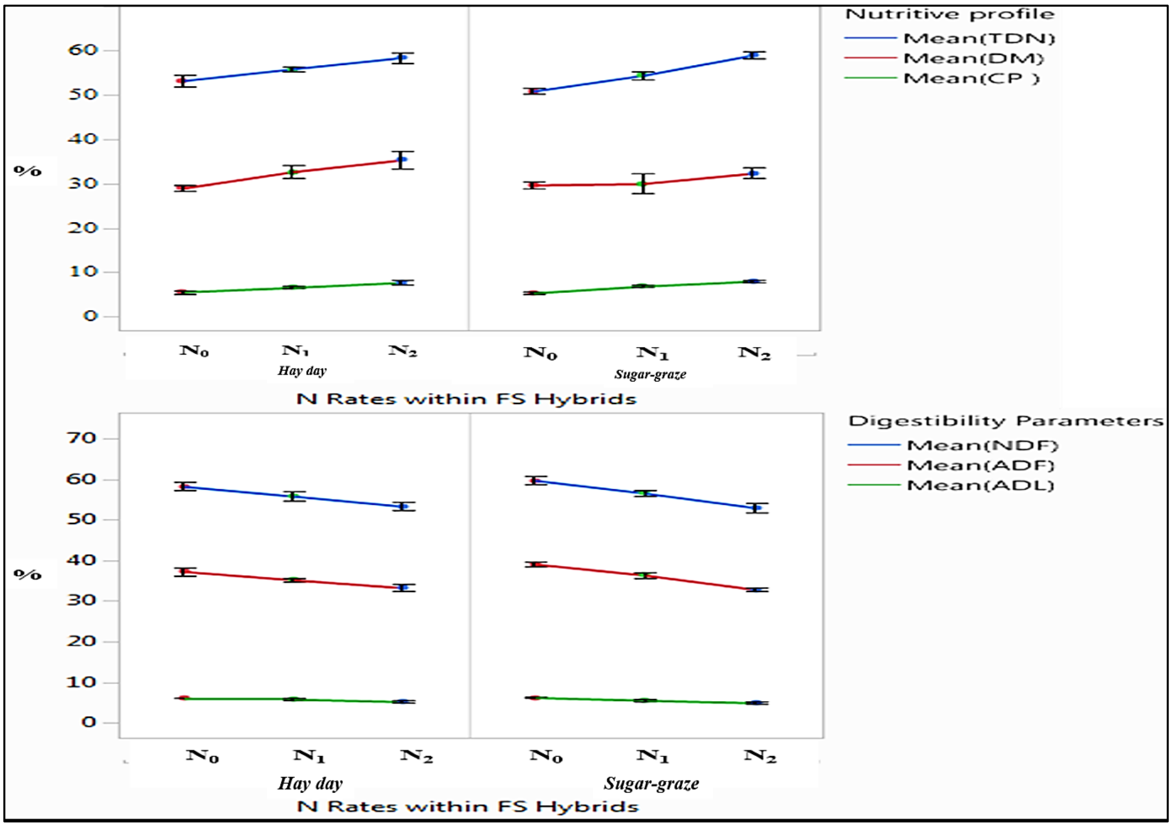
Nutritive Profile

The effect of the forage sorghum hybrids on nutritive profile; ADF, TDN, ME, and NE_p had significantly different ($P<0.05$), while DM, CP, NDF, and ADL were not (ns). The N rates effect on CP, ADF, NDF, ADL, TDN, ME, and NE_p detected significant differences ($P<0.01$) in accordance with one-way ANOVA, excluding DM ($P<0.05$). The interaction of forage sorghum hybrids × N fertilizer rates were found non-significant for all parameters. “Hay day” and “Sugar-graze” had more than 30% DM (32.53% and 30.67%) (Figure 3). The maximum CP content (6.77%), ADF (36.14%), NDF (56.43%) were obtained in “Sugar-graze”. “Hay day” had a maximum ADL (5.77%), TDN (55.78%), ME (2.03 Mcal kg^{-1} DM), and NE_p (1.30 Mcal kg^{-1} DM) compared to “Sugar-graze”.

It was indicated that the maximum of DM (33.83%), CP (7.90%), TDN (58.63%), ME (2.16 Mcal kg^{-1} DM), NE_p (1.38 Mcal kg^{-1} DM) in N_2 rate. N_0 had the maximum ADF (38.23%), NDF (58.98%), and ADL (6.21%). N_2 plots gave 30% more CP, %11 TDN, %14 ME, %13 NE, and less %13 ADF, %10 NDF, % and 17% ADL over non-fertilizing (N_0) plots (Figure3).

Forage sorghum hybrids did not affect DM content, while N fertilizer rates did [N_0 to N_2 (0 to 180 kg ha^{-1})]. “Hay day” and “Sugar-graze” showed a little variation of each other on DM content. In the contrast to current study; Costa *et al.* (2016) and Tolentino *et al.* (2016) indicated that 12 and 24 different sorghum genotypes might show wide variation in DM content (30.14-42.33%) (36.31-50.25%), in the same order. This type of wide variation makes predicting nutritive profile and dry matter intake non-confident. In addition, this is one of the main reasons why livestock producers choose corn rather than forage sorghum as silage for their total mixed rations (Govea *et al.*, 2010).

The nutritive quality increase when N fertilizers should be so properly applied that they should improve the efficient use of plant nutrients. This application preserves also feed digestibility as well as silage quality (Kaplan *et al.*, 2019). ADF, NDF, and ADL percentages are inversely related to DM intake, palatability and digestibility. Under these circumstances, low fiber contents (Ahmad *et al.*, 2016) and higher CP are more acceptable for better quality, and vice versa. The current study indicated that CP increased and digestibility parameters (ADF, NDF, ADL) decreased with applied N rates (Figure 3). The more digestible product, which had lower ADF (35.30%), NDF (55.82%) was "Hay day". The findings of Holman *et al.* (2019) and Damian *et al.* (2017) about increasing CP content with applied N rates showed similar trends to the current study. CP contents were also documented by Celik and Turk (2021) (10.72% in 5 cultivars), Costa *et al.* (2016) (8.03% in 15 genotypes), Tolentino *et al.* (2016) (8.14% in 24 genotypes).



N_0: control treatment, N_1: 120 kg ha^{-1} N, N_2: 180 kg ha^{-1} N, **DM**: Dry matter, **CP**: Crude protein, **TDN**: Total digestible nutrient, **ADF**: Acid detergent fiber, **NDF**: Neutral detergent fiber, **ADL**: Acid detergent lignin.

Figure 3. *Expression of nutritive profile and digestibility parameters.*

In the current study, there were variations among N fertilizer rates [N_0 to N_2 (0 to 180 kg ha^{-1})] in digestibility parameters, which were ADF, NDF, and ADL. Increasing N rates declined ADF, NDF, and ADL content, in contrast to the findings of Tang *et al.* (2018) and Marsalis *et al.* (2010). These rates in the current study also made forage sorghum hybrids more digestible for animal feed. The findings of Tang *et al.* (2018) (40.60% ADF, 65.20% NDF) and Sher *et al.* (2016) (43.80% ADF, 53.95% NDF) were higher than the current study. Kir and Sahan (2019) indicated the ADL content of "Sugar-graze" was 5.20%, which is lower for the same hybrid in the current study. TDN defines the available nutrients for livestock and the energy content of forages (Sayar *et al.*, 2014; Posada *et al.*, 2012); therefore, the highest TDN (55.78%) was observed in "Sugar-graze". N fertilizer rates [N_0 to N_2 (0 to 180 kg ha^{-1})] increased TDN in the current study. The TDN content of the current study had %11 more TDN (55.78%) compared to the finding of Bilen and Turk (2021) for "Sugar-graze" (49.52%). Tang *et al.* (2018) (49.00%) and Sher *et al.* (2016) (44.80%) also stated lower TDN content over the current study.

Similar to TDN, which is highly desirable quality components, ME and NEp are beneficial to forage quality by improving animals' ability to utilize the forage nutrients (Carmi *et al.*, 2006, Lithourgidis *et al.*, 2006). Applied N rates increased energy values in agreement with Kaplan *et al.*, 2016; Kaplan *et al.*, 2019). They also determined ME values as 2.11 and 1.56 Mcal kg^{-1} DM, which were higher compared to the control treatment and lower than N_2 rates in the current study.

Growing Degree Days (GDD) Relationship with Agronomic Profile, Yield Components and Nutritive Profile

Table 2. *Correlation co-efficients of some important parameters with growing degree days (GDD).*

	SD	PH	DM	GHY	DMY	CPY	MEY	NE_pY	CP	ADF	TDN	NDF	ADL
GDD	-0.44	0.71	0.41	0.50	0.54	0.38	0.45	0.45	0.17	-0.13	0.13	-0.10	-0.30
p level	0.03*	0.001***	0.09	0.03*	0.02*	0.13	0.06	0.06	0.50	0.60	0.61	0.72	0.23

SD: silking date, **DM**: dry matter, **GHY**: green herbage yield, **DMY**: dry matter yield, **CPY**: crude protein yield, **MEY**: metabolic energy yield, **NE_pY**: net energy production yield, **CP**: crude protein, **ADF**: acid detergent fibre, **TDN**: total digestible nutrient, **NDF**: neutral detergent fibre, **ADL**: acid detergent lignin

GDD had a relationship with some agronomic profiles and yield components, while all nutritive profiles were not (ns) (Table 2). Plant height (r = 0.71, P<0.001) showed a positive strong correlation. GHY (r = 0.50, P = 0.03), DM yield (r = 0.54, P = 0.02) showed a positive weak correlation, while silking date (r = -0.44, P = 0.03) showed a negative weak correlation with GDD (Table 2). Lyons *et al.* (2019) noted a positive weak relationship between GDD to sorghum biomass yield and DM yield, similar to the current study.

CONCLUSION

The two forage sorghum hybrids cultivated with three different N rates were evaluated for agronomic profiles, yield components, and nutritive profiles in Ankara. The results indicated that all agronomic profiles, yield components, and nutritive profiles were influenced by N fertilizing and its rates. But forage sorghum hybrids just affected GHY, ADF, TDN, ME, NE_P. "Hay day" was determined more nutritive in DM, ADF, NDF, TDN, ME, and NE_p compared to "Sugar-graze". In contrast to the nutritive profile, "Sugar-graze" had more GHY, DM yield, and CP yield. Forage sorghum hybrids were affected positively by applied N rates [N_0 to N_2 (0 to 180 kg ha^{-1})]. In the current study, more digestible forage was expressed by "Hayday" than "Sugar-graze" with lower ADF, NDF. In the progress of control treatment (N_0) to 180 kg ha^{-1} (N_2); digestibility and quality increased. This progress also positively affected the agronomic profiles and yield components of forage sorghum hybrids.

DECLARATION OF COMPETING INTEREST

Authors declare that they have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Ugur Ozkan: Investigation, methodology, formal analysis, writing, editing,
Nesim Yildiz: Investigation, data curation, writing,
Celal Peker: Data curation, laboratory analysis.

ETHICS COMMITTEE DECISION

This article does not require any ethical committee decision.

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