

THE EFFECT OF DIFFERENT IRRIGATION LEVEL AND NITROGEN DOSES ON THE SILAGE YIELD AND QUALITY OF SORGHUM × SUDAN GRASS HYBRID (Sorghum bicolor L. × Sorghum sudanese)

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ABSTRACT

This study aimed to determine the effects of different irrigation levels (I30, I60, and I100) and nitrogen doses (N0, N50, N100, and N150 kg ha⁻¹) on the silage yield and quality of sorghum × Sudan grass hybrids (*Sorghum bicolor* L. × *Sorghum sudanese*). The experiment was conducted via a split-plot trial design with three replicates over two years, 2021 and 2022. Silage yield was evaluated over two years, and quality traits were evaluated over one year. Silage yield, irrigation water use efficiency (IWUE), water use efficiency (WUE), dry matter, pH, organic acids (lactic and acetic), relative feed value (RFV), crude protein, potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), total phenolic and total flavonoid content, DPPH free radical scavenging activity, and condensed tannin content were determined in the silage samples. The highest silage yield was observed in the 1100×N1000 (64.3 t ha⁻¹) and 1100×N150 (61.8 t ha⁻¹) treatments. The highest WUE was obtained from the I30×N150 interaction, whereas the highest IWUE value was obtained from the I30×N150 interaction. The lactic acid and crude protein contents of the silages ranged from 2.29-4.38% and from 6.51-9.70%, respectively. As a result, the silage yield decreased, whereas the silage quality was not affected by stress conditions. Accordingly, the I100xN1000 interaction, which results in the highest silage yield, is recommended.

Keywords: Drought stress, Fertigation, Silage yield, Silage quality

INTRODUCTION

Natural resources and agricultural areas are being depleted due to global climate change and population growth. However, the need for natural resources and agricultural land is growing daily, particularly owing to population expansion. This necessitates the efficient use of agricultural land and natural resources. In particular, maintaining the continuation of human existence depends heavily on the proper use of water, which is becoming a valuable resource.

Because of global warming and fast population growth, freshwater supplies are being depleted domestically and globally. However, as a result of rising daily water demands, which are necessary for all living things to survive, the supply-demand balance is starting to breakdown. Food shortages and water crises are predicted to result from the world's rapidly growing population. Consequently, either more agricultural land must be allocated to agriculture or agricultural methods that maximize output per unit area must be adopted to fulfill the demands of the growing population. Studies should concentrate on maximizing the unit's efficiency because expanding the agricultural area is not an option.

Water efficiency has become the most important criterion, especially for the agriculture industry, which uses approximately 70% of the water worldwide. Therefore, research on agricultural practices that may be used in dry and hot climates as well as plant species and types that can withstand these circumstances has become more important in recent years, and this field of study has seen an increase in activity (Khoshouei et al. 2024). Deficit irrigation and semiwetting irrigation are two methods that may be applied to use water efficiently. According to Geerts and Raes (2009), deficit irrigation is a significant and sustainable production technique utilized in areas with limited water supplies. Reducing the quantity of irrigation water or irrigation frequency is intended to increase plant water usage efficiency. A plant undergoing restricted watering is anticipated to conserve irrigation water without noticeably reducing production since it is subjected to varying degrees of water stress at any point throughout the growing season

or during development (Kırda, 2002; Erkovan and Afacan, 2024).

One of the most important inputs for enhancing agricultural output is fertilization, along with irrigation. The use of chemical fertilizers has increased significantly in recent years worldwide, including in our own nation. Nevertheless, a portion of the fertilizers are gassed off or washed out of the soil, or they are fixed in the soil and lose their useful forms, which decreases their efficacy. Because of this, it is crucial to apply the right quantity of fertilizer to the soil for the plant rather than using too much fertilizer.

It is crucial to select plants that are suited for the right circumstances in addition to the agricultural practices that will be used. While silage and grasses that have been cut and dried from field areas are frequently utilized to meet animal feed demands during the winter, meadows and pastures serve as sources of food for animals during warmer weather. When used as silage feed, one of the plants with the highest nutritional value is maize. However, in dry and semiarid climate zones, as well as in situations where irrigation is not carried out, the high water requirements of maize during its growth and development phase result in a

decrease in productivity and a narrowing of the maize planting areas. Therefore, sorghum and its hybrids have become viable crops, especially in arid and semiarid areas, to replace maize (Tutar, 2024).

This study investigated the effects of different nitrogen doses and irrigation levels on the quality and yield of silage produced from a sorghum × Sudan grass hybrid.

MATERIALS AND METHODS

Experimental Site

Field experiments were conducted for two years, 2021 (03.06.2021) and 2022 (06.06.2022), at the University of Bilecik Seyh Edebali's application and Research Station (40° 6' N, 30°.0' E), in the province of Bilecik, Turkey. Bilecik is in a semiarid climate zone, and according to longterm climate data, the average temperature is 12°C, and the average annual rainfall is 459 mm. During the experimental plant growth period from 2021–2022, the total precipitation was 132.6 mm and 227.7 mm, respectively. The experimental area has a loamy soil texture suitable for agriculture, and some of its properties are given in Table 1.

| Table 1. Some | properties | of the | experimental | area soil |
|---------------|------------|--------|--------------|-----------|
| | | | | |

| Depth (cm) | Texture | Volume weight (g cm ⁻³) | Field capacity PW (%) | рН | Organic Matter (%) | Phosphorus P2O5 (kg ha ⁻¹) | Potassium K2O (kg ha ⁻¹) |
|---------------|---------|---|-----------------------------|------|-----------------------|--|---|
| 0-30 | CL | 1.26 | 27.87 | 7.77 | 1.18 | 267.40 | 1162.80 |
| 30-60 | L | 1.21 | 24.57 | 7.81 | 1.24 | 274.50 | 915.90 |
| 60-90 | L | 1.27 | 26.67 | 7.71 | 2.07 | 210.20 | 964.20 |

CL: Clay-loam; L: Loamy.

Experimental Design and Treatments

A sorghum × sudan grass hybrid (Sorghum bicolor L. × Sorghum sudanense "SS hybrid") was used as the crop material for the experiment. The experiment was conducted via a split-plot trial design with three replicates over two years. The plot size was $6 \times 2.8 \text{ m} (16.8 \text{ m}^2)$, the row spacing was 70 cm, the plant spacing was 5 cm, and each parcel had 4 rows. Four irrigation levels, 100% (I100), 60% (I60), and 30% (I30) 0% (I0) of the evaporation measured in Class A Pan, were placed in the main plots, and four nitrogen treatments (N0, N50, N100, and N150 kg ha⁻¹) were placed in the subplots. DAP base fertilizer was applied to all the plots at 8 kg P₂O₅ per decare during planting. After taking into account the amount of nitrogen we provided with the base fertilizer, we completed the missing amounts to 50, 100, and 150 kg per hectare and distributed them to the parcels. Irrigation was performed in 3 different critical growth periods for the plants as supplemented irrigation. Three irrigations were performed as follows: the first took place when the plants were 30 to 35 cm tall; the second was performed at the start of flowering; and the third was performed when the panicle appeared. The plants were irrigated with drip irrigation. A lateral line with a dripper spacing of 20 cm and a flow rate of 4 l h⁻¹ was placed in each row. The amount of irrigation water to be applied before each irrigation event was calculated via Equation 1

according to the amount of cumulative evaporation occurring in the Class A Pan. (Kanber, 1984).

$I = A \times kcp \times Ep \times P$

where I is the irrigation amount (mm), A is the parcel area (m^2) , kcp is the plant-pan coefficient (0, 0.30, 0.60, 1.00). Ep is the Class A Pan's total cumulative evaporation amount (mm), and P is the percentage of vegetation.

The soil water content was measured gravimetrically from 0.3 m depth to 1.2 m depth throughout the growing season. Crop evapotranspiration was calculated via a water balance equation (James, 1988).

$ETa = I + P - Dp - Rf \pm \Delta S$

where I is the irrigation amount (mm), P is the seasonal amount of precipitation (mm), Dp deep penetration is considered 0 (mm), Rf is the amount of surface runoff and ΔS is the change in soil moisture content between planting and harvest (mm). The P value in the equation is obtained from the Meteorological Station at Bilecik State. Deep penetration and surface runoff were considered insignificant because the irrigation water quantity was regulated.

The plants were harvested at the milk dough stage. Two cuttings were taken in both years. Therefore, silage yields are given as the sum of two harvests. In this study, silage yield was evaluated over two years, and quality traits were evaluated over a single year.

Measurements and analysis

Silage yield, preparation, ensiling, and silo opening

Following the harvest and weighing of the plants, the yield of green forage was computed as t ha⁻¹ on the basis of the fresh weight. The silage yield was calculated by reducing the green forage yield by 25%. The gathered plants were cut into 2 cm pieces and then packed into vacuum silage bags. The silages were stored under controlled conditions at $25 \pm 2^{\circ}$ C and opened after 45 days, and the necessary analyses were performed.

Water productivity

The most important indicators used to explain plant water yield relationships are water productivity and irrigation water productivity values. The irrigation water productivity shows the yield values obtained per unit of water applied to the plant, and the water productivity shows the yield values obtained against the seasonal plant water consumption. These values were calculated from the equations determined by Howell et al. (1990):

$$IWUE: \frac{Y}{I}$$
$$WUE: \frac{Y}{ETa}$$

where IWUE refers to the irrigation water use efficiency (kg m⁻³), Y refers to the yield (kg da⁻¹), I refers to the volume of seasonal irrigation water applied (m³ da⁻¹), WUE refers to the water use efficiency (kg m⁻³), and ETa refers to the actual seasonal evapotranspiration (m³ da⁻¹).

Dry matter and pH

The dry matter ratio (DM) (%) was computed after the silage samples were dried for 48 hours at 105°C in a hot-air oven. A digital pH meter was used to measure the pH of the silage samples.

Organic acid analyses

An electric blender was used to blend the 20-gram silage sample from the silage bags for 30 minutes, after which it was filtered. The mixture contained 100 mL of distilled water. Organic acid analyses (lactic acid, acetic acid and butyric acid) were performed via HPLC.

Crude protein

To determine the amount of crude protein, the nitrogen ratios of the samples were first determined via the Kjeldahl apparatus. The amount of crude protein was calculated by multiplying these determined nitrogen concentrations by the coefficient of 6.25 (FOSS 984.13).

Acid deterh-gent fiber (ADF), neutral detergent fiber (NDF) and mineral content analyses

The levels of ADF, NDF, and minerals (potassium, phosphorus, calcium, and magnesium) were measured via near-infrared reflectance spectroscopy (NIRS, 'Foss 6500').

Relative feed value (RFV)

The RFV was calculated via the following formulas from Rohweder et al. (1978).

Dry matter intake % (DMI) = 120/NDF

Digestibility of dry matter % (DDM) = $88.9 - (0.779 \times ADF)$

Relative feed value (RFV) = (DDM * DMI)/1.29

Total phenolic contents

The Folin–Ciocalteu technique (Singleton et al., 1999) was used to quantify the total phenolic content (TPC) in the SS hybrid extracts. Specifically, 200 μ L of each chicory extract was combined with 0.2 mL of Folin-Ciocalteu solution and 9 mL of distilled water. Finally, the volume was adjusted to 10 mL by adding 0.6 mL of a 20% sodium carbonate solution. Absorbance readings at 760 nm were taken after the combinations were incubated at room temperature in the dark for two hours. The data are presented as mg of gallic acid equivalent (GAE) per gram of extract. Nine distinct concentrations of the gallic acid standard were used to generate the calibration curve (y = 0.004x - 0.0138; R2 = 0.9995).

Total flavonoid content

The technique used by Arvouet-Grand et al. (1994) was slightly modified to assess the total flavonoid content (TFC) of the extracts. One milliliter of potassium acetate (1 M) and one milliliter of aluminum nitrate (10%) were combined with each 200 μ L of SS extract. Five milliliters, or 99% ethanol, was used to adjust the final volume. The absorbance was measured at 417 nm after the mixture was incubated for 40 minutes in the dark at room temperature. The quercetin standard's calibration curve (y = 0.0367x + 0.0003; R2 = 0.9900) was used to determine the extracts' total flavonoid content in μ g of quercetin equivalent (QE).

DPPH free radical scavenging activity

Yaman et al. (2020) examined how SS hybrid silage extracts affect the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. A 3.2 mL solution of DPPH in 0.004% methanol was mixed with 200 μ L of the sample. Absorbance measurements at 517 nm were taken after the combinations were allowed to stand at room temperature for 30 minutes in the dark.

Condensed tannins

The analysis of condensed tannins was performed in accordance with Bate-Smith (1975). A total of 0.01 g of the pulverized material was combined with 6 ml of the tannin mixture, placed in a tube, and vortexed. The samples were allowed to cool after the tubes were securely sealed and heated to 100°C for one hour. The samples were then measured with a spectrophotometer at 550 nm for absorbance. This formula was used to compute condensed tannins (CTs): dry weight (%)/absorbance (550 nm × 156.5 × dilution factor).

Statistical analysis

The yield and quality parameters were subjected to analysis of variance (ANOVA) via the Minitab 19 package program. An F test was performed to determine the significance of irrigation and nitrogen dose. In applications where the F test was significant, Tukey's (p<0.05) multiple comparison test was applied to irrigation, nitrogen dose and interactions.

RESULTS AND DISCUSSION

Variance analysis

The results of the variance analysis and significance levels of the effects of different irrigation levels and nitrogen doses on the quality traits of the sorghum–sudan grass hybrid are presented in Table 2. The effects of irrigation level on crude protein and nitrogen dose on total flavonoids were found to be statistically insignificant. However, the effects of irrigation level and nitrogen dose on other traits were found to be significant. Additionally, the interaction between irrigation level and nitrogen dose had a significant effect on all the examined traits.

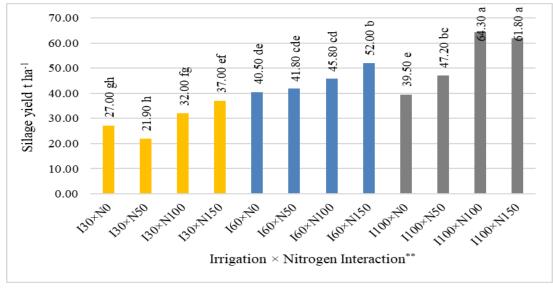
Table 2. Analysis of variance for SS hybrid silage under different irrigation levels and nitrogen doses

| | Mean of squares | | | | | | | | | | | | | | |
|-----------|-----------------|---------|---------|---------|---------|---------|----------|----------|---------|----------|----------|-----------|----------|-----------|----------|
| | DF | DM | рН | LA | AA | СР | RFV | Р | K | Mg | Ca | ТР | TF | DPPH | СТ |
| Rep | 2 | 0.686 | 0.00016 | 0.00172 | 0.00316 | 0.0432 | 1.914 | 0.00004 | 0.0026 | 0.0048 | 0.00067 | 8.338 | 0.091 | 0.00026 | 0.00006 |
| IL | 2 | 8.515** | 0.039** | 7.271** | 0.021* | 4.624 | 339.81** | 0.0068** | 2.530** | 0.0002* | 0.0038** | 817.747** | 16.692** | 2799.26** | 0.0582** |
| Error (1) | 4 | 0.209 | 0.0015 | 0.0035 | 0.0027 | 0.974 | 0.487 | 0.0003 | 0.00041 | 0.000024 | 0.000038 | Eyl.67 | 0.061 | 17.997 | 0.0006 |
| ND | 3 | 4.103** | 0.125** | 0.465** | 0.061** | 2.986** | 147.82** | 0.0037** | 0.047** | 0.0063** | 0.041** | 418.723** | 0.436 | 178.495** | 0.043** |
| IL×ND | 6 | 7.028** | 0.033** | 0.571** | 0.034** | 2.283** | 95.438** | 0.0016** | 0.360** | 0.0022** | 0.0064** | 204.625** | 7.179** | 71.113** | 0.079** |
| Error (2) | 18 | 2.168 | 0.031 | 0.061 | 0.021 | 5.008 | 31.812 | 0.0049 | 0.0567 | 0.0015 | 0.0099 | 135.065 | 2.534 | 132.663 | 0.02 |
| CV | - | 1.00% | 1.00% | 1.77% | 7.04% | 6.72% | 1.38% | 5.41% | 2.89% | 3.72% | 7.97% | 3.58% | 5.59% | 4.24% | 4.56% |

*: P≤0.05; **: P≤0.01; IL: Irrigation level; ND: Nitrogen dose; IL×ND: Irrigation level and nitrogen dose interaction; DF: Different degrees of freedom; DM: Dry matter; LA: Lactic acid; AA: Acetic acid; CP: Crude protein; RFV: Relative feed value; TP: Total phenolics; TF: Total flavonoids; CT: Condenced tannins

Silage yield

The irrigation level, nitrogen dose, interaction and year had significant (p<0.01) effects on the silage yield. According to the interactions, the silage yield of the SS hybrid ranged from 21.9 to 64.3 t ha⁻¹. The I100 × N100 and I100 × N150 treatments resulted in the highest silage yield, whereas the I30 × N50 treatment resulted in the lowest silage yield (Figure 1). This situation shows that the interaction effect between the irrigation level and nitrogen dose is strong. High irrigation levels eliminated the effectiveness of low nitrogen doses. Indeed, high irrigation levels and high nitrogen doses were more effective and increased the silage yield. Increasing the irrigation level and nitrogen dose alone increased the silage yield. However, although N100 and N150 resulted in significantly different groups, they presented similar values.



*: P≤0.05; **: P≤0.01

Figure 1. Silage yield of the SS hybrid according to the interaction

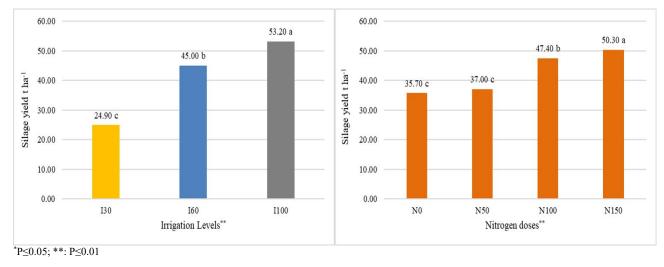


Figure 2. Silage yield of the SS hybrid according to the irrigation level and nitrogen dose

Figure 2 shows that the silage yield increased with increasing nitrogen dose and irrigation water level. While the yield differences between nitrogen doses were relatively small, the differences were significantly greater across the various irrigation water levels. Research has shown that nitrogenous fertilizer typically increases plant production, although these improvements are not consistent (Subedi and Ma 2005; Islam et al. 2010). According to

Kiziloglu et al. (2009), water deficiencies resulted in a lower output of green herbage. In a study that examined the effects of different irrigation levels and nitrogen doses on maize yield, Kaplan et al. (2016) reported that the herbage yield of maize ranged from 48.9 to 80.9 t ha⁻¹. Nematpour et al. (2021) reported that yield decreased significantly under water stress conditions.

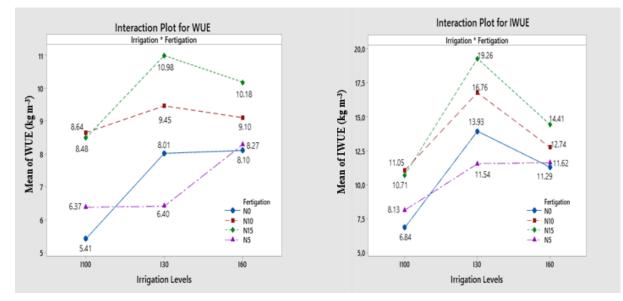


Figure 3. WUE and IWUE values of the SS hybrid silage yield

Water-producing SS hybrid silage

WUE and IWUE are crucial metrics for assessing irrigation practices. The WUE and IWUE values are given in Figure 3. The values varied according to the water levels used for irrigation. The lowest WUE values were obtained from the I100×N0 interaction, whereas the highest WUE values were obtained from the I30×N150 interaction. The lowest IWUE values were obtained from the I100×N0 interaction, whereas the highest values were obtained from the I30×N150 interaction. When we evaluated the averages of irrigation subjects separately, the highest IWUE value was obtained from the I30 irrigation subject, whereas the highest WUE value was obtained from the I60 irrigation subject. When we evaluated the fertilizer applications separately, the highest IWUE and WUE values were observed for the N150 fertilizer doses (Figure 3). The WUE and IWUE values increased as the irrigation level decreased, per the two-year mean results. This outcome demonstrates that under some irrigation application restrictions, the WUE and IWUE values can increase. Deficit irrigation water may therefore be appropriate in areas where it is scarce. In other studies, researchers obtained similar results. They reported that the WUE and IWUE values increased with water shortage (Kaplan et al., 2019; Aydınsakir and Erdurmus, 2021; Farhadi et al., 2022; Khalaf et al., 2019; Akcay and Dagdelen, 2016).

Dry matter and pH of the SS hybrid silage

The pH and silage dry matter ratio of the SS hybrid were significantly (p<0.01) affected by irrigation level, nitrogen dosage, and their interaction (Tables 1 and 3). According to Ball et al. (1996), ripening duration, temperature, irrigation, fertilization, and ratios of leaves to stems may affect a cultivar's dry matter ratio. Regional climate, soil conditions, plant genetics, sowing time and cultural

practices significantly influence dry matter yield (Cacan et al., 2018). Water stress can stop the buildup of dry matter in plants by reducing nutrient transport (Kruse et al., 2008; Setter and Parra, 2010). In our study, the dry matter content decreased with increasing irrigation level and nitrogen dosage. Additionally, the present dry matter ratios (32.02–37.13%) were between 25% and 40% of the desired ratios (Panyasak and Tumwasorn, 2013). According to Filya (2001), silage should have a pH of less than 5 to prevent Enterobacteria and Clostridial spores from growing and interfering with fermentation. The pH of the SS hybrid silage ranged from 4.34--4.77 and was below the critical level. Esen et al. (2022) reported that the average dry matter ratio and pH value of sorghum silage were 24.7% and 3.90, respectively.

| Table 3. Dry matter ratio and | pH values of the | SS hybrid silage |
|-------------------------------|------------------|------------------|
| | | |

| | Dry matter ratio (%) | | | | | | | | pН | | |
|----------------|----------------------|----------|----------|---------|--------|------|---------|--------|------------|---------|-------|
| Nitrogen doses | | | | | | | | N | itrogen do | oses | |
| IL | NO | N50 | N100 | N150 | Ave. | IL | N0 | N50 | N100 | N150 | Ave. |
| 130 | 37.13a | 32.95ef | 35.59bc | 35.28bc | 35.23a | I30 | 4.73ab | 4.50ef | 4.40 fg | 4.60cde | 4.56b |
| I60 | 34.38cd | 33.74de | 34.81bcd | 35.90ab | 34.70b | I60 | 4.79a | 4.57de | 4.55de | 4.77a | 4.67a |
| I100 | 34.86bcd | 34.88bcd | 32.59ef | 32.02f | 33.58c | I100 | 4.70abc | 4.34 g | 4.70abc | 4.63bcd | 4.59b |
| Ave. | 35.45a | 33.85b | 34.33b | 34.40b | | Ave. | 4.74a | 4.47d | 4.55c | 4.66b | |

IL: Irrigation level

Organic acids of SS hybrid silage

The lactic and acetic acid contents of the SS hybrid were significantly (p<0.01) affected by irrigation level, nitrogen dosage, and their interactions (Tables 1 and 4). Butyric acid was not found in the present study. A lactic acid level greater than 2.0% is required for the development of high-

quality silage. As a result, in comparison with the crucial value, the lactic acid contents (2.29-4.38%) of the silage samples used in this investigation were rather high. On the other hand, because acetic acid signals deterioration in silage, the percentage of acetic acid in the silage should not be greater than 0.8%. In the present study, the acetic acid content (0.30--0.67%) of the silages was below this value.

Table 4. Lactic and acetic acid contents of the SS hybrid silage

| Lactic acid (%) | | | | | | | | Acetic acid (%) | | | | | |
|-----------------|-------------------|---------|--------|-------|-------|------|--------|-----------------|------------|--------|-------|--|--|
| | Nitrogen doses IL | | | | | | | Ni | trogen dos | es | | | |
| IL | NO | N50 | N100 | N150 | Ave. | IL | NO | N50 | N100 | N150 | Ave. | | |
| I30 | 3.87c | 4.29ab | 4.38a | 4.14b | 4.17a | I30 | 0.54bc | 0.54bc | 0.30e | 0.40de | 0.44b | | |
| I60 | 2.88e | 2.55 fg | 2.41gh | 3.56d | 2.85b | I60 | 0.60ab | 0.39de | 0.43cd | 0.44cd | 0.47b | | |
| I100 | 2.29 h | 2.64f | 3.41d | 2.84e | 2.79b | I100 | 0.67a | 0.37de | 0.63ab | 0.43cd | 0.52a | | |
| Ave. | 3.01d | 3.16c | 3.40b | 3.51a | | Ave. | 0.60a | 0.45b | 0.43b | 0.43b | | | |

IL: Irrigation level

Crude protein content and RFV of SS hybrid silage

The RFV of the SS hybrid was significantly (p<0.01) affected by the irrigation level, nitrogen dosage, and their interactions. The effects of nitrogen dose and its interaction with irrigation level on crude protein were found to be significant, whereas the effect of irrigation level alone was not significant. (Tables 1 and 5). The crude protein percentage of the silage samples ranged from 6.51% to 9.70% and increased as the nitrogen dose increased but decreased as the irrigation level increased. The RFV ratio is the same in this regard. The value of RFV is established via NDF and ADF. This means that RFV decreases as ADF and NDF increase. In addition, increases in the ratios of ADF and NDF make digestion more challenging, which ultimately lowers crude protein. The temperature,

irrigation, fertilization, and ratio of leaves to stems may affect the crude protein content. Water stress can stop the buildup of dry matter in plants by reducing nutrient transport; therefore, the crude protein content may decrease (Ball et al., 1996; Kruse et al., 2008; Setter and Parra, 2010). In addition, in plants, nitrogen is essential for the production of enzymes and proteins (Islam et al., 2010). In this context, nitrogen increases the protein content of plants.

The most extensively used feed quality metric in the world, relative feed value (RFV), is based on estimations of feed intake from the NDF content and digestibility from the ADF content. The RFV value, which represents the forage quality, was therefore > 151 for the beginning quality standard, 151-125 for the first quality standard, 124-103

for the second quality standard, 102-87 for the third quality standard, 86-75 for the fourth quality standard, and < 75 for the fifth quality standard. The RFV values determined in the present study revealed that silages between the fourth

and second quality classes were examined. Celik and Turk (2021) reported that the RFV of the Aneto SS hybrid was 94.55.

| Crude protein content (%) | | | | | | | | Relative feed value (RFV) | | | | | |
|---------------------------|---------|---------|---------|---------|------|------------|----------|---------------------------|-------------|---------|---------|--|--|
| Nitrogen doses | | | | | | | | Ν | itrogen dos | es | | | |
| IL | NO | N50 | N100 | N150 | Ave. | IL | NO | N50 | N100 | N150 | Ave. | | |
| 130 | 7.33b-d | 8.96ab | 9.70a | 8.22a-d | 8.55 | I30 | 95.46cd | 107.99a | 102.91b | 102.32b | 102.17a | | |
| I60 | 6.51d | 7.51bcd | 6.71d | 8.88abc | 7.40 | I60 | 91.35ef | 85.74 g | 104.21b | 101.56b | 95.72b | | |
| I100 | 7.11cd | 7.94a-d | 8.19a-d | 7.05d | 7.57 | I100 | 87.94 fg | 90.59ef | 91.86de | 96.06c | 91.61c | | |
| Ave. | 6.98b | 8.13a | 8.20a | 8.05a | | Ave. | 91.58c | 94.77b | 99.66a | 99.98a | | | |

Table 5. Crude protein content and RFV of SS hybrid silage

IL: Irrigation level

The macronutrients of the SS hybrid were significantly (p < 0.01) impacted by irrigation level, nitrogen dosage, and their interactions (Tables 1 and 6). K is crucial for maintaining an animal's water balance and osmotic pressure, whereas P is involved in every metabolic reaction and energy transfer inside the body. Calcium, the primary building block of bones and teeth, is the most common mineral in the body. In addition, the minerals that most affect a cow's ability to produce and reproduce. In the feed of dairy cows, magnesium is utilized to maintain proper

blood magnesium levels, ideal ruminal pH values (6.2– 6.5), and proper operation of the ruminal digestive processes. Consequently, at least 0.21% P, 0.8% K, 0.3% Ca, and 0.1% Mg are needed for roughage (Kidambi et al. 1993; Tekeli and Ates 2005). All the silages in the present study presented P, K, Mg and Ca contents within the acceptable range. In addition, as the irrigation level and nitrogen dose increased in the present study, the P and Mg contents of the silages increased, whereas the K and Mg contents decreased. (Table 6).

| Table 6. Macronut | rient contents | of the SS | hvbrid silage |
|-------------------|----------------|-----------|---------------|
| | | | |

| | | Pho | osphorus (| %) | | | | Ро | otassium (9 | %) | | |
|---------|--------|---------|------------|---------|-------|-------------|----------------|---------|-------------|----------|-------|--|
| | | Ni | trogen dos | es | | | Nitrogen doses | | | | | |
| IL | NO | N50 | N100 | N150 | Ave. | IL | N0 | N50 | N100 | N150 | Ave. | |
| I30 | 0.23de | 0.26d | 0.35b | 0.35b | 0.29b | I30 | 2.74a | 2.44b | 2.03ef | 1.93 fg | 2.29a | |
| I60 | 0.24de | 0.25de | 0.34bc | 0.28cd | 0.27b | I60 | 2.10de | 2.26c | 1.83 g | 2.22cd | 2.10b | |
| I100 | 0.19e | 0.24de | 0.33bc | 0.44a | 0.31a | I100 | 1.161 | 1.28hı | 1.80 g | 1.43 h | 1.42c | |
| Average | 0.22c | 0.25b | 0.34a | 0.36a | | Average | 2.00a | 1.99a | 1.89b | 1.86b | | |
| | | Ma | gnesium (| %) | | Calcium (%) | | | | | | |
| | | Ni | trogen dos | es | | | | Ni | itrogen dos | ses | | |
| IL | NO | N50 | N100 | N150 | Ave. | IL | N0 | N50 | N100 | N150 | Ave. | |
| I30 | 0.26bc | 0.24cde | 0.21f | 0.29ab | 0.25a | I30 | 0.33a | 0.34a | 0.33ab | 0.31abc | 0.33a | |
| I60 | 0.21f | 0.22ef | 0.27abc | 0.28abc | 0.24a | I60 | 0.32ab | 0.31abc | 0.28b-e | 0.30abcd | 0.30b | |
| I100 | 0.20f | 0.26bcd | 0.23def | 0.30a | 0.24a | I100 | 0.34a | 0.26de | 0.27cde | 0.24e | 0.28c | |
| Average | 0.22c | 0.24b | 0.23bc | 0.28a | | Average | 0.33a | 0.30b | 0.29bc | 0.28c | | |

IL: Irrigation level

The secondary metabolites of the SS hybrid, with the exception of total flavonoids, were significantly (p<0.01) impacted by the irrigation level and nitrogen dosage, and the effects of their interactions with the nitrogen dose on the total flavonoid content were not significant (Tables 1 and 7). When plants are under stress, they produce

secondary metabolites, which are crucial for their ability to adapt to shifting environmental conditions (Edreva et al., 2008). In the present study, the highest secondary metabolite contents were obtained from the least-irrigationlevel (I30) treatments. Plants may have been stressed and produced secondary metabolites (Table 7).

| | | Tota | al phenolic (| %) | | | Total flavonoid (%) | | | | |
|------------|-----------------------------|-----------|---------------|---------------|--------|------|----------------------|--------|------------|--------|-------|
| | Nitrogen doses | | | | | | | | trogen dos | es | |
| IL | IL NO N50 N100 N150 Ave. IL | | | | | IL | NO | N50 | N100 | N150 | Ave. |
| I30 | 86.11ab | 91.10a | 72.46cd | 83.94ab | 83.40a | I30 | 9.31a | 8.74a | 6.74bc | 6.98b | 7.94a |
| I60 | 79.21bc | 81.11b | 72.46cd | 82.72b | 78.87b | I60 | 6.83bc | 6.95b | 5.80c | 6.79bc | 6.59b |
| I100 | 54.35f | 66.10de | 62.19ef | 86.92ab | 67.39c | I100 | 3.59e | 4.69d | 6.92b | 7.16b | 5.59c |
| Ave. | 73.22c | 79.44b | 69.03d | 84.53a | | Ave. | 6.57 | 6.79 | 6.48 | 6.97 | |
| | DF | PH Radica | l Scavenging | g Activity (% | 6) | | Condensed tannin (%) | | | | |
| | | N | itrogen dose | s | | | | Ni | trogen dos | es | |
| IL | NO | N50 | N100 | N150 | Ave. | IL | NO | N50 | N100 | N150 | Ave. |
| I30 | 77.58a | 77.83a | 74.79a | 72.85a | 75.76a | I30 | 0.56f | 0.84bc | 0.94a | 0.90ab | 0.81a |
| I60 | 74.17a | 63.36bc | 70.37ab | 70.54ab | 69.61b | I60 | 0.64ef | 0.56f | 0.93ab | 0.59f | 0.68b |
| I100 | 57.32c | 35.39e | 48.03d | 46.37d | 46.77c | I100 | 0.75cd | 0.73de | 0.57f | 0.76cd | 0.70b |
| Ave. | 69.69a | 63.25b | 63.25b | 58.86c | | Ave. | 0.65d | 0.71c | 0.81a | 0.75b | |

Table 7. Secondary metabolite contents of SS hybrid silage

IL: Irrigation level

Research on the nutrition of ruminants has demonstrated the importance of secondary metabolites for the health of the rumen and the production of the animals. These substances have antibacterial and antioxidant properties and may significantly increase the quantity and quality of animals produced. Furthermore, several studies have shown the benefits of these compounds for animal production and health, rumen fermentation, and the management of nutritional stressors such as bloat and acidosis (O'Connell and Fox, 2001; Robbins, 2003; Rochfort et al., 2008; Santos Neto et al., 2009; Frozza et al., 2013; Seradj et al., 2014; Patra et al., 2006; Paula et al.,

CONLUSION

While the silage yield increased with increasing irrigation level and nitrogen dose, the silage quality decreased. Accordingly, in terms of silage yield, the I30xN100 treatment resulted in the best results in terms of silage quality, and the I60xN150 treatment resulted in the best results in terms of IWUE and WUE. As a result, the I60xN100 treatment was determined to be suitable for improving the silage yield and quality of the SS hybrid.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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2016; Lee et al., 2017). On the other hand, condensed tannins lower greenhouse gas emissions by inhibiting some protozoans and methane-producing organisms that consume hydrogen directly in the rumen. In addition, condensed tannins also have anthelmintic effects, lessening internal parasites in animals and increasing animal output. Consequently, plants must have a condensed tannin concentration of no more than 2-3% (Kumar and Singh, 1984; Bary, 1987; Luscher et al., 2016). The condensed tannin concentration in the present study was below the essential threshold, ranging between 0.56% and 0.90% in the combined years (Table 6).

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