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Water and nitrogen impacts on water use, forage yield and quality of annual ryegrass

Ihsan Serkan Varol[1✉], Beyza Ciftci[2], Engin Kaymaz[3] & Mahmut Kaplan[2]

In this study, the effects of different irrigation levels (0 (rainfed), 25, 50, 75 and 100%) and nitrogen doses (100, 150 and 200 kg ha^{-1}) on water use, morphological characteristics, herbage yield and herbage quality of annual ryegrass were investigated. Experiments were carried out in randomized blocks–split plots experimental design with 3 replications in 2021–2022 and 2022–2023 for 2 years. Applied irrigation water quantities varied between 190 and 762 mm in 2021–2022 and between 204 and 816 mm in 2022–2023. Crop water consumptions (ETc values) were calculated as between 179 and 872 mm in the first year and 170–916 mm in the second year. The highest green-dry herbage yields, plant height, plant diameter and number of tillers were obtained from IT100xN200 treatments, and the highest protein yield was obtained from IT50xN200 treatments. While ADF, NDF, ADL, crude fiber and crude ash contents increased, crude protein content decreased with increasing irrigation water levels. Crude protein and crude ash contents increased, and cell fiber content decreased with increasing nitrogen doses. Mineral contents of ryegrass increased with increasing irrigation water levels and nitrogen doses. It was concluded based on present findings that IT100xN200 treatments could be applied for herbage yield and mineral contents and IT50xN200 treatments could be applied for protein yield and quality herbage production in ryegrass production.

Keywords Annual ryegrass, Irrigation, Nitrogen, Forage yield, Feed quality

Annual ryegrass (*Lolium multiflorum* L.) is a commercially important forage crop widely grown worldwide for grass and silage production[1]. It is a fast-growing, tall, abundant forage crop that can produce abundant grass. It can be mown more than once under favorable climate conditions, has rich protein, mineral and water-soluble carbohydrate contents, is tasty, easily digestible and has high metabolic energy[2]. It has quite a positive response to fertilization under irrigated conditions, it can be winter-sown, it can efficiently be incorporated into crop rotations. Therefore, it has been increasingly grown as a forage crop worldwide[2]. It has a high potential to meet quality roughage deficit of ruminants[3].

Nitrogen plays a critical role in plant growth, development and yield formation. Sufficient soil nitrogen levels are crucial to sustain the morphological and metabolic processes of plants, including nutrient uptake, antioxidant activities, photosynthesis and respiration[4]. However, excessive use of nitrogen is a serious problem in intensive agricultural production, as it leads to soil acidification, an increase of reactive nitrogen compounds in the environment, as well as changes in the nitrogen structure of the soil and consequent ecosystem degradation[5]. Widespread use of nitrogen in modern agriculture intensively pollutes the natural environment[6].

Irrigation and fertilization are interrelated agricultural practices. Over-fertilization combined with improper irrigation can significantly increase nutrient losses through leaching and cause serious pollution of soil and water resources. Therefore, the application of nitrogen at an appropriate rate can clearly regulate plant growth and yield, and nitrogen pollution can be avoided[7]. Drought stress causes significant changes in morphological and physiological characteristics of plants, such as root development, plant height, stem diameter, and number of leaves[8,9]. Several other properties of plants such as mineral content, carbohydrate content, protein properties and cellulose content also vary with the amount of irrigation[10,11]. Climate change has altered the amount and distribution of precipitation and eventually negatively affected the dynamics and quantity of water resources[12]. Climate change has not only limited water resources but also led to other causes of drought such as high temperatures, high light intensity and dry wind, all of which accelerate evaporation from the soil[13]. Drought stress is one of the most researched environmental stresses by researchers around the world[14]. The fact that water use will decrease day by day due to global climate change[15] makes this optimization mandatory.

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This study was conducted to determine the effects of different irrigation levels and nitrogen doses on water use, forage yield and forage quality of annual ryegrass.

Materials and methods

Climate and soil characteristics of experimental site

The study was conducted in 2021–2022 and 2022–2023 in Kayseri under Central Anatolian conditions of Turkiye. Kayseri province has a semi-arid climate with an long term average annual temperature of 10.8 °C. Avarage seasonal maximum temperature is 18.0 °C and minimum temperature is 3.1 °C. The average total seasonal precipitation is 389.0 mm and the relative humidity is 63.6% (Table 1).

Experimental soils were sandy-loam in texture, low in lime and salt, slightly alkaline in pH and poor in organic matter (Table 2). The electrical conductivity of irrigation water was 242 µS cm^{-1} and pH was 7.60.

Field experiments

Annual ryegrass cultivar 'Devis' was used as plant material. The ryegrass cultivar was purchased from a commercial company for the study. The seeds were sown in 8 rows of 5 m long with a row spacing of 20 cm. The amount of seed used was set as 20 kg ha^{-1}. In order to carry out the sowing process, seedbeds were prepared using a marker, and after sowing, the seeds were covered with soil to complete the procedure. The seeds were sown on 20th of September in 2021 of the experiment and on 25th of September in2022. In addition to nitrogen fertilization based on soil analysis, triple superphosphate was applied at the rate of 100 kg ha^{-1} P_2O_5. Nitrogen fertilizers were applied three times. In 100 kg N ha^{-1} nitrogen treatment (N100), 40 kg N ha^{-1} was applied with sowing, 30 kg N ha^{-1} was applied at emergence and 30 kg N ha^{-1} was applied after the first mowing. In 150 kg N ha^{-1} nitrogen treatment (N150), 50 kg N ha^{-1} was applied with sowing, 50 kg N ha^{-1} was applied at emergence and 50 kg N ha^{-1} was applied after the first mowing. In 200 kg N ha^{-1} nitrogen treatment (N200), 60 kg N ha^{-1} was applied at sowing, 70 kg N ha^{-1} at emergence and 70 kg N ha^{-1} after the first mowing. The first fertilization was practiced by mixing with the soil at sowing and the other fertilizations were practiced through fertigation. Hoeing was done 3–4 times from the time the plants reached about 10 cm height until harvest.

	Mean temperature (℃)				Min. temperature (℃)				Max. temperature (℃)			
Months	2021	2022	2023	LT*	2021	2022	2023	LT	2021	2022	2023	LT
January	2.5	1.9	2.2	− 1.7	− 1.8	− 2.7	− 1.2	− 6.9	8.3	5.3	5.8	4.1
February	2.8	2.1	− 1.4	0.1	− 2.9	− 4.9	− 5.3	− 5.2	10.0	5.7	3.7	6.1
March	5.0	6.2	6.6	4.7	− 0.2	1.4	2.4	− 1.4	10.7	10.9	11.5	11.4
April	10.7	13.6	13.4	11.3	4.4	6.4	4.8	4.9	16.8	20.8	15.0	17.7
May	15.9	14.1	13.8	14.7	7.4	7.5	6.8	6.9	23.6	20.7	19.8	22.5
June	18.8	20.5	19.9	18.3	12.0	11.8	13.2	9.8	25.8	27.3	24.1	26.9
July	23.7	22.2	21.6	20.4	14.6	12.8	13.9	11.9	31.8	27.5	28.9	30.6
August	21.9	25.4	24.8	21.9	13.4	16.9	16.5	11.4	30.6	33.9	32.5	30.7
September	16.9	18.2	18.2	16.9	10.3	9.8	10.8	7.3	24.3	26.6	25.9	26.5
October	11.8	12.3	18.0	11.8	4.0	4.8	8.9	3.5	20.4	19.8	27.8	20.4
November	7.9	11.6	12.9	7.9	2.2	3.6	5.1	− 0.9	15.6	15.9	22.7	13.0
December	2.9	4.5	5.0	2.9	− 1.0	− 2.1	− 0.3	− 4.5	9.0	9.7	10.5	6.4
Total	–	–	–	–	–	–	–	–	–	–	–	–
Mean	11.7	12.7	12.9	10.8	5.2	5.4	6.3	3.1	18.9	18.7	19.0	18.0
	Wind speed (m s^{-1})				Precipitation (mm)				Relative humidity (%)			
Months	2021	2022	2023	LT*	2021	2022	2023	LT	2021	2022	2023	LT
January	1.9	1.7	1.7	1.1	41.2	29.1	30.4	35.2	72.0	75.9	74.7	76.6
February	1.6	1.6	1.7	1.3	7.7	21.2	3.3	36.5	64.9	67.8	68.3	74.0
March	2.2	1.6	2.6	1.6	38.3	19.8	22.4	41.8	64.6	67.7	67.6	68.3
April	2.2	1.9	1.9	1.7	22.7	15.4	37.0	52.1	63.5	67.6	64.5	62.3
May	2.1	1.5	1.7	1.7	21.3	44.9	20.3	51.8	56.4	61.8	61.8	61.2
June	2.0	1.5	1.6	1.5	37.2	52.5	44.5	39.5	53.0	59.3	65.7	55.8
July	2.4	1.6	1.5	1.4	0.0	0.6	2.7	10.5	44.6	46.9	48.0	49.5
August	1.4	1.5	2.0	1.2	17.1	0.0	7.5	8.8	53.0	42.5	35.6	49.2
September	1.6	1.6	1.8	1.1	16.7	58.1	27.3	15	59.2	52.0	47.5	54.1
October	1.4	1.0	1.4	1.0	0.2	17.9	12.2	28	53.0	66.3	49.7	63.6
November	1.3	1.2	1.2	1.0	15.7	29.7	21.5	32.4	69.8	70.4	61.8	71.7
December	1.7	1.6	1.6	1.1	13.3	30.8	9.0	37.4	72.6	76.5	71.5	77.1
Total		–	–	–	231.4	320.0	238.1	389.0		–	–	–
Mean	1.8	1.5	1.7	1.3	–	–	–	–	60.6	62.9	59.7	63.6

Table 1. Meteorological data of the experimental area. *LT: Long Term (1965–2020).

Properties	2021–2022		2022–2023	
	0–30 cm	30–60 cm	0–30 cm	30–60 cm
Clay (%)	13.58	9.06	14.01	9.38
Silt (%)	5.71	10.8	5.62	10.22
Sand (%)	80.71	80.1	80.37	80.40
Class	Sandy-Loam	Sandy-Loam	Sandy-Loam	Sandy-Loam
pH	7.76	7.61	7.82	7.72
Organic matter (%)	1.09	1.15	1.02	1.13
$CaCO_3$ (%)	0.26	0.28	0.27	0.26
K_2O (kg ha^{-1})	1122	827	1165	858
P_2O_5 (kg ha^{-1})	104	12.38	93.78	12.94
EC (mmhos cm^{-1})	0.81	0.22	0.80	0.27
Field capacity (P_w) (%)	23.64	23.76	23.68	23.76
Wilting point (P_w) (%)	12.55	11.23	11.76	11.23
Bulk density (g cm^{-3})	1.27	1.23	1.28	1.24

Table 2. Physical and chemical characteristics of soils of the experimental site.

Irrigation treatments

Drip irrigation system was used for irrigation of annual ryegrass plants. A PE (Polyethylene) lateral pipeline with 16 mm outer diameter and 1 atm operating pressure was laid in 8 rows, 20 cm apart, one for each plant row. In-line drippers were used on the lateral pipeline with a flow rate of 2 l h^{-1} and 25 cm spacing to obtain at least 100% wetted area ratio according to soil characteristics[16]. A water meter was placed at the beginning of each block to measure the amount of irrigation water. Irrigation scheduling was done on a weekly basis to bring the moisture deficit at the effective root depth to the field capacity moisture level. Moisture in the plots was monitored at 7-day intervals to a depth of 90 cm along the soil profile by means of a neutron meter.

Five different irrigation levels were arranged based on field capacity: 0% (rainfed - IT0), 25% (IT25), 50% (IT50), 75% (IT75) and 100% (IT100). Three different nitrogen doses were used as 100–N100, 150–N150 and 200–N200 kg ha^{-1} N.

Irrigations were started when the plants emerged from winter dormancy and started to grow. Hoeing was carried out to control weeds during the growth period of the plants. Morphological observations were taken during the 10% flowering period and the yield was determined by harvesting after removing the side effects.

Amount of applied irrigation water (mm) was determined with the use of Eq. (1);

$$d = \frac{(Pwfc - Pwm) \bullet \gamma}{100} \bullet D \bullet P \tag{1}$$

where; d: Applied irrigation water quantity (mm), P_{wfc}: Field capacity (%), P_{wm}: Permanent wilting point (%), γ: Soil bulk density (g cm^{-3}), D: Soil depth to be wetted (mm), P: Wetted area ratio.

Water budget equation given in James[17] was used to determine crop water consumption (ET) values of experimental treatments (Eq. 2);

$$ET = I + R + Cr - Dp - Rf \pm \Delta s \tag{2}$$

where; ET: Crop water consumption (mm), I: Irrigation water (mm), R: Effective rainfall (mm), C_r: Capillary rise (mm), D_p: Deep percolation (mm), R_f: Runoff (mm), Δs: Change in moisture along the soil profile (mm).

Stewart model[18] was used to assess the relationships between relative crop water consumption deficit and relative yield reduction.

$$\left(1 - \frac{Y_a}{Y_m}\right) = k_y \times \left(1 - \frac{ET_a}{ET_m}\right) \tag{3}$$

where; Y_a: Actual yield (kg ha^{-1}), Y_m: Maximum yield (kg ha^{-1}), k_y: Yield response factor, ET_a: Actual crop water consumption (mm), ET_m: Maximum water consumption (mm).

Crop water productivity values were determined with the use the equations given in Howell et al.[19] (Eqs. 4 and 5):

$$CWP = \frac{Y}{ET} \times 100 \tag{4}$$

$$IWP = \frac{Y}{I} \times 100 \tag{5}$$

$$NPFP = \frac{Y}{N} \times 100 \tag{6}$$

where; CWP: Crop water productivity (kg m^{-3}), IWP: Irrigation water productivity (kg m^{-3}), I: Irrigation water (mm), NPFP: Nitrogen partial factor productivity (kg kg^{-1} N), ET: Crop water consumption (mm), Y: Yield (kg ha^{-1}), and N: Nitrogen application rate (kg ha^{-1}).

Morphological observations

Plants were harvested at the beginning of flowering after taking morphological observations. In harvests, two rows and 50 cm at the beginning and bottom of each plot were considered side effects and observations, and green herbage yields (kg ha^{-1}) were taken from the remaining part of the plots. For plant height (cm), number of tillers, and plant diameter (mm), 10 randomly selected plants from each plot were used, and the average was taken. Plant height was determined by measuring the plants from the bottom to the tip. To determine the plant diameter, the main stem of the plants was measured with a caliper. The dry matter ratio was determined by drying approximately 500 g of green grass samples taken from each plot at 70 °C until they reached a constant weight. Dry herbage yield (kg ha^{-1}) was determined by multiplying green herbage yield by the dry matter ratio. Crude protein yield (kg ha^{-1}) was obtained by multiplying dry herbage yield by crude protein content.

Biochemical and mineral analysis

The dried grass samples were ground using a mill equipped with a 1 mm sieve and prepared for subsequent chemical analyses. The crude ash content of the annual ryegrass samples was determined by incinerating the samples in a muffle furnace at 550 °C for 8 h[20]. Nitrogen (N) content was measured using the Kjeldahl method, and crude protein content was calculated using the formula N x 6.25[20]. Analyses for crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) were conducted following the[21]. For the determination of mineral contents such as Na, Ca, P, S, K, Mg, Fe, Mn, Ni, Cu, Zn, and B, ryegrass samples were subjected to wet digestion in a nitric acid and perchloric acid mixture. Elemental readings were subsequently performed using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) device (Agilent 5800)[22].

Statistical analysis

The experiments were arranged in a randomized block design with split plots and included three replications. Irrigation treatments were assigned to the main plots, while nitrogen doses were allocated to the sub-plots. The experimental data were analyzed using analysis of variance (ANOVA) with the SAS software package[23]. Mean differences deemed significant were further evaluated using the LSD test for comparisons.

Results

Crop water consumption and irrigation

In both years, irrigation of winter-sown annual ryegrass plants was started in the first week of April when the plants emerged from winter dormancy and started to grow. In 2022, the amount of irrigation water given to the irrigation treatments varied between 191 mm (IT25) and 762 mm (IT100). In 2021–2022 and 2022–2023, 762 and 816 mm irrigation water was given to the same treatments, respectively (Table 3). Due to the low total available water capacity of the soil, soil moisture levels of the irrigation treatments varied between readily available water (RAW) and wilting point (WP) in both years. The IT100 treatment fell below the RAW level during the periods when plant water consumption increased, but the soil moisture did not decrease enough to stress the plant in both years and remained above the WP (Fig. 1).

Plant water consumption calculations were started with soil samples taken from the moment of irrigation and the available soil moisture determined at the beginning was included in ET calculations. ETc values varied between 179 mm (IT0) and 872 mm (IT100) in the first year of the study. In the second year, it was determined as 170 mm in IT0 treatment grown under rainfed conditions, while it was calculated as 916 mm in IT100, which was the control treatment (Table 3).

CWP and IWP values were calculated separately for the average green herbage yield, dry herbage yield and protein yield of the years obtained at all three nitrogen doses. It was observed that water productivity of annual ryegrass increased with increasing nitrogen doses. Especially in IT0 grown under rainfed conditions, water productivity was high. The increase in protein ratio with stress conditions may explain this situation. However, it was seen that the plants need to be irrigated in order to obtain high yield and respond well to irrigation (Fig. 2).

NPFP for green and dry herbage yields increased with increasing irrigation water. This indicates that increasing the irrigation amount can improve the absorption and utilization of nitrogen fertilizer, but the increasing effect weakens when a certain irrigation amount is reached. Protein yield showed an increasing trend

Application	2021–2022			2022–2023		
	I (mm)	*P* (mm)	Etc (mm season^{-1})	I (mm)	*P* (mm)	Etc (mm season^{-1})
IT_0	0	113	179	0	104	170
IT_{25}	191	113	361	204	104	365
IT_{50}	381	113	521	408	104	538
IT_{75}	572	113	695	612	104	726
IT_{100}	762	113	872	816	104	916

Table 3. Irrigation, precipitation and evapotranspiration.

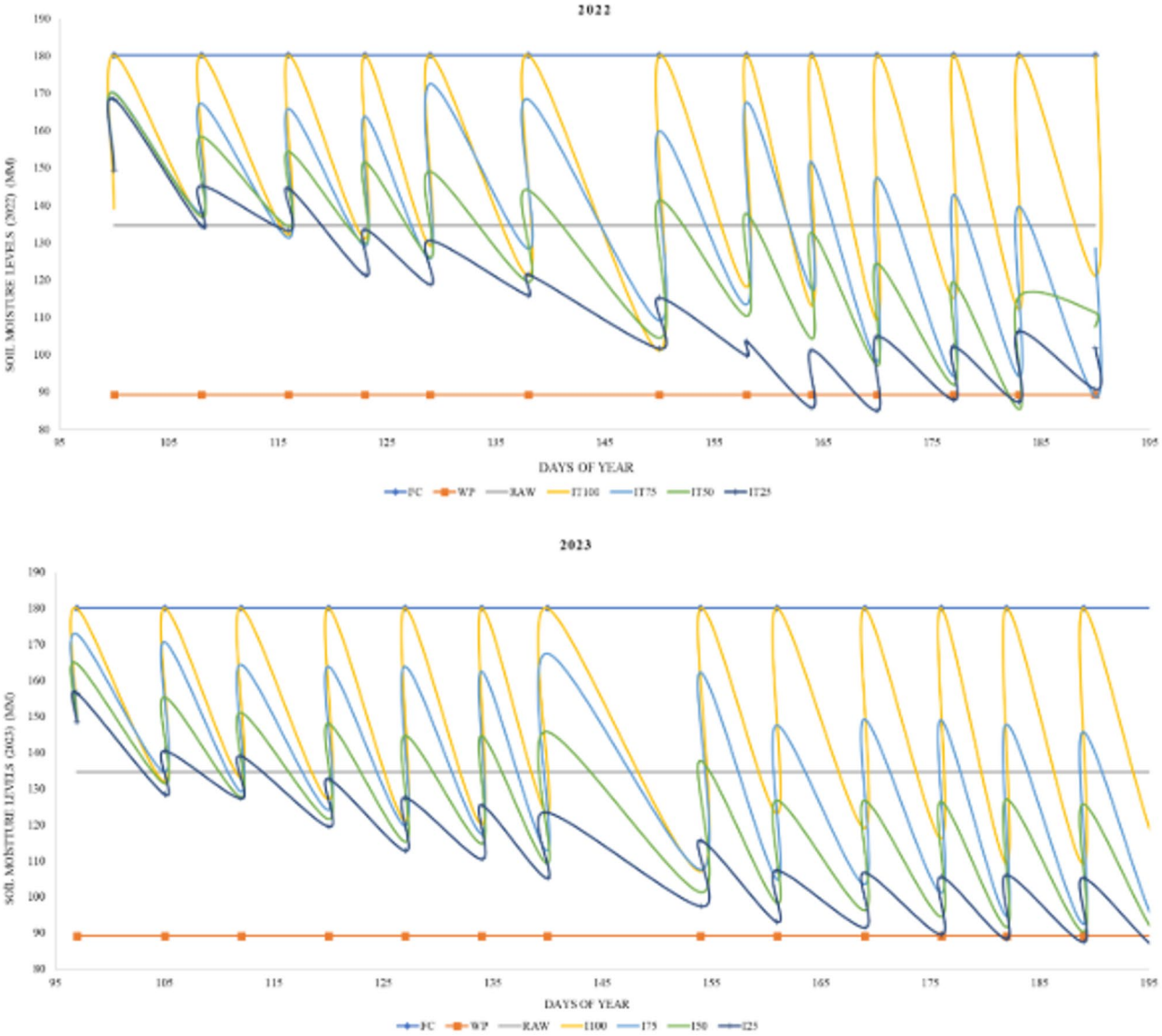


Fig. 1. Changes in soil moisture levels in 2022 and 2023 (soil moisture level monitoring started with irrigations and ended at the harvest time).

at first and then a decreasing trend. The highest NFPF value was observed in N100 treatments in all three yield groups and the rate of increase in nitrogen dose remained below the rate of increase in yield (Fig. 3).

Yield response factors (ky) obtained from two-year average values are given in Fig. 4. In general, in semi-arid climate conditions, the sensitivity of annual ryegrass plants, which is found to be tolerant to water stress, increased more from green herbage yield to dry herbage yield, while it became the most tolerant in protein yield. Yield response factors and linear R^2 relationships are given in Fig. 4.

Yields and morphological observations

The effects of irrigation treatments, nitrogen doses and irrigation x nitrogen interactions on morphological characteristics and yield values were found to be highly ($p \leq 0.01$) (Table 4). All morphological parameters increased with increasing nitrogen doses. Except for crude protein yield, all other parameters increased significantly with increasing amount of irrigation water. Green herbage yields varied between 11,533 kg ha^{-1} (IT0xN100) and 57,227 kg ha^{-1} (IT100xN200). Similarly, dry herbage yield was the lowest in IT0xN100, where the nitrogen dose was the lowest and water stress was the most intense, and remained at the level of 5000 kg ha^{-1}. The highest dry herbage yield reached 16,330 kg ha^{-1} in IT100xN200, where no stress was experienced. Stress conditions had a positive effect on crude protein yield and the highest crude protein yield was 1662 kg ha^{-1} in IT50xN200 treatment. The lowest protein yield was obtained from IT0xN100 treatment with 525 kg ha^{-1} (Table 4).

The lowest and highest values for the number of tillers were 8.58 and 26.92 in IT100xN200 and IT0xN100 treatments, respectively. The main stem thickness also reached the maximum level in IT100xN200 and reached

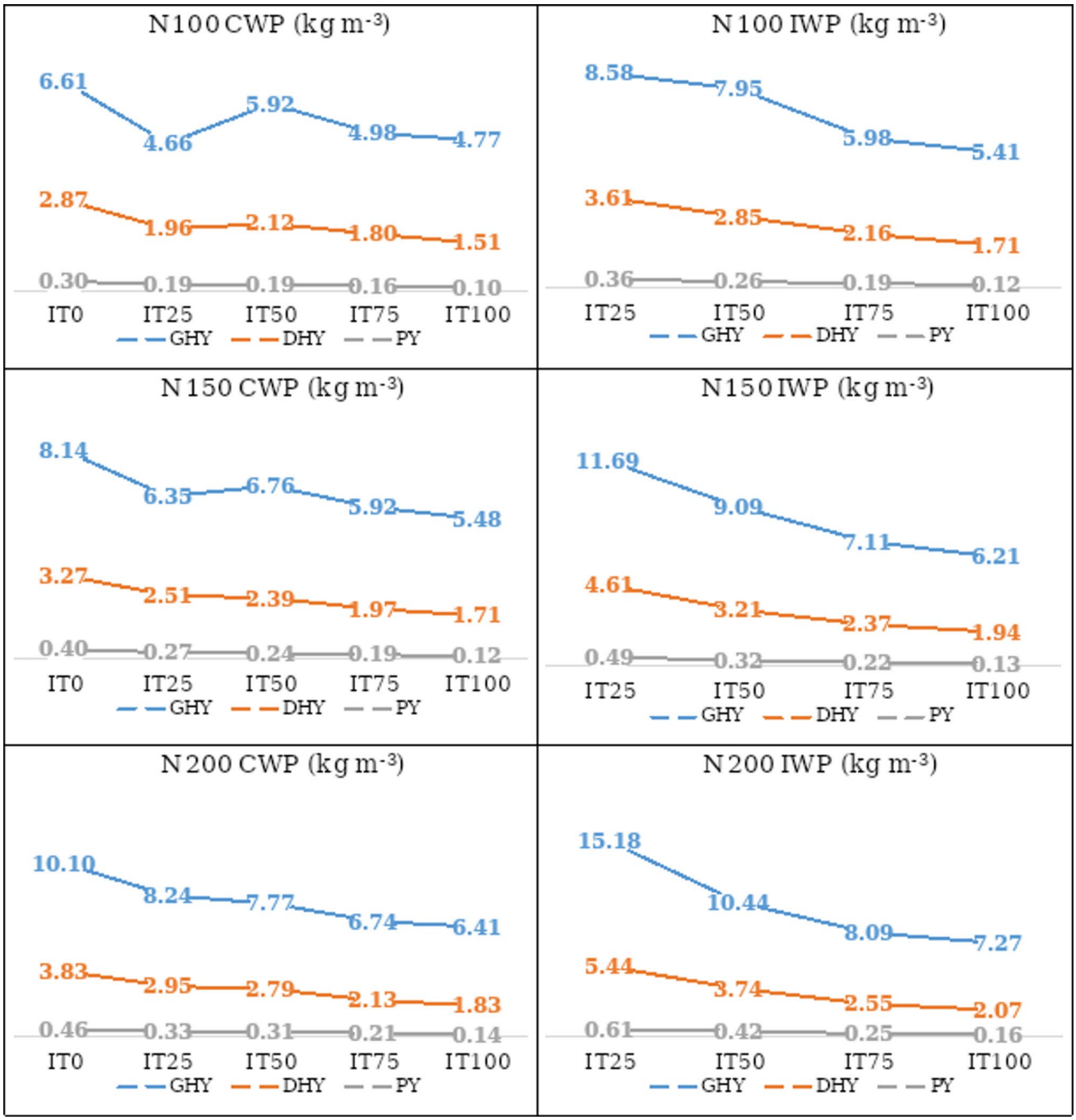


Fig. 2. CWP and IWP values of green herbage, dry herbage and protein yields (the values in figure are presented as the average of 2-year research results).

3.22 mm thickness. The lowest plant height was obtained from IT0xN100 treatment with 57.08 cm, while the highest plant height was measured as 95.00 cm in IT100xN200 treatment.

Biochemical observations

Biochemical analyses result of annual ryegrass are given in Table 5. The effects of irrigation treatments and nitrogen doses on ADF, NDF, ADL, crude fiber, crude ash and crude protein contents were found to be highly significant ($P \leq 0.01$). While the effects of irrigation × nitrogen interactions on crude fiber and crude protein contents were significant at 1% level, no significant effect was found on other biochemical properties.

ADF, NDF, ADL and crude ash contents increased with increasing irrigation water levels and the highest values were 41.27%, 61.36%, 7.46% and 10.45%, respectively, in IT100 treatments. The lowest values were observed in IT0 treatments. The opposite was observed in the crude protein content and the protein ratio increased with increasing water stress. The highest crude protein content was 11.82% in IT0xN200 and the lowest was 6.96%

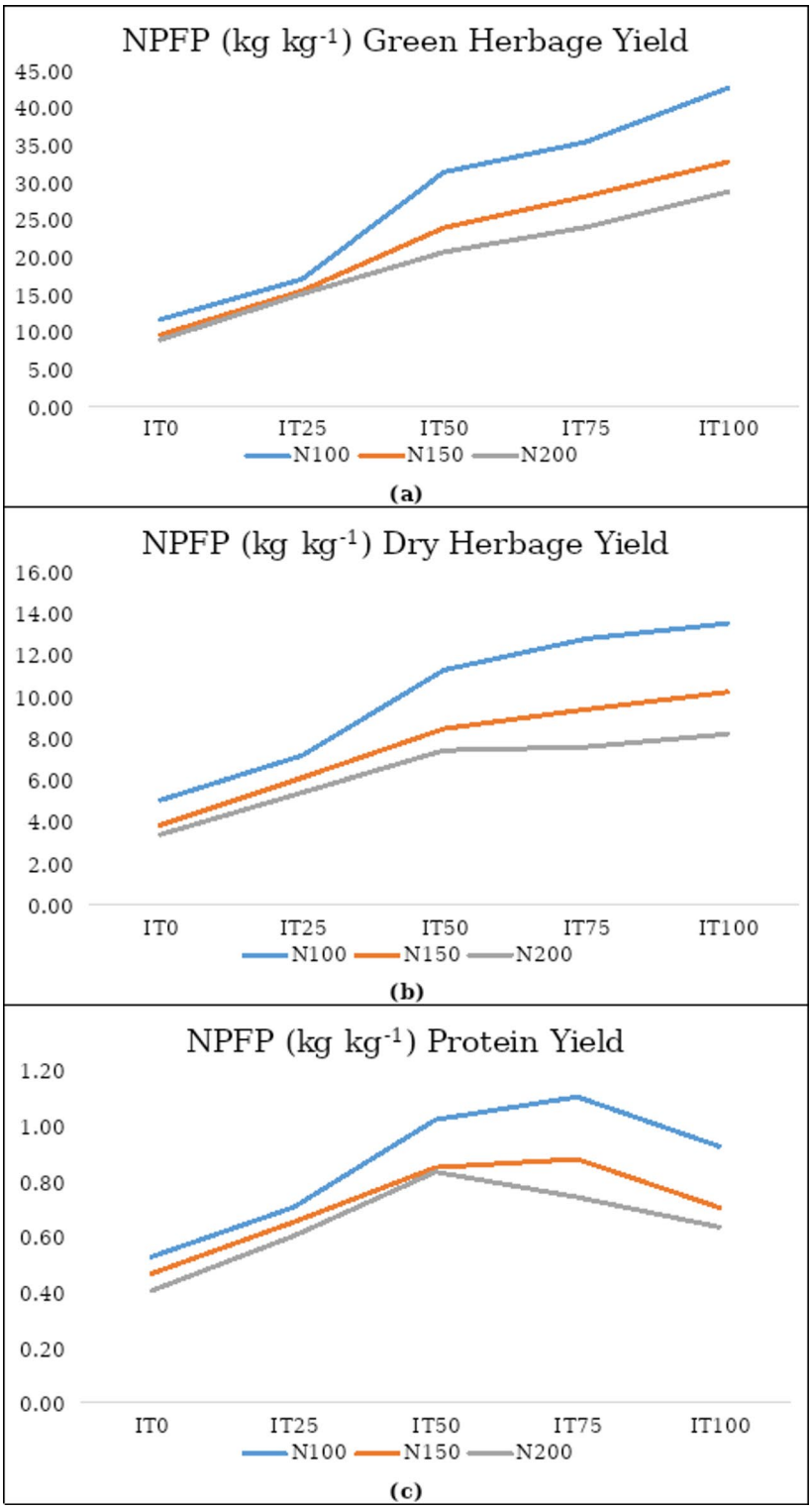


Fig. 3. Effects of different irrigation treatments and nitrogen doses on nitrogen partial factor productivity (*NPFP*) of *Lolium multiflorum* (The values in figure are presented as the average of 2-year research results).

in IT100xN100 treatments. The crude fiber content reached the highest level (32.99%) in IT100xN100 and the lowest level (24.96%) in IT0xN200 treatments.

Mineral content of herbage

The mean values for mineral contents of the samples are given in Table 6. Effects of irrigation treatments, nitrogen doses and water x nitrogen interactions on all minerals were found to be highly significant ($P \leq 0.01$).

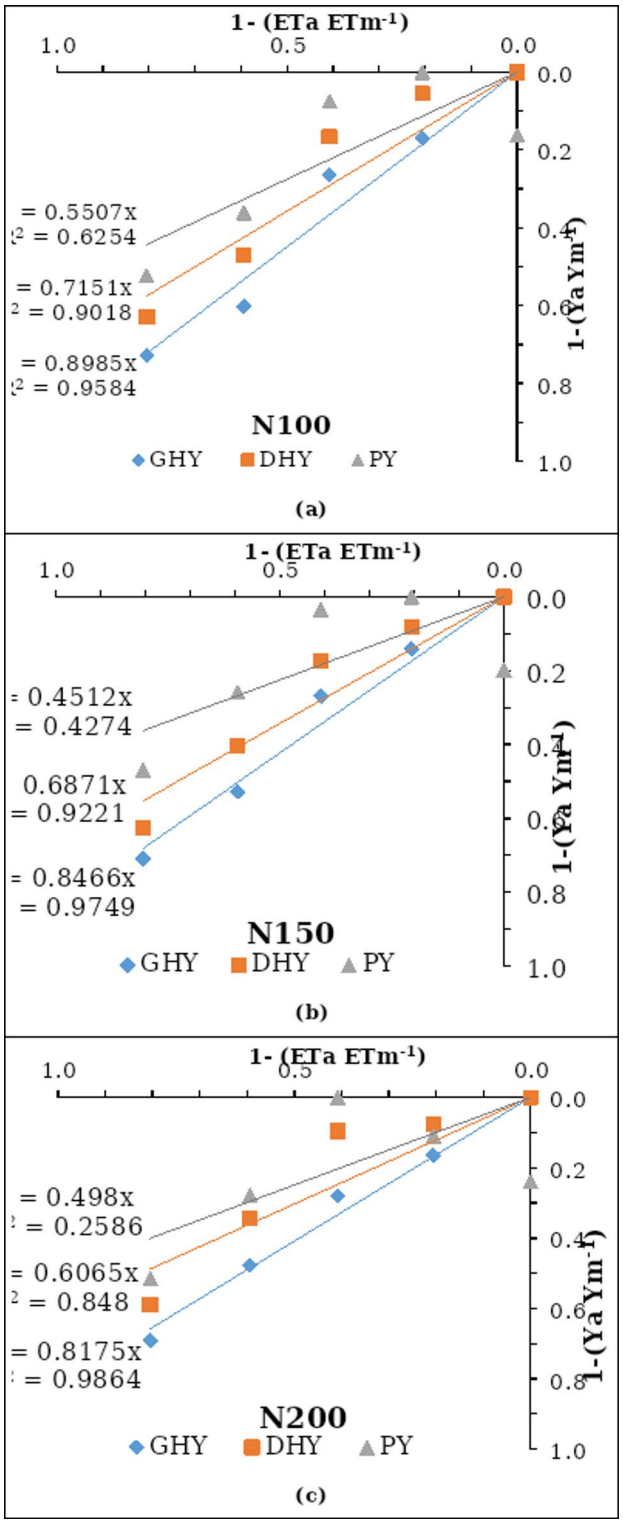


Fig. 4. Yield response factors (ky) of green herbage, dry herbage and protein yields (*GHY* Green herbage yield, *DHY* Dry herbage yield, *Py* Protein yield) (The values in figure are presented as the average of 2-year research results).

The increase in nitrogen dose caused significant increases in all minerals, except for Na. While the change in irrigation level did not cause a regular change in Ca and Na, the decrease in stress level increased the other minerals. The lowest contents of K, Mg, P, S, B, Cu, Fe, Mn, Zn and Ni were found as 4130.76, 770.22, 1262.38, 673.73, 5.77, 5.23, 57.16, 41.73, 18.31 and 1.50 mg kg^{-1}, respectively, in IT0xN100, while the highest values were found in IT100xN200 treatments. The highest Ca content was obtained from IT75xN200 (4406.89 mg kg^{-1}) and

Number of tillers					Main stem thickness (mm)				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	8.58^{j}	10.17^{ij}	11.42^{hi}	10.06^{e}	IT0	1.24^{j}	1.35^{ij}	1.57^{hi}	1.39^{e}
IT25	10.17^{ij}	12.08^{gh}	13.00^{fg}	11.75^{d}	IT25	1.55^{h}	1.89^{g}	2.16^{ef}	1.87^{d}
IT50	12.25^{fgh}	13.75^{ef}	15.25^{d}	13.75^{c}	IT50	1.92^{fg}	2.26^{de}	2.56^{c}	2.25^{c}
IT75	14.50^{fg}	19.08^{c}	21.17^{b}	18.25^{b}	IT75	2.32^{d}	2.71^{c}	2.97^{ab}	2.67^{b}
IT100	16.25^{de}	23.67^{b}	26.92^{a}	22.28^{a}	IT100	2.63^{c}	3.05^{b}	3.22^{a}	2.97^{a}
Mean	12.35^{c}	15.75b	17.55^{a}		Mean	1.93^{c}	2.25^{b}	2.49^{a}	

Plant height (cm)					Green herbage yield (kg ha^{-1})				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	57.08^{j}	61.50^{i}	63.75^{i}	60.78^{e}	IT0	11,533^{i}	14,217^{hi}	17,653^{h}	14,468^{e}
IT25	67.17^{h}	73.83^{f}	79.17^{d}	73.39^{d}	IT25	16,939^{g}	23,077^{f}	29,919^{e}	23,312^{d}
IT50	70.17^{g}	76.00^{e}	83.25^{c}	76.47^{c}	IT50	31,286^{e}	35,745^{d}	41,137^{c}	36,056^{c}
IT75	77.83^{e}	85.25^{c}	91.17^{b}	84.75^{b}	IT75	35,325^{d}	42,058^{c}	47,799^{b}	41,728^{b}
IT100	83.83^{c}	91.42^{b}	95.00^{a}	90.08^{a}	IT100	42,520^{c}	48,938^{b}	57,227^{a}	49,562^{a}
Mean	71.22^{c}	77.60^{b}	82.47^{a}		Mean	27,521^{c}	32,807^{b}	38,747^{a}	

Dry herbage yield (kg ha^{-1})					Crude protein yield (kg ha^{-1})				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	5000^{g}	5714^{g}	6696^{g}	5804^{e}	IT0	525^{i}	697^{hi}	806^{ghi}	676^{c}
IT25	7129^{f}	9106^{e}	10,732^{e}	8989^{d}	IT25	704^{fgh}	975^{def}	1200^{cd}	959^{b}
IT50	11,237^{de}	12,626^{cd}	14,762^{b}	12,875^{c}	IT50	1018^{efg}	1270^{cd}	1662^{a}	1317^{a}
IT75	12,736^{c}	14,016^{b}	15,086^{ab}	13,944^{b}	IT75	1101^{def}	1315^{bc}	1479^{ab}	1299^{a}
IT100	13,472^{c}	15,268^{ab}	16,330^{a}	15,024^{a}	IT100	923^{def}	1055de	1264^{bc}	1080^{b}
Mean	9915^{c}	11,346^{b}	12,720^{a}		Mean	854^{c}	1062^{b}	1282^{a}	

Table 4. Morphological properties and herbage yield of *Lolium multiflorum* under different irrigation levels and nitrogen doses. *The values in Table 4 are presented as the average of two-year research results.

the lowest in IT0xN100 (3469.17 mg kg^{-1}) treatments. Sodium content reached the highest level with 566.77 mg kg^{-1} in IT25xN150 and the lowest level with 355.27 mg kg^{-1} in IT0xN200 treatments (Table 6).

Discussion

In this study, different irrigation levels and nitrogen doses were applied and morphological characteristics, green-dry herbage yield, quality characteristics and mineral content of annual ryegrass plants were determined. The effects of the treatments on the analyzed traits were found to be significant. Previous studies on forage crops revealed that environmental conditions, agricultural techniques, irrigation methods, genotype and climate parameters altered the crop water consumptions[24]. Water consumption of annual grass may vary with the length of growth period and climate conditions. Complying with the values of Neal et al.[25], present plant water consumption values varied between 872 and 916 mm. Critical decreases in soil moisture level occurred in this study. Researchers have stated that the root system of annual grass plants in sandy-loam soil can go down to a depth of 80 cm[26] and this may explain the depletion of moisture in the soil, especially under water-stressed conditions. CWP values are usually influenced by climate, plant species, plant variety, fertilization and irrigation practices[25]. While irrigation decreases the CWP value, N applications increase this CWP value[27]. A similar case was found to be related to the IWP value of the present study. Present CWP values were similar to those obtained under semi-arid conditions[28] but were lower than those obtained in sub-tropical regions[29]. The higher IWP obtained in sub-tropical environments is probably a result of the lower vapor pressure deficit in these environments. It is known that an increase in the amount of irrigation water increases the NPFP value[30,31]. In the present study, an increase in the amount of irrigation water increased the NPFP value as it was in green and dry herbage yields. However, while some increase in protein yield was observed at first, the NPFP value decreased slightly with increasing irrigation water levels. Such a case can be attributed to decreasing protein values under water-stressed conditions.

Yield decreases with increasing water stress in forage crops[24,32]. In the study, the values of morphological characteristics such as plant height, number of tillers and stem diameter decreased with decreasing water and nitrogen doses, which caused a decrease in green and dry herbage yield (Table 4). The morphological characteristics, yield and herbage quality characteristics and mineral contents obtained from annual ryegrass were similar to the results of previous studies on annual ryegrass[33–35].

It was reported that plant height and number of leaves, leaf area, leaf dry weight, stem diameters and herbage yields increased with increasing nitrogen doses[9]. However, it is known that the increase in plant height is not continuous, and the lack of sufficient nitrogen in the soil causes shortening of plant height and decreases in

ADF (%)					NDF (%)				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	36.81	36.30	34.99	36.56^{e}	IT0	55.24	54.23	53.73	54.40^{e}
IT25	38.78	38.10	36.50	37.79^{d}	IT25	56.82	55.89	54.82	55.84^{d}
IT50	39.85	38.78	37.90	38.84^{c}	IT50	58.92	58.28	57.45	57.45^{c}
IT75	42.14	40.23	39.54	40.63^{b}	IT75	61.29	59.77	59.09	60.05^{b}
IT100	42.33	41.02	40.47	41.27^{a}	IT100	62.14	61.67	60.26	61.36^{a}
Mean	39.98^{a}	38.88^{b}	37.88^{c}		Mean	58.88^{a}	57.97^{b}	57.07^{c}	

ADL (%)					Crude fiber (%)				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	6.44	6.32	5.95	6.38^{e}	IT0	27.31^{g}	26.24^{h}	24.96^{i}	26.17^{e}
IT25	6.94	6.75	6.34	6.84^{d}	IT25	28.76^{ef}	28.04^{fg}	26.30^{h}	27.70^{d}
IT50	7.24	6.96	6.78	6.87^{c}	IT50	29.84^{d}	29.37^{de}	28.28^{f}	29.16^{c}
IT75	7.59	7.24	7.01	7.41^{b}	IT75	31.89^{b}	30.86^{c}	29.69^{d}	30.81^{b}
IT100	7.72	7.43	7.23	7.46^{a}	IT100	32.99^{a}	32.41^{ab}	32.16^{b}	32.52^{a}
Mean	7.19^{a}	6.94^{b}	6.66^{c}		Mean	30.16^{a}	29.38^{b}	28.28^{c}	

Crude ash (%)					Crude protein (%)				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	6.51	6.80	7.38	6.90^{e}	IT0	10.36^{cd}	11.19^{ab}	11.82^{a}	11.12^{a}
IT25	7.60	8.15	8.75	8.17^{d}	IT25	9.74^{de}	10.43^{c}	10.88^{bc}	10.35^{b}
IT50	8.45	8.71	9.24	8.80^{c}	IT50	8.85^{gh}	9.64^{ef}	10.54^{bc}	9.68^{c}
IT75	9.04	9.55	9.93	9.51^{b}	IT75	8.49^{h}	9.04^{fgh}	9.38^{efg}	8.97^{d}
IT100	10.03	10.44	10.86	10.45^{a}	IT100	6.96^{i}	7.08^{i}	7.49^{i}	7.17^{e}
Mean	8.33^{c}	8.73^{b}	9.23^{a}		Mean	8.88^{c}	9.48^{b}	10.02^{a}	

Table 5. Biochemical properties of *Lolium multiflorum* under different irrigation levels and nitrogen doses. *The values in Table 5 are presented as the average of 2-year research results.

biomass yield[36]. Nitrogen deficiency in forage crops negatively affects the vegetative structure and causes the plant to grow undersized[37]. When the plant cannot find the nitrogen it needs in the environment, it is forced to bloom and the development period is shortened. This situation causes yield losses. In dry conditions, the movement of nutrients toward the plant's root system is restricted, thereby limiting nutrient uptake and ultimately reducing dry matter production[38]. Despite this, drought conditions have been observed to delay plant maturity while increasing the leaf-to-stem weight ratio, resulting in an enhancement of herbage quality[39]. Additionally, plant height and stem diameter are recognized as sensitive indicators of water availability and are reported to play a crucial role in determining herbage yield[40]. Present findings are in agreement with the researchers who reported that water stress negatively affected leaf and stem elongation and limited root development[41]. In plants under water stress, dry matter accumulation stops as a result of reduced nutrient transport[42]. Dry matter content increases with increasing water content and decreases with increasing nitrogen doses, as reported by Yosef et al.[25] and Turgut et al.[44]. This can be said to be the reason for the lack of an expected increase in dry herbage yield.

Crude protein is a key determinant of feed quality and plays a vital role in animal nutrition. It is a critical parameter for assessing the quality of roughages[45,46]. Variations in dry matter and protein contents among plant varieties have been attributed to genetic factors and are influenced by leaf, spike, and stem ratios, ripening stages, irrigation practices, temperature, and fertilization[8,47]. Nitrogen, as an essential element for protein and enzyme synthesis, is fundamental to plant metabolism. Enzymes regulate all metabolic processes in plants, while nitrogen is also an integral part of chlorophyll synthesis, enabling the absorption of light energy necessary for photosynthesis[48]. Under water stress, stomatal closure reduces carbon dioxide uptake and lowers the photosynthetic rate[49]. This stress condition leads to the accumulation of carbohydrate and protein metabolites, such as proline and glycine, in plant leaves due to reduced stomatal activity[50]. Adequate nitrogen availability promotes protein synthesis, consequently enhancing crude protein content[51]. Research findings indicate that increased nitrogen application elevates crude protein levels in annual ryegrass[34,52]. However, water stress negatively impacts photosynthetic efficiency, leading to alterations in the chemical composition of plants[53]. Conversely, increased irrigation has been reported to decrease crude protein content in various crops, including fenugreek[54], maize[48], annual grass[33], millet[55], and sorghum sudan grass[8].

In this study, increasing irrigation water levels increased the contents of ADF, NDF, ADL and crude fiber constituting cell wall components, while increasing nitrogen doses caused a decrease in these parameters. Increasing water levels increased the contents of cell wall components including ADF, NDF, ADL and crude fiber[54]. The increase in ADF, NDF, ADL and crude fiber contents, which make digestion difficult, leads to a decrease in the amount of crude protein, metabolic energy and digestible organic matter in the plant[56]. Kaplan

Ca (mg kg^{-1})					K (mg kg^{-1})				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	3469.17^{i}	3657.00^{h}	3691.92^{gh}	3606.03^{d}	IT0	4130.76^{i}	4530.60^{h}	5115.06^{g}	4592.14^{d}
IT25	3668.34^{gh}	3819.89^{efgh}	3911.46^{def}	3799.89^{c}	IT25	4963.33^{g}	5998.14^{e}	6340.06^{d}	5767.18^{c}
IT50	3758.09^{fgh}	3984.32^{bcd}	4141.23^{b}	3871.20^{b}	IT50	6270.12^{de}	7055.99^{c}	6937.17^{c}	6754.43^{b}
IT75	3820.90^{efg}	3977.90^{cde}	4406.89^{a}	4068.56^{a}	IT75	5435.96^{f}	7190.21^{c}	7532.22^{b}	6719.47^{b}
IT100	3799.46^{fgh}	3908.44^{def}	4080.96^{bc}	3929.62^{b}	IT100	6440.69^{d}	7894.81^{a}	8194.43^{a}	7509.98^{a}
Mean	3703.19^{c}	3869.51^{b}	4135.13^{a}		Mean	5448.17^{c}	6533.95^{b}	6823.79^{a}	

Mg (mg kg^{-1})					P (mg kg^{-1})				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	770.22^{i}	891.45^{h}	1009.00^{g}	890.22^{e}	IT0	1262.38^{h}	1638.39^{ef}	1598.21^{f}	1499.66^{d}
IT25	901.74^{h}	1070.90^{ef}	1097.97^{de}	1023.54^{d}	IT25	1463.84^{g}	1698.56^{e}	1857.33^{cd}	1673.24^{c}
IT50	1018.51^{g}	1129.53^{d}	1176.25^{c}	1108.10^{c}	IT50	1684.50^{e}	2015.09^{a}	1932.60^{b}	1877.40^{a}
IT75	1057.45^{f}	1129.36^{d}	1270.72^{b}	1152.51^{b}	IT75	1632.88^{ef}	1827.81^{d}	1927.36^{bc}	1796.02^{b}
IT100	1101.19^{de}	1269.03^{b}	1320.27^{a}	1230.16^{a}	IT100	1657.47^{ef}	1807.55^{d}	2020.52^{a}	1828.51^{b}
Mean	969.82^{c}	1098.05^{b}	1174.84^{a}		Mean	1609.67^{c}	1837.25^{b}	1934.46^{a}	

S (mg kg^{-1})					Na (mg kg^{-1})				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	673.73^{i}	827.21^{h}	903.37^{fg}	890.22^{d}	IT0	391.82^{f}	426.24^{e}	355.27^{i}	409.03^{d}
IT25	803.59^{h}	948.51^{de}	968.68^{cd}	906.92^{c}	IT25	468.27^{c}	566.77^{a}	439.82^{d}	491.62^{a}
IT50	888.16^{g}	982.57^{cd}	1024.49^{b}	965.07^{b}	IT50	459.36^{c}	461.27^{c}	509.81^{b}	476.81^{b}
IT75	929.54^{ef}	980.00^{cd}	1116.89^{a}	1008.81^{a}	IT75	421.02^{e}	417.89^{e}	459.86^{c}	419.45^{c}
IT100	871.44^{g}	999.36^{bc}	1118.95^{a}	996.58^{a}	IT100	378.41^{gh}	381.99^{fg}	367.50^{hi}	375.97^{e}
Mean	907.76^{c}	1029.63^{b}	1107.40^{a}		Mean	423.78^{b}	450.83^{a}	426.45^{b}	

B (mg kg^{-1})					Cu (mg kg^{-1})				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	5.77^{h}	5.82^{h}	6.16^{g}	5.92^{d}	IT0	5.23^{h}	5.90^{g}	6.31^{f}	5.81^{e}
IT25	6.37^{g}	7.49^{e}	8.43^{c}	7.43^{c}	IT25	6.00^{g}	6.38^{f}	6.73^{e}	6.37^{d}
IT50	7.23^{f}	7.99^{d}	8.86^{b}	8.02^{b}	IT50	5.93^{g}	6.96^{d}	7.23^{c}	6.71^{c}
IT75	7.44^{ef}	8.08^{d}	8.95^{ab}	8.16^{a}	IT75	6.33^{f}	7.24^{c}	7.91^{b}	7.16^{b}
IT100	7.40^{ef}	7.94^{d}	9.17^{a}	8.17^{a}	IT100	6.68^{e}	7.74^{b}	8.39^{a}	7.61^{a}
Mean	6.84^{c}	7.47^{b}	8.31^{a}		Mean	6.04^{c}	6.84^{b}	7.31^{a}	

Fe (mg kg^{-1})					Mn (mg kg^{-1})				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	57.16^{k}	66.36^{j}	76.79^{i}	66.77^{e}	IT0	41.73^{k}	45.76^{j}	54.38^{h}	47.29^{e}
IT25	66.43^{j}	76.36^{i}	83.33^{g}	75.37^{d}	IT25	50.39^{i}	57.71^{fg}	62.71^{e}	56.94^{d}
IT50	79.68^{h}	89.21^{f}	95.30^{e}	88.06^{c}	IT50	50.63^{i}	59.62^{f}	70.64^{c}	60.30^{c}
IT75	88.62^{f}	98.32^{d}	105.18^{c}	97.37^{b}	IT75	56.76^{g}	63.39^{e}	74.16^{b}	64.77^{b}
IT100	107.23^{c}	126.70^{b}	130.30^{a}	121.41^{a}	IT100	63.54^{e}	67.87^{d}	83.98^{a}	71.80^{a}
Mean	79.82^{c}	91.39^{b}	98.18^{a}		Mean	55.33^{c}	62.15^{b}	72.88^{a}	

Zn (mg kg^{-1})					Ni (mg kg^{-1})				
	Nitrogen doses					Nitrogen doses			
Irrigation	N100	N150	N200	Mean	Irrigation	N100	N150	N200	Mean
IT0	18.31^{j}	21.88^{h}	22.33^{h}	20.84^{e}	IT0	1.50^{i}	1.59^{h}	1.97^{e}	1.54^{e}
IT25	19.85^{i}	22.10^{h}	23.96^{g}	21.97^{d}	IT25	1.88^{f}	2.14^{e}	2.21^{cd}	2.07^{d}
IT50	22.54^{h}	26.29^{e}	28.01^{c}	25.62^{c}	IT50	1.77^{g}	2.31^{b}	2.14^{d}	2.07^{c}
IT75	24.93^{f}	27.48^{cd}	29.74^{b}	27.39^{b}	IT75	1.91^{ef}	2.28^{bc}	2.41^{a}	2.10^{b}
IT100	26.95^{de}	30.40^{b}	33.34^{a}	30.23^{a}	IT100	2.24^{bc}	2.46^{a}	2.29^{b}	2.33^{a}
Mean	22.52^{c}	25.63^{b}	27.48^{a}		Mean	1.86^{c}	2.16^{b}	2.20^{a}	

Table 6. Mineral content of *Lolium multiflorum* dry herbage under different irrigation levels and nitrogen doses. *The values in Table 6 are presented as the average of two-year research results.

et al.[8] in sorghum, Islam et al.[48] in maize, Wang et al.[40] and Varol et al.[24] in alfalfa, Kaplan et al.[54] in fenugreek, Stone et al.[57] in bermudagrass reported that the increase in water level increased ADF and NDF contents and decreased feed digestibility. Abraha et al.[33] reported that water deficits did not significantly affect NDF content in annual grass. Water restriction improves forage quality in forage crops by decreasing ADF and NDF content and increasing crude protein content[58]. Jafarian et al.[59] reported that crude protein content increased significantly as the severity of deficit irrigation increased, while other traits were not affected under stress conditions. In present study, protein content also increased with increasing water deficits. There are different opinions about the effect of nitrogen application on cell wall components in forage crops. Türk et al.[52] and Ertekin et al.[34] reported that ADF and NDF contents decreased in grass, Kaplan et al.[8] in sorghum Sudan grass, Kering et al.[60] in bermuda grass with increasing nitrogen doses. Pinho et al.[61] reported that the response of pearl millet to nitrogen doses in terms of NDF varied depending on genotypes. Ul-Allah et al.[62] reported that nitrogen fertilization and irrigation had no effect on ADF content of grass. Sidhu et al.[63] determined that the effect of nitrogen fertilization on ADF and NDF content of grass was not significant. However, the researcher also stated that the increased protein content due to the increase in the leaf biomass of the grass compared to the stem with nitrogen fertilization may cause a decrease in ADF and NDF content.

Water availability is positively correlated with the nutrient uptake efficiency of roots[64]. In arid conditions, root nutrient absorption is significantly reduced, while the restricted transpiration rates and impaired active transport mechanisms further limit the movement of nutrients from roots to shoots. A poorly developed root system exacerbates the issue by restricting the uptake of essential soil nutrients[65]. Consequently, these limitations alter the mineral composition of plants and lead to a reduction in ash content. While drought affects the absorption of various nutrients, low soil moisture conditions specifically hinder the uptake of these elements by roots. Water deficits have been shown to decrease the levels of key elements such as Na, Mn, Mg, Zn, and Fe, all of which play critical roles in enzyme activity, protein synthesis, and photosynthesis in plants[66]. On the other hand, practices like fertilization and irrigation are known to enhance the uptake of Zn, Mn, and B by plants[67]. Drought stress also results in reduced total phosphorus (P) accumulation, which adversely impacts plant growth and development[68]. Moreover, magnesium (Mg) deficiency under drought stress conditions diminishes chlorophyll content, stomatal conductance, and photosynthetic performance[69]. Interestingly, under water stress, increases in calcium (Ca) and reductions in potassium (K) concentrations have been observed in plants[70]. In this study, water stress was found to elevate Ca levels while reducing K levels. Calcium, which plays a vital role in cell wall integrity and various physiological processes, is recognized for its protective function against stress conditions in plants[71]. Meanwhile, drought-induced reductions in total phosphorus accumulation further exacerbate developmental challenges in plants experiencing water stress.

Fertilization and irrigation increase plant uptake of Zn, Mn and B[67]. Urea application often causes soil acidification, helps accelerate nutrient leaching and alters nutrient forms/availability[72]. Increasing N application rates may reverse the case. However, it contributes to the nutrient uptake of plants from slightly alkaline soils such as the soils of the research area. Nitrogen application increases the amounts of N, P, K and S in plants[73]. S requirement and metabolism in plants are closely related to nitrogen fertilization and N metabolism also affects the amount of S in the plant. As a result, N deficiency reduces the efficiency of sulphur utilization in plants[74]. Similarly, nitrogen fertilization increases Fe and Zn uptake from the soil and increases Fe and Zn availability by loading the phloem for nutrient transport[75]. N concentrations have significant and positive correlation with Cu, Fe, Mn, Mg and P[76]. In this study, increases in mineral contents were observed with the increase in nitrogen fertilization. The differences in mineral contents in plants may be mostly due to the different response of plants to drought stress and nitrogen utilization capacities. It was reported that the mineral contents of drought-resistant plants with high nitrogen utilization had higher values than plants with low mineral contents[77].

Conclusion

In this study, plant height, number of tillers, green and dry herbage yields increased with increasing irrigation water levels and nitrogen doses. While water stress increased crude protein content, irrigations increased crude ash, ADF, NDF, ADL and crude fiber contents. Increasing nitrogen doses increased crude protein and crude ash contents but decreased the amount of cell wall components (ADF, NDF, ADL and crude fiber). Although irrigation and fertilization caused different responses in mineral contents, in general, increasing irrigation water levels and nitrogen doses increased mineral contents. The highest crude protein yield was obtained from 75% irrigation and 200 kg ha^{-1} N dose. It is recommended that 100% irrigation and 200 kg ha^{-1} N fertilization could be practiced for high yield and quality herbage production in annual ryegrass plants. However, 75% irrigation and 200 kg ha^{-1} N application with high protein yield can also be recommended. Further research is also recommended to be conducted under field conditions and with different soil textures.

Data availability

Data will be made available on request (Contact: ihsanserkanvarol@gmail.com IS Varol).

Received: 29 December 2024; Accepted: 23 June 2025
Published online: 01 October 2025

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Acknowledgements

This research was supported by The Scientific and Technological Research Council of Turkiye (TUBITAK) (Project Code: 123O006).

Author contributions

İ.S.V.: Field experiment, data curation, formal analysis, writing-original draft, writing-review and editing. B.C.: Field experiment, data curation, formal analysis, writing-original draft. E.K.: Field experiment, data curation. M.K.: Supervision, funding acquisition, data curation, methodology, writing-original draft, writing-review and editing.

Declarations

Competing interests

The authors declare no competing interests.

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