EFFECTS OF NITROGEN FERTILIZATION RATES ON FORAGE YIELD AND QUALITY OF ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM* L.) IN CENTRAL BLACK SEA CLIMATIC ZONE IN TURKEY

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Abstract. In this research, we aimed to determine the effects of nitrogen fertilization on forage yield and forage quality of annual ryegrass (*Lolium multiflorum* L.) in Kazova, Tokat/Turkey conditions (the Central Black Sea Climate Zone) in 2014/2015 and 2015/2016 growing years. The experiment consisted of four replications in randomized complete block design to test effect of seven nitrogen rates (0, 50, 100, 150, 200, 250, 300 kg ha^{-1}) on plant height (PH), fresh forage yield (FFY), dry matter yield (DMY), crude protein ratio (CP), crude protein yield (CPY), Acid Detergent Fiber (ADF) ratio, Neutral Detergent Fiber (NDF) ratio, digestible dry matter ratio (DDM), digestible dry matter yield (DDMY), and relative feed value (RFV). The results revealed that the highest plant height (86.7 cm) was at the 200 kg ha^{-1} of nitrogen rate, highest fresh forage yield (48360 kg ha^{-1}), dry matter yield (13325 kg ha^{-1}), crude protein yield (1870 kg ha^{-1}) and digestible dry matter yield (8340 kg ha^{-1}) was obtained at the 250 kg ha^{-1} of nitrogen rate, and the highest digestible dry matter rate (62.72%) was determined at the 150 kg ha^{-1} nitrogen rate. Thus, 250 kg ha^{-1} nitrogen rate was the optimal dose for high forage yield and increasing nitrogen doses did not affect the ADF and NDF rates. Nevertheless, application of 300 kg ha^{-1} of nitrogen decreased yield but increased crude protein yield. Therefore, a nitrogen fertilization rate of 250 kg ha^{-1} is recommended for high forage yield of annual ryegrass (*Lolium multiflorum* L.) in the Central Black Sea Climate Zone or similar climatic conditions.
Keywords: *ADF, NDF, DMY, DDMY, RFV*

Introduction

Cost of animal derived food and food products are high in Turkey. One of the most important reasons for this is the insufficient production of high quality roughage. In order to meet the high quality roughage requirement in Turkey, it is necessary to improve the meadow pastures, increase the forage crop production areas, introduce other cheap and alternative roughage resources to animal production systems, and transfer the quality roughage production techniques to the producers (Serin and Tan, 2001).

Forage crop cultivation is one of the strategies to meet the high quality roughage need of the country's livestock. Forage legumes are the source of protein and forage grasses are the source of carbohydrates in animal diet. The annual forage grass *Lolium*

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multiflorum L. is one of the grasses with highest forage production potential and fertilizer use efficiency (Acıkgoz, 2001). The annual ryegrass is also known as annual ryegrass since it was initially cultivated in Italy as a annual forage crop.

Annual ryegrass, which is a grassy forage plant of Southern Europe origin, is a one-year species cultivated in the genera *Lolium* (Genckan, 1983). It is an important alternative source of roughage in cool and temperate climates, in areas where barley and oats from winter cool climate cereals are grown for feed production. Under normal conditions, green yields varying from 15000 to 25000 kg ha^{-1} per hectare and hay yields of 5000-8000 kg ha^{-1} can be obtained. In areas where water is sufficient, it is harvested 2-3 times, 40000-60000 kg ha^{-1} green, 7500-15000 kg ha^{-1} dry grass can be taken (Baytekin et al. 2009).

In general, the crop is harvested and fed to livestock freshly; however, it can also be utilized as hay or silage. Annual forage grass production in Turkey has increased in recent years owing to the government subsidies for forage crops. The cultivation area of 4.832 da and green grass production of 17.023 tons in 2014 has increased to a cultivation area 103.410 da and green grass production of 448.086 tons in 2019 (TUIK, 2019).

In order to achieve the expected yield and quality of forage crops, the plants should be fertilized with the appropriate combination and rate in the required period. In addition to yield, forage quality is also of great importance for animal health. Nitrogen, which is the most important nutrient for plants, constitutes the majority of dry matter. In addition, nitrogen is incorporated to proteins, chlorophyll, enzymes, and vitamins in plants. Nitrogen is the most commonly used nutrient in grasses. Appropriate amounts of nitrogenous fertilizers increase the protein content in grasses, but the use of excess nitrogen in plants also leads to the accumulation of nitrate and alkaloids. A positive response was reported in annual ryegrass with the application of nitrogen fertilizers (Colak, 2015; Ozdemir et al., 2019).

Different results have been obtained in studies on fertilizer use on annual ryegrass. Celen (1991) reported that the highest yields were achieved at 100 and 150 kg ha^{-1} nitrogen (N) application in Bornova, İzmir/Turkey conditions. Seker (1992) realized highest dry matter and crude protein yields 200 kg ha^{-1} N and the highest crude protein ratio at the rate of 250 kg ha^{-1} N application rate in trials in Erzurum/Turkey. Parlak et al. (2007) indicated the highest fresh forage yield (11630 kg ha^{-1}), dry matter (3840 kg ha^{-1}) and crude protein yield (800 kg ha^{-1}) at 200 kg ha^{-1} N application in Ankara/Turkey. In a study carried out in Serbia, Simic et al. (2009) reported that the highest dry matter yield varied between years and achieved in N application rates of 50-150 kg ha^{-1}. Kesiktas (2010) reported the highest dry matter yield at the application of 100 to 150 kg ha^{-1} of N and the highest crude protein ratio at the 150 kg ha^{-1} N applications in Karaman/Turkey conditions. Pavinato et al. (2014) reported that the increase in the N rate increased dry matter and crude protein yield and the best results were attained from the dose of 120 kg ha^{-1} of N in Brazil. Colak (2015) declared 80 kg ha^{-1} of N being sufficient to achieve high yield in Ankara and Ozdemir et al. (2019) reported that 500 kg ha^{-1} of N was suitable for high yield and high quality product of annual forage grass in in a study conducted in Bursa/Turkey.

Annual ryegrass, high growth rate and nitrogen absorption in fertilization due to the ability to (Ozkul et al., 2012) is an alternative forage crops can be obtained high yield. Turkey and Turkey's Central Black Sea region is a plant that has the potential to close the fodder deficit in the region.

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The purpose of the study, Turkey's Central Black Sea Region in the efficiency and high level of quality forage in closing the deficit will be an alternative forage crops in one annual ryegrass (*Lolium multiflorum* L.) to determine the appropriate nitrogen levels.

Materials and Method

Materials

The experimental trial was established in the Research and Application Center of Faculty of Agriculture located at Tasliciftlik Campus of Gaziosmanpasa University in Tokat Turkey for two years, 2014-2015 and 2015-2016 growing seasons. Trial area was 598 m above the sea level, at the 40°19’58.17 North latitude and 36°28’05 East longitude (*Figure 1*).

Figure 1. *Geographic location of the field trial*

Long term average rainfall, temperature, and relative humidity records of the research place was gathered compared to the temperature (°C), monthly total rainfall (mm) and monthly average relative humidity (%) of the breeding periods in which the experiment was conducted. The average temperatures of breeding periods (2014-2015, 2015-2016) (13.6, 12.0°C) in which the experiment was carried out were higher than the average long-term temperature (11.7°C), and total rainfall in the first breeding period (419.4 mm) is lower than the long year average (428.5 mm) and higher in the second breeding period (463.4 mm) than long year average. In addition, the relative humidity in the in the first breeding period (57.0%) is lower than the long year average (59.6%) and higher in the second breeding period (63.7%) than long year average (Anonymous, 2017). According to this, it can be concluded that the years in which the experiment was conducted were hotter and rainfall and humidity also different than the long-term averages (*Table 1*).

Chemical analyses of soil samples taken from 0-20 and 20-40 cm depths in the research area were carried out in Gaziosmanpasa University, Faculty of Agriculture, Department of Soil Science. According to the analysis results, the soil of the trial area was found to be poor in terms of organic matter, potassium, and lime, and it was clayey alkaline (Kacar, 2016).

In the research, Caramba cultivar (*Lolium multiflorum* cv. Caramba) was used and seven doses of nitrogen (0, 50, 100, 150, 200, 250, 300 kg ha^{-1}) were evaluated.

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Table 1. *Climate data for breeding periods (2014-2015, 2015-2016) and long year average of the region where the research was conducted*

Aylar	Temperature (°C)			Precipitation (mm)			Relative Humidity (%)		
	2014-2015	2015-2016	LYA	2014-2015	2015-2016	LYA	2014-2015	2015-2016	LYA
September	20.2	22.9	18.8	39.0	0.2	18.5	54.2	49.6	56.2
October	14.1	15.1	13.7	51.6	55.6	38.8	68.5	69.2	58.6
November	7.1	8.6	7.9	63.1	15.8	44.1	73.1	65.6	52.2
December	7.0	1.0	3.8	39.4	35.5	46.6	75.6	81.6	70.1
January	4.1	2.4	1.9	38.4	104.6	41.4	68.6	69.0	74.8
February	8.0	5.2	3.5	25.8	42.6	34.0	49.6	62.5	65.0
March	11.1	8.1	7.4	57.0	49.4	40.7	50.7	65.6	54.8
April	16.2	10.0	12.5	34.5	23.4	55.4	43.0	58.3	47.8
May	17.5	16.9	16.5	34.8	89.5	58.5	57.4	57.1	62.4
June	20.3	19.9	19.9	35.6	33.1	38.3	57.0	63.7	59.6
July	24.2	22.1	22.3	0.2	13.7	12.2	49.4	55.0	55.4
Average/Total	13.6	12.0	11.7	419.4	463.4	428.5	58.8	63.3	59.7

LYA: Long Year Average

Method

The experiment consisted of four replications in randomized complete block design (RCBD) with seven plots in each replication. Each plot consisted of 6 rows with 5 m length and 20 cm row spacing. Plants were seeded at 2-3 cm planting depth in 1st year on 16 October 2014 and 2nd year on 12 October 2015. In the experiment, the plot sizes were determined as 1.2x5 m = 6 m^2 (Avcioglu and Geren, 1996).

Sowing was based on 30 kg ha^{-1} seed amount (Acikgoz, 2001). Seeds weighed for each row were planted by hand in rows opened by marker. About ¼ of the predetermined nitrogen rate was applied after planting, another ¼ was applied during the tillering, ¼ was applied after the first harvest, and last ¼ was applied after the second harvest (Kesiktas, 2010). TSP (Triple Super Phosphate) was used as phosphorus fertilizer and urea was used as nitrogen fertilizer in the experiment. Pure 50 kg ha^{-1} phosphorus was applied to each parcel in planting (Kusvuran and Tansi, 2005). In both years, plots were harvested three times and irrigation was performed after the harvests.

The general view of the trial parcels is shown in *Figure 2*.

Figure 2. *General views from trial parcels in different periods*

The height of the 10 plants randomly selected from each plot were measured from the soil surface to the highest point of the plant and the plot based plant height was determined by taking the mean of the measured plants. The plots were harvested during

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the beginning of heading (10% heading). One row from the edges of each plot, top and bottom 0.5 m was mowed and removed, the remaining area was harvested. The fresh biomass obtained from each plot was weighed and converted to the fresh forage yield per ha (Anonymous, 2001). A randomly selected 500 g fresh forage sample was dried to a constant weight at 60ºC and dry weights ratios were determined based on dried samples (Sleugh et al., 2000). Samples of 5 g from the dried herbages were dried further for 24 hours in the oven set at 105ºC, cooled in a desiccator, and subsequently weighed on sensitive a balance to determine the dry matter ratios. Dry matter yields were calculated based on the obtained dry matter ratio. Some of the samples dried at 60ºC were ground and nitrogen was determined in the grass samples by Kjeldahl method. Determined nitrogen values were multiplied by 6.25 conversion coefficient to determine crude protein contents (%) of the of samples (Tan, 1995). In the experiment, ADF (Acid Detergent Fiber) and NDF (Neutral Detergent Fiber) analyses were performed, according to Van Soest et al. (1991) using the ANKOM 200/220 device.

Digestible dry matter ratio, ADF composition, relative feed value (RFV), dry matter consumption values were calculated using formulas (*Eq.1, Eq.2, Eq.3*) provided by Sheaffer et al. (1995).

$$\text{Digestible Dry Matter (DDM)} = 88.9 - (0.779 \times \%\ \text{ADF}) \qquad \text{(Eq.1)}$$

$$\text{Dry Matter Intake (DMI)} = 120 / (\%\ \text{NDF}) \qquad \text{(Eq.2)}$$

$$\text{Relative Feed Value} = (\%\text{DDM} \times \%\text{DMI})/1.29 \qquad \text{(Eq.3)}$$

The digestible dry matter yield was obtained by multiplying the dry matter yield with the digestible dry matter ratio and the crude protein yield was obtained by multiplying the crude protein ratios and the dry matter yield.

The variance analysis (ANOVA) of the data was performed in the first year, second year and combined randomized block experiment design in MSTATC statistical program. Mean separations of significant traits were conducted using Duncan's multiple comparison test (Duzgunes et al., 1987).

Results and Discussion

The table of the variance analysis of the combined data is as follows (*Table 2*).

Table 2. *Results of analysis of variance and mean squares of the traits determined*

	DF	PH	FFY	DMY	CP	CPY	ADF	NDF	DDM	DDMY	RFV
Year (Y)	1	45.2	1998788.8	352126.9*	0.03	6393.3*	4.7*	9.8	2.9*	153911.4*	90.6
Error 1	6	8.8	640803.8	30728.0	0.70	844.7	0.7	5.7	0.4	12838.03	34.9
Nitrogen (N)	6	39.3**	1342460.5**	102591.8**	6.32**	3626.2**	2.2*	10.2*	1.3*	40573.4**	65.8**
NxY	6	5.5	92145.4	7161.7	0.20	153.21	1.0	3.5	0.6	2486.7	14.7
Error 2	36	11.8	1980817.8	9564.9	0.38	265.7	0.9	3.4	0.6	3458.7	18.5
CV		4.1	10.2	8.5	4.5	10.4	2.8	3.4	1.2	8.2	4.0

DF: Degree of freedom; * $P<0.05$ and ** $P<0.01$

According to the variance analysis, combined data of two years made a significant statistical difference in nitrogen applications, plant height, fresh forage yield, dry matter

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yield, crude protein yield, ADF, NDF, DDM, crude protein yield, DDMY and RFV. Nitrogen applications were found to be statistically significant in dry matter yield, crude protein yield, ADF, DDM, DDMY between years. Nitrogen x year interaction was not statistically significant in any parameter.

Plant Height (cm)

According to the ANOVA results, the nitrogen rate has a significant effect on plant height values of the first year and two-year average. However, nitrogen rates did not affect plant height of annual ryegrass in the second year. The average plant height values determined in the plots with different nitrogen rates are shown in *Table 3*.

Although the average plant height in the second year was higher than the first year, it was found that year effect was not statistically significant. In the first year and two-year averages, plant height was significantly higher in the plots where nitrogen was applied compare to the control group. Nitrogen dose applied to annual ryegrass increased the plant height (*Table 3*). This increase can be attributed to the vegetative growth stimulating effect of nitrogen fertilizers in plants (Kun, 1994; Gokmen et al., 2001).

Table 3. *Average plant height values (cm) of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen (kg ha^{-1})	Years		
	2015	**2016**	**Mean**
0	76.9 b**	82.1	79.5 b
50	83.2 a	84.3	83.7 a
100	83.0 a	85.3	84.1 a
150	83.8 a	85.7	84.8 a
200	86.6 a	86.8	86.7 a
250	84.8 a	85.5	85.2 a
300	83.0 a	84.4	83.7 a
Mean	83.1	84.9	

** Values within the same columns followed by different letters are significantly different based on Duncan's test at P < 0.01

The average plant height of annual ryegrass was reported to be 60-90 cm by Erkun (1954), 65.7-68.6 cm by Kusvuran and Tansi (2005), 48.4 cm by Demiroglu et al. (2007), 64.5 cm by Kesiktas (2010), 88.4 cm by Cinar et al. (2011), 50.1-68.3 cm by Colak and Sancak (2017). The plant height measurement in the present study was closer to the values reported in the study of Cinar et al. (2011) and deviated from the other studies. This may be due to the variations in ecological conditions, cultivation techniques and the cultivars tested.

Fresh Forage Yield (kg ha^{-1})

Difference in the nitrogen rate had a significant effect on fresh forage yield of annual ryegrass in both years and in the mean of the two years. The average fresh forage yield values based on the nitrogen rates are given in *Table 4*.

As shown in *Table 4*, fresh forage yield ranged from 32330 kg ha^{-1} to 46150 kg ha^{-1} in the first year, from 40420 kg ha^{-1} to 50570 kg ha^{-1} in the second year, and from 36375 kg ha^{-1} to 48360 kg ha^{-1} in the two-year average depending on the fertilizer rate applied. In the first year, second year and two-year average, the highest fresh forage yield was obtained from the application of nitrogen rate of 250 kg ha^{-1}. However, fresh

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forage yield obtained from the nitrogen rates between 50-200 kg ha^{-1} in the first year, from the nitrogen rates of 150-200 kg ha^{-1} in the second year, and from the nitrogen rates between 150-200 kg ha^{-1} at the two-year averages were not significantly different from that of the nitrogen rate of 250 kg ha^{-1}. In both years separately and on a two-year average, the nitrogen rate of 300 kg ha^{-1} decreased fresh forage weight.

Table 4. *Fresh forage yield values (kg ha^{-1}) of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen (kg ha^{-1})	**Years**		
	2015	**2016**	**Mean**
0	32330 b**	40420 d	36375 d
50	41150 a	44520 bcd	42835 bc
100	38510 ab	42670 bcd	40590 cd
150	43790 a	45790 abc	44790 abc
200	45930 a	47760 ab	46845 ab
250	46150 a	50570 a	48360 a
300	39660 ab	42230 cd	40945 cd
Mean	41074	44851	

** Values within the same columns followed by different small letters are significantly different based on Duncan's test at P < 0.01

The fresh forage yield of the annual ryegrass was reported to be 5500 kg ha^{-1} with the application of 150 kg ha^{-1} nitrogen (Alvim and Moojen, 1984), 13500.0 kg ha^{-1} with 55 kg ha^{-1} nitrogen application (Kallenbach et al., 2003), 11630 kg ha^{-1} with 200 kg ha^{-1} nitrogen application (Bright et al., 2006), 15430 kg ha^{-1} with 65 kg ha^{-1} nitrogen application (Piskin, 2007), 32450 kg ha^{-1} with 200 kg ha^{-1} nitrogen application (Kusvuran and Tansi, 2005), 19270, 19320, 19320 kg ha^{-1} with 40, 80, 120 kg ha^{-1} of nitrogen dose applications, respectively (Colak and Sancak, 2017).

When compared with the abovementioned previous research, it is evident that both fresh forage yield values and nitrogen rates vary greatly. This discrepancy may be due to differences in the nature of the studies, ecology, nitrogen rate, and the cultivar tested.

Dry Matter Yield (kg ha^{-1})

According to the ANOVA results for dry matter yield values, different nitrogen fertilizer rates had a significant effect on dry matter yield in the first year, second year, and two-year average. Average dry matter yield values in respect to nitrogen rates are given in *Table 5.*

Depending on the fertilizer rates applied, dry matter yield ranged from 8590 kg ha^{-1} to 12410 kg ha^{-1} in the first year, 11340 kg ha^{-1} to 14240 kg ha^{-1} in the second year, and 9965 kg ha^{-1} to 13325 kg ha^{-1} when means were averaged over two years. The highest dry matter yield in the first year, second year and two-year average was obtained from the application of 250 kg ha^{-1} nitrogen rate. Dry matter yield decreased at 300 kg ha^{-1} nitrogen rate for each of the two years and on average. Dry matter yield in the second year was significantly higher than that in the first year (*Table 4*). The reason for the higher average dry matter yield in the second year is higher rainfall in the second year (*Table 1*).

In general, dry matter yield results were parallel to fresh forage yield (*Table 4*) as expected. The earlier research revealed that dry matter yield of annual ryegrass was 9600 kg ha^{-1} when 350 kg ha^{-1} nitrogen fertilizer is applied (Corainville et al., 1973),

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was 8760 kg ha^{-1} with 90 kg ha^{-1} nitrogen application (Bartholomew and Williams, 1978), was 2020 kg ha^{-1} with 55 kg ha^{-1} nitrogen application (Piskin, 2007). In the research reported here, the highest dry matter yield was obtained with the application of 250 kg ha^{-1} pure nitrogen. There is a discrepancy between our results and the previous results listed above. Different ecological conditions, variation in the rate of fertilizer, management differences and the difference in cultivar may have led the discrepancy among reported results.

Table 5. *Average dry matter yield values (kg ha^{-1}) of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen (kg ha^{-1})	Years		
	2015	2016	Mean
0	8590 d**	11340 c	9965 e
50	10510 bc	12070 bc	11290 cd
100	9830 cd	11400 c	10615 de
150	11190 abc	12550 bc	11870 bc
200	11870 ab	12980 ab	12425 ab
250	12410 a	14240 a	13325 a
300	10620 bc	11540 bc	11080 cd
Mean	10717 B+	12303 A	

** Values within the same columns followed by different letters are significantly different based on Duncan's test at $P < 0.01$.
+ Values within the same row followed by different capital letters are significantly different based on Duncan's test at $P < 0.05$

Crude Protein Ratio (%)

ANOVA results for crude protein ratio based on different nitrogen rates revealed that nitrogen rates had a significant effect on the crude protein ratio in the first year, second year and on the two-year averages. Average crude protein ratios determined according to nitrogen rates are provided in *Table 6*.

The ratio of crude protein, one of the most important forage quality parameter, increased in parallel to the increase in the nitrogen rate in annual ryegrass. The highest crude protein content was obtained from 300 kg ha^{-1} nitrogen rate in both trial years and in two-year average. It has been stated by many researchers that nitrogen increases the crude protein content of annual grasses (Colak, 2015).

In the previous research, the highest crude protein ratio was reported at 200 and 250 kg ha^{-1} nitrogen application in Kusvuran and Tansi (2005), at 150 kg ha^{-1} nitrogen application in Simic et al. (2009) and Kesiktas (2010), at 470 kg ha^{-1} nitrogen application in Kusvuran (2011), and at 200 kg ha^{-1} nitrogen application in Colak (2015). When the results obtained here are compared with the abovementioned studies, no concordance is observed. Different ecological conditions, variation in the rate of fertilizer, management differences along with the difference in tested cultivars may have led this difference.

Crude Protein Yield (kg ha^{-1})

One way ANOVA results discerned that the crude protein yield significantly differ with the various fertilizer rates in the first year, second year, and two-year average. Average crude protein yields determined according to nitrogen rates are listed in *Table 7*.

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Table 6. *Determination of the crude protein ratio (%) of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen ($kg\ ha^{-1}$)	**Years**		
	2015	**2016**	**Mean**
0	11.91 b**	12.37 d	12.14 d
50	12.70 b	12.99 cd	12.85 c
100	13.93 a	13.61 bc	13.77 b
150	14.12 a	14.15 ab	14.13 b
200	13.83 a	13.43 bc	13.63 b
250	13.96 a	14.02 b	13.99 b
300	14.75 a	14.96 a	14.86 a
Mean	13.60	13.65	

** Values within the same columns followed by different small letters are significantly different based on Duncan's test at $P < 0.01$

Table 7. *Average crude protein yield values ($kg\ ha^{-1}$) of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen ($kg\ ha^{-1}$)	**Years**		
	2015	**2016**	**Mean**
0	1030 c**	1400 c	1220 d
50	1340 b	1570 bc	1460 c
100	1370 b	1550 bc	1460 c
150	1580 ab	1780 ab	1680 b
200	1650 a	1750 b	1700 b
250	1730 a	2000 a	1870 a
300	1570 ab	1730 b	1650 b
Mean	1467 B+	1682 A	

** Values within the same columns followed by different small letters are significantly different based on Duncan's test at $P < 0.01$.
+ Values within the same row followed by different capital letters are significantly different based on Duncan's test at $P < 0.05$

The crude protein yield was calculated by multiplying the crude protein ratio and dry matter yield and the highest crude protein yield was obtained at the nitrogen rate of 250 $kg\ ha^{-1}$ in the first year, in the second year and in two-year average. In general, the crude protein yields of the plots with high crude protein content and dry matter yield were high as expected (*Table 5*).

Crude protein yield of annual ryegrass was reported as 1200 $kg\ ha^{-1}$ by Alvim and Moojen (1984), as 1290 $kg\ ha^{-1}$ by Basbug (1990), as 470 $kg\ ha^{-1}$ by Karakurt and Ekiz (1991), as 1150 $kg\ ha^{-1}$ by Kusvuran and Tansi (2005), as 800 $kg\ ha^{-1}$ by Bright et al. (2007), as 920 $kg\ ha^{-1}$ by Kesiktas (2010), as 820 $kg\ ha^{-1}$ by Kusvuran et al. (2014), and as 680 $kg\ ha^{-1}$ by Colak and Sancak (2017). The results obtained in this study do not comply with the results listed above. It can be concluded that the difference in crude protein yields is not surprised as the trials were carried out in regions with different ecological characteristics, distinctive applications were performed, and different cultivars were tested.

ADF Concentration (%)

According to the one way ANOVA results, ADF concentration was not affected by the nitrogen fertilizer rate in the first year and in the second year. Nonetheless, nitrogen fertilizer application rates had a significant effect on two-year average ADF

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concentration values. The average ADF concentration values determined according to the nitrogen rates are given in *Table 8*.

According to the two-year averages, the ADF content was significantly higher at nitrogen rates of 50 kg ha^{-1} and 100 kg ha^{-1} compared to 150 kg ha^{-1} nitrogen application rate. However, the ADF concentrations at 0, 200, 250, and 300 kg ha^{-1} nitrogen rates were not different from both higher or lower concentrations of 50, 100 or 150 kg ha^{-1} doses. The mean ADF concentration in the first year was significantly higher than in the second year (*Table 8*). This may be due to the fact that the second falling rainfall and relative humidity are higher than the first year (*Table 1*).

Table 8. *Average ADF concentration (%) values of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen (kg ha^{-1})	Years		
	2015	**2016**	**Mean**
0	33.64	33.65	33.64 ab*
50	34.72	34.66	34.69 a
100	34.62	34.81	34.71 a
150	33.86	33.33	33.59 b
200	35.95	33.72	34.65 ab
250	34.36	33.31	33.83 ab
300	34.15	33.40	33.78 ab
Mean	34.42 A+	33.84 B	

* Values within the same columns followed by different small letters are significantly different based on Duncan’s test at $P < 0.05$.
+ Values within the same row followed by different capital letters are significantly different based on Duncan’s test at $P < 0.05$

ADF concentrations of the annual ryegrass reported by Caddel and Allen (1997) were 31.0-35.0%, by Meeske et al. (2009) were 31.4%-32.3, by Kusvuran et al. (2014) was 37.4%, by Colak and Sancak (2017) were 31.1-32.1%, and by Ozdemir et al. (2019) were 30.5-34.2. The results obtained in this study are in agreement with Caddel and Allen (1997) and Ozdemir et al. (2019) but not with other studies. The difference can be attributed to the change in the cellulose and lignin contents of the cultivars along with the different ecological conditions and harvest regimen.

NDF Concentration (%)

According to the ANOVA results of NDF concentrations based on different nitrogen rates, the first year and the second year nitrogen rates did not affect the NDF concentrations. Nevertheless, nitrogen rates had a significant effect on the two-year average NDF concentrations in annual ryegrass (*Table 9*).

According to the two-year averages, the NDF composition of the herbage at 200 kg ha^{-1} nitrogen rate was significantly higher than that of the control and 250 to 300 kg ha^{-1} nitrogen rates (*Table 9*).

Van Soest (1985) stated that in order to ensure optimum milk yield in dairy cattle, NDF composition should be 36%. Yavuz (2005) affirmed that the amount of feed digested by the animal decreased with the increase in NDF ratio and the increase in NDF ratio in roughage caused a decrease of 1-2% of milk yield. Thus, it can be concluded that due to the high NDF composition, annual ryegrass alone is not suitable for feeding dairy cattle.

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NDF composition in annual ryegrass was reported to be in the range of 47.7-54.7% by Viviani Rossi et al. (1994), was in the range of 40-46% according to Caddel and Allen (1997), was in the range of 42.2-50.6% in Teutsch and Smith (2001), was 47% in Meeske (2009), was 58.7% in Kusvuran and Tansi (2005), was 59.6% in Simsek (2015), and was in the range of 56.01-54.14% in Colak (2015). The results obtained in the current research did not show compliance with Caddel and Allen (1997), Meeske (2009), Kusvuran et al. (2014), Simsek (2015): however, they were in agreement with the Viviani Rossi et al. (1994), Teutsch and Smith (2001) and Colak (2015). It can be concluded that the differences in NDF composition can be attributed to the fact that the trials were carried out in regions with different ecological characteristics, with distinctive applications, and different cultivars.

Table 9. *Average NDF concentrations (%) of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen (kg ha^{-1})	Years		
	2015	**2016**	**Mean**
0	54.85	51.35	53.10 b*
50	55.23	55.02	55.12 ab
100	55.40	54.34	54.87 ab
150	55.43	54.47	54.95 ab
200	56.38	56.45	56.42 a
250	54.23	53.39	53.81 b
300	53.28	53.90	53.59 b
Mean	54.97	54.13	

* Values within the same columns followed by different small letters are significantly different based on Duncan’s test at P < 0.05

Digestible Dry Matter Ratio (%)

One way ANOVA analyses targeting the effect of nitrogen rate on digestible dry matter ratio revealed that different nitrogen rate did not lead a significant difference in the digestible dry matter ratios in the first and in the second years whereas a significant difference was observed among the nitrogen rates for the analysis of values averaged over two years (*Table 10*).

As indicated in *Table 10*, the average digestible dry matter ratio varied between 61.85% and 62.72% for the two-year averages depending on the nitrogen rates applied. The highest DDM ratio was found at 150 kg ha^{-1} nitrogen dose and the lowest was at 100 kg ha^{-1} nitrogen dose. The mean DDM ratio in the second year was significantly higher than the one in the first year. As expected, the rate of digestible dry matter, which was negatively correlated with the ADF composition of the herbage, was higher in the nitrogen dose with low ADF and higher in the year with the low ADF composition (*Table 8*).

Digestible Dry Matter Yield (kg ha^{-1})

Nitrogen rate significantly affected digestible dry matter yield in the first year, in the second year, in average over the two years (*Table 11*).

The digestible dry matter yield increased in annual ryegrass in parallel to nitrogen application rate. In the first year, the digestible dry matter yield at 250 kg ha^{-1} nitrogen rate was significantly higher than the those of rates other than 150 and 200 kg ha^{-1}. In the second year, the digestible dry matter yield at 250 kg ha^{-1} nitrogen rate was

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significantly higher than those in rates other than 200 kg ha^{-1}. In the two-year average values, the digestible dry matter yield at 250 kg ha^{-1} nitrogen rate was significantly higher than in all other nitrogen rates (*Table 11*).

Digestible dry matter yield is a variable that depends on digestible dry matter content and dry matter yield. Therefore, digestible dry matter yield was high when both digestible dry matter ratio and dry matter yield were high (*Table 5, Table 10*).

Table 10. *Digestible dry matter ratios (%) of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen (kg ha^{-1})	Years		
	2015	**2016**	**Mean**
0	62.69	62.68	62.68 ab*
50	61.85	61.89	61.87 b
100	61.92	61.78	61.85 b
150	62.51	62.93	62.72 a
200	61.16	62.63	61.90 ab
250	62.13	62.95	62.54 ab
300	62.29	62.87	62.58 ab
Mean	62.08 B$^+$	62.53 A	

* Values within the same columns followed by different small letters are significantly different based on Duncan’s test at P < 0.05.
$^+$ Values within the same row followed by different capital letters are significantly different based on Duncan’s test at P < 0.05

Table 11. *Average digestible dry matter yield values (kg ha^{-1}) of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen (kg ha^{-1})	Years		
	2015	**2016**	**Mean**
0	5370 d**	7110 c	6240 e
50	6500 bc	7470 bc	6985 cd
100	6080 cd	7040 c	6560 de
150	7000 abc	7900 bc	7450 bc
200	7260 ab	8130 ab	7695 b
250	7710 a	8970 a	8340 a
300	6620 bc	7260 bc	6940 cd
Mean	6648 B$^+$	7697 A	

** Values within the same columns followed by different small letters are significantly different based on Duncan’s test at P < 0.01.
$^+$ Values within the same row followed by different capital letters are significantly different based on Duncan’s test at P < 0.05

Relative Feed Value (RFV)

According to the ANOVA results, nitrogen rates did not significantly affect the relative feed value in the first year. Nonetheless, relative feed values of the second year and two-year averages indicated that nitrogen rates had a significant effect on relative feed values in annual ryegrass (*Table 12*).

In the second year of the study and at the two-year averages, the lowest RFV was obtained from 200 kg ha^{-1} nitrogen application. The relative feed value is calculated using the ADF and NDF ratios and has a negative correlation with the ADF and NDF ratios. Therefore, a high relative feed value is expected at low ADF and NDF composition values.

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Colak and Sancak (2017) reported RFV of 111.2 in annual ryegrass at 240 kg ha^{-1} nitrogen rate in a study conducted in Ankara. Kusvuran et al. (2014) reported RFV of 94.0 in annual ryegrass. RFV obtained from the present study is lower than the values reported in Colak and Sancak (2017) and higher than those reported in Kusvuran et al. (2014).

Caddel and Allen (1997) reported the relative feed value of completely headed wheat as lower than 77. Schroeder (2004) stated that the relative feed value decreases as the harvest time delayed, and Linn and Martin (1999) argued that the relative feed value of forage for high-milk yield dairy cows should be at least 124. The RFV obtained from the current study is below the stated value. RFV is a value obtained from ADF and NDF values. It is inversely proportional to ADF and NDF, and low RFV is obtained from high ADF and NDF values.

Table 12. *The average RFV of annual ryegrass based on nitrogen fertilizer rates*

Nitrogen (kg ha^{-1})	**Years**		
	2015	**2016**	**Mean**
0	106.4	114.5 a*	110.4 a**
50	104.2	104.6 b	104.4 bc
100	104.0	105.8 b	104.9 bc
150	105.0	107.5 ab	106.2 abc
200	100.9	103.3 b	102.1 c
250	106.5	109.6 ab	108.1 ab
300	108.9	108.5 ab	108.7 ab
Mean	105.1	107.6	

** Values within the same columns followed by different small letters are significantly different based on Duncan's test at $P < 0.01$

Conclusions

Forage crops produced in Turkey are not enough for animals. Therefore, meat and milk production is low. Annual ryegrass is a high yield and high quality alternative fodder plant due to its high growth rate and ability to absorb nitrogen. Annual ryegrass, fertilizer is a forage plant with high yield. The production of forage crops may increase with the determination of suitable fertilizer feeds. Meat and milk production can be increased by increasing forage crop production.

In this research, we aimed to determine the optimum rate of nitrogen fertilization to achieve high yield and high quality forage from annual ryegrass (*Lolium multiflorum* L.) in the Central Black Sea Climate Zone in Turkey. According to the results of the research, we conclude that (i) it is possible to obtain high yield and high forage quality with nitrogen fertilization under the conditions of Central Black Sea Climate Zone, (ii) application of 250 kg ha^{-1} nitrogen rate is appropriate for high yield, (iii) increasing nitrogen doses do not affect the ADF and NDF rates, and (iv) yield decreases with 300 kg ha^{-1} nitrogen application rate but the crude protein yield increases. For the annual ryegrass, 250 kg ha^{-1} nitrogen rate is recommended to achieve high yield in the Central Black Sea Climate Zone in Turkey and possibly in similar ecological conditions.

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