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Original Research

The Effect of Different Doses of Nitrogen and Mixtures with Berseem Clover on Yield and Quality of Italian Ryegrass

Mahmut Kamacı[1], Abdullah Özköse[2]*

[1]Çumra District Directorate of Agriculture and Forestry, Konya, Türkiye
[2]Selçuk University, Faculty of Agriculture, Department of Field Crops, Konya, Türkiye

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Abstract

Considering the impact of producing and using nitrogen-based fertilizer on the environment, as well as the economic cost of nitrogen-based fertilizer application, growing high-protein grass such as Italian ryegrass mixed with berseem clover as an alternative for sustainable production could be an option. This study was carried out in 2020 to determine the effects of different nitrogen doses and berseem clover mixtures on the yield and quality of Italian ryegrass in Konya ecological conditions. Within the scope of the study, different nitrogen doses (0, 5, 10, 15 and 20 g m^{-2} as pure substance) and different mixtures of Italian ryegrass + berseem clover (monoculture Italian ryegrass, 75% + 25%, 50% + 50% and 25% + 75%) were conducted as two separate trials. As a result, it was found that as the amount of nitrogen given in Italian ryegrass increased, yield and quality increased, and in the mixtures, yield and quality increased due to the increase in the ratio of berseem clover. In Konya conditions, we can recommend a nitrogen dose of 15-20 g m^{-2} for Italian ryegrass, and 25% + 75% for mixtures of Italian ryegrass and berseem clover. In order to get high green grass yield from Italian ryegrass, it would be more appropriate to grow it in a mixture with berseem clover and to increase the ratio of berseem clover in the mixture instead of fertilizing with high doses of nitrogen.

Keywords: berseem clover, forage, intercropping, mixture ratios, reduce fertilizer use

Introduction

Feed is the most important input in livestock farms. Feeding expenses make up 70-75% of the costs in animal production [1]. The feed used in animal feeding directly affects both the cost of the farm and the health of the animals. In good animal husbandry, concentrates and roughages need to be in balance. Italian ryegrass (*Lolium multiflorum* Lam.) is a valuable forage plant that meets the need for high-quality roughage, with high nutritional value and high yield, and is eagerly eaten by animals [2]. It mainly grows in temperate regions and is a cold-season forage species [3].

Italian ryegrass is valuable for fast establishment, long growth period, high yield with proper fertilization and maintenance in suitable climatic conditions [4], multiple harvest in one-year, high feeding value, eagerly eaten by animals, suitable for grazing and frequent

*e-mail: aozkose@selcuk.edu.tr

cutting, and the ability to make silage as a mixture or pure. While Italian ryegrass cultivation area in Turkey was only 483 ha in 2014, its cultivation area and production have increased exponentially every year. In 2022, Italian ryegrass cultivation area was 53994 ha, production was 2122105 tons and grass yield were 39.3 tons/ha [5].

Nitrogen fertilization increases yield and quality of Italian ryegrass. Deficient nitrogen fertilization will reduce the amount of product obtained per unit area and quality in Italian ryegrass. Excessive nitrogen fertilization will increase production costs. Besides, chemical fertilizer not only pollutes the environment but also leaches down and contaminates the groundwater table [6]. Yield of Italian ryegrass varies greatly depending on variety, climate, soil, and agricultural practices. Therefore, the amount of nitrogen fertilizer to be applied per hectare must be determined according to the region, variety, and cultivation technique.

In Italian ryegrass grown under monoculture conditions, it is necessary to apply significant amounts of nitrogen fertilizers to increase both yield and crude protein content of the forage. The production of ammonia is a carbon emissions and energy-intensive process, relying primarily on natural gas and coal as feed stocks, accounts for approximate 90% of the fertilizer industry's total energy consumption and about 2% of the world's total energy consumption [7]. In recent years, nitrogen fertilizer prices have risen very high due to energy prices. Farmers' ability to buy and apply nitrogen fertilizer has been limited. There has been an increased search for different agricultural practices to reduce the need for nitrogen fertilizer. Nitrogen fertilizer requirements are low due to rhizobia bacteria in the roots of legume plants. Rhizobium improves sustainable production by boosting organic nitrogen content [8]. Legume forage plants can be grown in monoculture or mixed with other forages to reduce the need for nitrogen fertilizer. Additionally, the use of cover crops, such as clover or alfalfa, can also help to reduce the need for nitrogen fertilization in Italian ryegrass. These cover crops can fix nitrogen from the air and transfer it to the soil, making it available for the ryegrass to use. This can not only reduce the cost of fertilization for the farmer but also reduce the environmental impact of excess nitrogen in the soil. [9].

Forage plants belonging to the *Poaceae* and *Fabaceae* families may have advantages over each other. Intercropping is an old cropping practice, possibly as old as the settled agriculture [10]. Legumes in symbiosis for nitrogen fixing with rhizobia provide beneficial input for complimentary plant [11]. When legume and grass forage plants are grown in a mixture that is compatible with climate and growth conditions, the roughage obtained is much more beneficial for animal husbandry. An example of a grass-legume mixture is Italian ryegrass and berseem clover. Berseem clover (*Trifolium alexandrinum* L.) varieties, which can be cut many times during a growing season, show good compatibility with Italian ryegrass. The mixture of berseem clover with Italian ryegrass not only increases forage yield and crude protein yield, but also has the potential to reduce the need for nitrogen fertilization due to the rhizobia bacteria in its roots [12-14].

Within the scope of the study, nitrogen fertilizer application in Italian ryegrass, which is widely used in agriculture in recent years, and a mixture of Italian ryegrass + berseem clover will be tested. The potential for achieving yield and quality in Italian ryegrass using nitrogen fertilizer and a legume forage plant mixture of berseem clover will be researched. If the desired yield and quality can be obtained from berseem clover mixtures, it is thought that chemical fertilizer use can be reduced, which will also provide an economic benefit.

For these reasons, three objectives have been established in this study. The first is to determine the most suitable mixture ratio of Italian ryegrass and berseem clover. The second is to determine the most suitable nitrogen fertilizer dose for Italian ryegrass cultivation. The third is to determine the potential of obtaining the dry grass and crude protein yield per square meter that can be obtained from Italian ryegrass monoculture planted with nitrogen fertilizer application, with the mixture of Italian grass + berseem clover without using nitrogen fertilizer. The results obtained from the study will provide important information to roughage producers, livestock farms, and seed companies operating in the region. Additionally, it will provide guidance to other researchers working on the similar issue.

Materials and Methods

The study was carried out in the 2020 growing season (April-October) in the experimental field of the Department of Field Crops, Faculty of Agriculture, Selçuk University in Konya (Türkiye) and the laboratory studies were conducted in the Laboratories of the Department of Field Crops. The climate of Konya, where the study was conducted, is continental. Winters are harsh, cold, and snowy, summers are hot and dry. The altitude of the area where the study was conducted is 1130 meters above sea level (38°02'07"N 32°30'55" E). According to soil analysis results, the soils of the experimental area are clay-loamy in structure and alkaline in properties. The amount of organic matter was 1.12%, EC (µS/cm) = 190, pH = 7.5, P_2O_5 = 10.58 ppm, K_2O = 242.36 ppm, N = 20.9 ppm, Na = 67.02 ppm, Ca = 5600 ppm, $CaCO_3$= 37.6%, Zn = 2.13 ppm, Mn = 4.80 ppm, Cu = 0.81 ppm, and Fe = 1.30 ppm. The climate data for the months in which the study was conducted in 2020 and the long-term averages are given in Table 1. In 2020, the total precipitation was 115.0 mm, the average temperature was 19.5ºC and the relative humidity was 45.4% while the long-term average was 157.4 mm, an average temperature of 17.1ºC and a relative humidity of 50.2%. The study year received

less rainfall, was warmer and had lower relative humidity compared to the long-term averages.

In the study, registered varieties of Italian ryegrass (*Lolium multiflorum* Lam.) (cv: Barmultra II) and berseem clover (*Trifolium alexandrinum* L.) (cv: Efsane) were used. The study was set up based on a randomized block design with three replications. It was held in sowing was done in spring on April 15, 2020. Each plot was 2x5 = 10 m^2 and consist of 10 rows with a length of 5 m and 20 cm inter-row spacing. Sowing process; made by hand on the rows opened with a marker. Irrigation was done by sprinkler irrigation method. The effective root depth was wetted at each irrigation. Irrigation was infrequent in spring and more frequent in summer. Irrigation was applied immediately after fertilization and mowing. After emergence, depending on the development of the plants, weed control was done by hand hoeing. Hoeing was carried out at a depth (3 cm) sufficient to break and dry the subsoil roots of weeds.

One of the subjects of study is the different mixing ratios of Italian ryegrass + berseem clover. The number of seed to be planted varies according to the mixing ratios. The number of seed to be planted was determined as 4 kg (approx. 1700 seeds m^{-2}) for Italian ryegrass and 3 kg (approx. 1000 seeds m^{-2}) for berseem clover in monoculture sowing. The number of seeds to be planted in the mixtures was calculated by multiplying the number of seeds used in monoculture planting with the mix ratio. The sole plantings were sown alone, and the mixes were sown in the same row.

Another study subject is the application of different N doses in Italian ryegrass monoculture (Table 2). Prior to sowing, it was ploughed to produce a fine seedbed, and fertilized with 150 kg ha^{-1} of diammonium phosphate (DAP; 18% N + 46% P_2O_5). No fertilizer application was applied to the mixtures of Italian ryegrass (IR) + berseem clover (BC), except DAP. In Italian ryegrass monoculture (IRM), the doses of 5, 10, 15 and 20 g m^{-2} were determined by pure substance calculation as nitrogen (N) application. The determined nitrogen doses are given in two equal parts. The first fertilization was given before irrigation when the plants were 15-20 cm tall (on 06 June 2020). The second fertilization was done after the first mowing and before irrigation (on 23 July 2020. Within the scope of the study, both mixture and nitrogen fertilizer applications are given in Table 2. The study consisted of 8 applications and 3 replications, a total of 24 plots.

Mowing was done at the beginning of flowering of berseem clover in mixtures (on July 23, September 5, and October 19), and in the application of lean nitrogen fertilizer according to the development of Italian grass (on July 23 and October 19) [15, 16]. The mowing was made at a height of 5 cm from the soil surface. Observations and measurements were taken at each mowing, considering the edge effect. One row of the sides and 0.5 meters from the heads in each parcel were taken as the edge effect and were excluded from the evaluation. Accordingly, the harvested area was 6.4 m^2 (8 rows x 0.20 cm x 4 m).

In the research, plant height (cm), stem thickness (mm), green fodder yield (g m^{-2}), hay yield (g m^{-2}), dry matter ratio (%) and yield (g m^{-2}), crude protein ratio (%) and yield (g m^{-2}) were obtained. Two separate trials were conducted within the scope of this study. First, different levels of nitrogen fertilization were applied to Italian ryegrass. Secondly, mixtures of Italian grass and berseem clover were tried in different proportions. The levels of both factors were analyzed together as the levels of a single factor. The data of the experiment were evaluated by using the MSTAT-C computer program. LSD (Least Significant Difference) tests at 5% or 1% significance were applied to compare the means for the characteristics that showed significant differences because of the analysis of variance.

Table 1. Climate data of Konya Province for 2020 and long-term (LT) average*.

Month	Total Precipitation (mm)		Air Temperature ºC				Relative Humidity (%)	
	2020	LT (1930-2021)	2020			LT (1930-2021)	2020	LT (1930-2021)
			Min.	Max.	Mean			
Apr	35.3	32.31	7.8	18.1	10.80	11.06	59.5	58.04
May	43.5	42.51	11.8	24.7	15.90	15.85	53.6	55.88
Jun	23.9	25.90	15.9	28.2	20.30	20.14	47.9	49.11
Jul	0.9	7.50	20.6	33.0	25.50	23.56	36.4	41.21
Aug	0.4	6.48	19.1	31.5	24.20	23.29	31.4	40.84
Sep	6.9	12.90	17.7	30.7	22.60	18.81	42.6	46.77
Oct	4.1	29.78	12.4	25.7	17.10	12.84	46.4	59.29
Total	115.0	157.4	-	-	-	-	-	-
Mean	-	-	-	-	19.5	17.9	45.4	50.2

*: Konya Climate Data for 2020. T.C. Ministry of Agriculture and Forestry 8th Regional Directorate of Meteorology, Konya.

Table 2. Applications within the scope of the study.

Treatment No / abbr.	Treatment
1. IRM N0	0 g m^{-2} N as Control (Italian ryegrass monoculture)
2. IRM N5	5 g m^{-21} N (Italian ryegrass monoculture)
3. IRM N10	10 g m^{-2} N (Italian ryegrass monoculture)
4. IRM N15	15 g m^{-2} N (Italian ryegrass monoculture)
5. IRM N20	20 g m^{-2} N (Italian ryegrass monoculture)
6. 25%IR+75%BC	25% Italian ryegrass + 75% berseem clover
7. 50%IR+50%BC	50% Italian ryegrass + 50% berseem clover
8. 75%IR+25%BC	75% Italian ryegrass + 25% berseem clover

Results and Discussion

Plant Height

In the study, variance analysis was performed with data on plant height of Italian ryegrass obtained from different levels of nitrogen fertilizer application and mixtures of Italian grass and berseem clover at different rates. As a result of variance analysis, the difference between the average plant height of Italian ryegrass was found to be statistically significant at the $p \leq 0.01$ level. To determine the significance level of the differences between the averages of plant height in Italian ryegrass, the LSD test was performed, and the averages and LSD groups are given in Table 3.

When we consider the ratio of berseem clover in the mixtures as nitrogen doses and evaluate them together, the differences between the mean plant height values were found to be statistically significant. The highest plant height group was measured from 20, 15 and 10 g m^{-2} nitrogen applied plots and Italian ryegrass (68.3, 61.9, 61.1 and 59.4 cm, respectively) in plots with 75% berseem clover. In other words, the plant height of Italian ryegrass decreased depending on the decreasing nitrogen dose and the decreasing clover ratio in the mixtures. In Italian ryegrass, the shortest plant height was measured in the control plot (44.8 cm) without nitrogen fertilizer and in the plot (51.9 cm) with the lowest rate of berseem clover (25%) in the mixtures.

In Italian ryegrass, plant height was positively affected by increasing nitrogen doses and increasing berseem clover ratio in mixtures. Depending on the increase in the rate of berseem clover, we can think that the increase in plant height of Italian ryegrass is since some of the nitrogen it needs is met from berseem clover. Again, we can state that the plant height may have increased in Italian ryegrass due to light competition with berseem clover.

Many factors affect plant height in Italian ryegrass. One of the researchers working with Italian ryegrass, [17] stated that the plant height in Italian ryegrass increased with the increase of the applied fertilizer doses, decreased with the increase of the spacing between the rows, and the lowest plant height was 86.17 cm

Table 3. Plant height (cm), stem thickness (mm), green fodder yield (kg da^{-1}) and hay yield (kg da^{-1}) related mean values and LSD groups in Italian ryegrass.

Treatment	Plant Height (cm)**	Stem Thickness (mm)*	Green Fodder Yield (g m^{-2})**	Hay Yield (g m^{-2})**
IRM N0	44.8 d	2.79 bc	3047 e	800.3 c
IRM N5	57.1 bc	3.13 abc	3247 de	825.3 c
IRM N10	61.1 abc	3.28 ab	4021 cde	1035.7 bc
IRM N15	61.9 ab	2.95 bc	4983 bc	1250.3 bc
IRM N20	68.3 a	3.50 a	5070 bc	1178.7 bc
25%IR+75%BC	59.4 abc	2.68 c	7029 a	1819.0 a
50%IR+50%BC	56.4 bc	2.76 c	5917 ab	1429.0 ab
75%IR+25%BC	51.9 cd	2.69 c	4639 bcd	1167.7 bc
Mean	57.6	2.97	4744	1188.3

*: The means with the same capital letter in the same column are not statistically significant different from each other according to the LSD test at $p \leq 0.05$

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(between the control and 40 cm rows) and 96.17 cm (15 g m^{-2} nitrogen dose and 20 cm row spacing). [18] found the plant height to be between 50.1 cm and 63.8 cm depending on the nitrogen dose applied in Italian ryegrass, and the plant height increased up to 4, 8, 12 and 16 g m^{-2} nitrogen dose from the control, a nd the plant height increased at later doses (20 and 24 g m^{-2} N) decreased and even shorter than the control at 24 g m^{-2} nitrogen dose. [19] found that the average plant height in Italian ryegrass varies between 76.9 cm and 86.6 cm, and the effect of nitrogen doses on plant height averages is significant. [20] determined that the effect of nitrogen doses on plant height in Italian ryegrass was positive, but the difference between increasing nitrogen doses was statistically insignificant and the average plant height changed from 48.09 cm to 62.92 cm. [21] showed that the plant height varies between 84.69 cm and 95.11 cm depending on the nitrogen dose applied in the Italian ryegrass, the average plant height increased up to 20 g m^{-2} nitrogen dose from the control, 10, 15, 20 and 25 in the highest plant height maintenance. He found that the nitrogen doses in g m^{-2} were in the same statistical group. [13], in his study of mixture of Italian ryegrass and berseem clover, determined that the effect of mixture ratios on plant height of Italian ryegrass was statistically significant and varied between 88.13-123.76 cm. [16] found the plant height of Italian ryegrass to be 99.14-103.17 cm in its mixture with Anatolian clover, and as 47.94-50.78 cm in its mixture with [22] red clover. [23] plant height was 100-107.3 cm, [24] 76.0-82.7 cm, and [25] 48.7-65.7 cm, which made a variety trial on Italian ryegrass. The data we obtained from our study were generally lower than the results of the researchers. The reason for this can be shown that because of the interaction of climate, soil, agricultural practices, and the variety used, the variety we use does not the stem elongation due to the inability to meet the vernalization need.

Stem Thickness

As a result of variance analysis, the difference between the average stem thickness of Italian ryegrass was found to be statistically significant at the $p \leq 0.05$ level. The stem thickness of Italian ryegrass varied between 2.69 mm (75%IR+25%BC) – 3.50 mm (IRM N20) according to the applications (Table 3). While the stem thickness was high in nitrogen fertilizer applications, it was low in the mixtures. Competing with berseem clover in mixtures, Italian ryegrass tended to be slender and tall stem.

[13], who studied the mixture of Italian ryegrass and berseem clover, found that the stem thickness of Italian ryegrass varied between 2.42 and 3.19 mm and the effect of the mixtures on the stem thickness was statistically significant. [26] determined the stem thickness in Italian grass between 1.69-3.75 cm, [18] 3.04-3.69 mm, [19] 3.2-3.8 mm and [21] 2.74-3.32 mm. While there are similarities between the results of our study and the results of the researchers, there are also differences. The reason for this may be the different varieties used in the studies, the effect of the study subjects, the climate, soil, and growing conditions in which the experiments were carried out.

Green Fodder Yield

When different nitrogen doses in Italian ryegrass and different berseem clover mixtures were evaluated together as the levels of the same application, the differences between the green fodder yield averages were found to be statistically significant at the $p \leq 0.01$ level. Green fodder yields ranged from 3047 to 7029 g m^{-2} (Table 3). Green fodder yields generally increased due to the increase in the ratio of berseem clover in the mixtures and the increase in the nitrogen dose in nitrogen fertilization. While making an evaluation here, it should not be ignored that a significant part of the green fodder yield in the mixtures comes from the berseem clover. However, if the green fodder yield per square meter is thought to be high, it would be more appropriate to increase the ratio of berseem clover in the mixture by growing Italian ryegrass in a mixture with berseem clover instead of giving high nitrogen fertilizer.

In the study, the total green fodder yield varied between 3046.7-7029.0 g m^{-2}. One of the researchers [21] determined the green fodder yield between 2252.6 g m^{-2} (control 0 g m^{-2} N) and 2982.0 g m^{-2} (25 g m^{-2} N). [17] found the average green forage yield between 1555.4 (control) - 2034.0 g m^{-2} (15 g m^{-2} N) depending on nitrogen doses, although he found an increase depending on nitrogen doses, this difference was not statistically significant. [27] determined the average green forage yield between 622.34 (control) – 1162.72 g m^{-2} (20 g m^{-2} N) and an increase in green forage yield depending on the increasing nitrogen dose. [28] who studied mixtures of Italian ryegrass and berseem at different rates, obtained the green fodder yield between 4153.0 g m^{-2} (20%IR+80%BC) and 6318.66 g m^{-2} (80%IR+20%BC) in the mixtures. they have determined. [13] found the effect of mixing ratios on green fodder yield in mixtures of Italian ryegrass and berseem clover important and determined green fodder yield at 2930.6-3455.6 g m^{-2}. [29] found that the green fodder yield in Italian ryegrass and berseem clover mixtures increased due to the increase in berseem clover, this increase was statistically significant, and the green fodder yield in the mixtures was 3178.8 g m^{-2} (20%IR + 80%BC) and 4123.8 g m^{-2} (80%IR + 20%BC). [12] obtained the highest green fodder yield with 1732. 2 g m^{-2} from 80%IR+20%AC mixture. In the Italian ryegrass variety trials, [23] determined the green fodder yield between 3108-5550 g m^{-2}, [24] 3377.3-4457.7 g m^{-2} and [25] 1978.1-2764.8 g m^{-2}. When we compare the results of the above-mentioned researchers with the results of the studies, we see that the results we obtained are higher, close, or lower than some.

The reason for the differences may be due to the different genotypes of Italian ryegrass and berseem clover used in the studies, the soil, climate, planting time, vegetation period of the study area and the agricultural practices applied.

Hay Yield

When different nitrogen doses in Italian ryegrass and different berseem clover mixtures were evaluated together as the levels of the same application, the differences between hay yield averages were found to be statistically significant at the p≤0.01 level. Hay yields ranged from 800.3 to 1819.0 g m^{-2}. Hay yields generally increased due to the increase in the ratio of berseem clover in the mixtures and the increase in the nitrogen dose in nitrogen fertilization. While a significant part of the hay yield in the mixtures came from the berseem clover, the increase in hay yield in Italian ryegrass monoculture was due to the application of nitrogen fertilization. The opinions reported on green fodder yield is valid for dry grass yield.

The hay yield in Italian ryegrass according to the nitrogen doses, [21] determined between 733.4 g m^{-2} (control) – 947.7 g m^{-2} (25 g m^{-2} N) and [18] 224.4 (24 g m^{-2} N) – 455.9 (8 g m^{-2} N) [27] stated that it varies between 213.7 (control) – 383.6 g m^{-2} (20 g m^{-2} N) and [17] 346.1 (control) – 523.1 g m^{-2} (15 g m^{-2} N). Among these researchers, [21], [18] and [27] found the effect of nitrogen doses on hay yield to be statistically significant, while [17] found it insignificant. The researchers who studied the mixture of Italian grass and berseem clover in different proportions, [28] obtained between the hay yield with 680 g m^{-2} (BCM) – 1187 g m^{-2} (80%BC + 20%IR), [13] 769.3 g m^{-2} (BCM) – 1038.8 g m^{-2} (80%BC + %20IR), [29] 670.2 g m^{-2} (BCM) – 1659.2 g m^{-2} (80%BC + 20%IR). When all the above research results and our research results are evaluated together, it can be said that the yield varies according to the monoculture or mixture ratios, the varieties used, the climate and soil conditions of the regions where the studies are carried out, and in most of the studies, the hay yield increases depending on the increase in the berseem clover in the mixture or the increase in the applied nitrogen dose.

Dry Matter Content

As a result of the analysis of variance, the difference between the dry matter ratio averages was not statistically significant. Dry matter content ranged from 21.9% (IRM N20) to 24.0% (IRM N0). The effect of applying different doses of nitrogen fertilizer or mixing with berseem clover at different rates on the dry matter ratio was insignificant. Dry matter ratio was seen as a very stable property.

[12] obtained the highest dry matter ratio from a mixture of 40%BC + 60%IR. [16] found that the dry matter ratio of Italian ryegrass is higher than that of Italian ryegrass + Anatolian clover mixtures and Anatolian clover monoculture. Similarly, [22] found the dry matter ratio of Italian ryegrass sown to be higher than that of Italian ryegrass + red clover mixtures and red clover monoculture. In our study, it is like the results of the researchers in that the dry matter ratio increases due to the increase in the ratio of Italian ryegrass in the mixtures and that the highest dry matter ratio is obtained from the Italian ryegrass monoculture.

Dry Matter Yield

As a result of the analysis of variance, the difference between the dry matter yield averages was found to be statistically significant at the p≤0.01 level. Dry matter yields varied between 733.2-1681.7 g m^{-2} (Table 4). Dry matter yields generally increased due to the increase

Table 4. Dry matter content (%), dry matter yield (kg da^{-1}), crude protein percentage (%) and crude protein yield (kg da^{-1}) related mean values (cm) and LSD groups in Italian ryegrass.

Treatment	Dry matter (%)	Dry matter yield (g m^{-2})**	Crude protein percentage (%)**	Crude protein yield (g m^{-2})**
IRM N0	24.0	733.2 c	8.73 e	50.10 d
IRM N5	23.9	771.2 c	9.00 e	69.67 cd
IRM N10	23.9	966.4 bc	10.40 d	100.50 bcd
IRM N15	23.7	1186.8 abc	10.33 d	122.43 bc
IRM N20	21.9	1106.8 bc	14.50 a	160.97 b
25%IR+75%BC	23.7	1681.7 a	14.03 b	234.07 a
50%IR+50%BC	22.4	1322.9 ab	12.83 c	166.07 ab
75%IR+25%BC	23.8	1082.6 bc	12.60 c	132.30 bc
Mean	23.4	1106.5	11.55	129.51

**: The means with the same capital letter in the same column are not statistically significant different from each other according to the LSD test at p≤0.01

in the berseem clover ratio in the mixtures and the increase in the nitrogen dose in nitrogen fertilization. While a significant part of the dry matter yield in the mixtures came from the berseem clover, the increase in dry matter yield in monoculture was due to the application of nitrogen fertilization. The effects of the applications on dry matter ratios were found to be insignificant, and the factors affecting the green fodder yield were reflected to the dry matter yield. For this reason, the opinions reported on green fodder yield are valid for dry matter yield.

[19] found the effect of nitrogen doses on dry matter yield in Italian ryegrass to be significant, with the lowest control plots (781.9 g m^{-2}) and the highest 25 kg nitrogen applied per square meter (1222.6 g m^{-2}), but the differences between the plots fertilized with 25, 20 and 15 kg nitrogen per square meter were statistically in the same importance group. Similarly, in our study, the highest dry matter yields were obtained from the parcels that fertilized with 15 kg (1237.4 g m^{-2}) and 20 kg (1106.8 g m^{-2}) nitrogen per square meter. [20] found an increase in dry matter yield (518.9, 957.4, 1130.2, 1368.5, 1419.8, 1644.9 and 1773.9 g m^{-2}, respectively) due to the increase in nitrogen doses (0, 10, 20, 30, 40, 50 and 60 g m^{-2} N) in Italian ryegrass. The increase in dry matter yield depending on the nitrogen doses and the yields obtained from the application of 10, 20 kg nitrogen doses per square meter are like our study. In mixtures of Italian ryegrass and berseem clover, [12] obtained the highest dry matter yield at 371.13 g m^{-2} from the mixture of 20%BC + 80%IR. [30] reported that the highest dry matter yield was obtained from the seed mixture of 60%BC-40% IR. [16] obtained the highest dry matter yield from monoculture of Anatolian clover, and the lowest from monoculture of Italian ryegrass, in mixtures of Italian ryegrass and Anatolian clover. In this respect, it is like our study.

Crude Protein Percentage

As a result of the analysis of variance, the difference between the crude protein ratio averages was found to be statistically significant at the $p \leq 0.01$ level. When we evaluate the effect of nitrogen fertilization and the mixture together, the highest crude protein ratio was obtained from IRM N20 with 14.5% and the lowest was obtained from IRM N0 with 8.73% (Table 4). In general, the crude protein ratio increased with the increase in the applied nitrogen dose. And again, as the ratio of berseem clover in the mixture increased, the crude protein ratio increased. For this reason, we can recommend increasing the nitrogen fertilizer dose to increase the crude protein ratio in Italian ryegrass monoculture and increasing the ratio of berseem clover in the mixture. Considering the environmental impact of producing and using nitrogen fertilizer, as well as the economic cost of nitrogen fertilizer application, growing Italian ryegrass in a mixture with berseem clover for forage production with high crude protein content may be an alternative for sustainability.

They found that the crude protein ratio of [25] 10.03-12.13%, [24] 14.63-21.13%, and [23] 11.21-15.47%, according to Italian ryegrass varieties, and these changes were statistically significant. [21] found statistically significant increases in crude protein ratio in Italian ryegrass depending on the increase in the dose of nitrogen fertilizer applied, the lowest crude protein content was obtained from the control with 8.13%, while the highest was obtained from the application of 30 kg nitrogen per square meter with 13.90%. [20] found significant increases in crude protein ratio depending on the nitrogen doses applied in Italian ryegrass. The crude protein ratio, which was 12.65% in the control plots, increased to 18.37% in the plots where 60 kg nitrogen was applied per square meter [20]. [19], who carried out a nitrogen fertilizer study in Italian ryegrass, found the effect of nitrogen doses on the crude protein percentage to be statistically significant and obtained the crude protein percentage from the control plots with the lowest 12.9%, the highest from the plots applied 30 g m^{-2} N with 15.8%. When we compare the results of the research with the results of our research, it is generally like the fact that the crude protein ratio increases at a significant level depending on the amount of nitrogen given per square meter in Italian ryegrass and it is the lowest in the control plots. In the mixtures of Italian ryegrass and berseem clover, the crude protein ratio was determined by [13] 12.94% (IRM) – 20.26% (BCM), [31] 13.5% (IRM) – 22.0% (BCM) and [29] found it to be between 10.71% (20%BC + 80%IR) – 12.99% (BCM). [12] obtained the highest crude protein ratio as 14.84% from a mixture of 80%BC + 20%IR. Crude protein ratio of berseem clover, which is a legume plant, is higher than Italian ryegrass. Therefore, in general, as the ratio of berseem clover to the mixtures increases, the crude protein ratio of the mixture also increases. In this respect, our research results are like the results of other researchers. [15] state that the crude protein content of the grass obtained from the mixtures decreases as the legume ratios decrease in the mixtures. While the crude protein ratio was close to the results of some of the other researchers in our study, it was lower than the results of some. The reason for this can be shown as the effect of nitrogen doses applied, berseem clover mixture ratios, the variety used, climate, soil and agricultural practices in Italian grass.

Crude Protein Yield

As a result of the analysis of variance, the difference between the crude protein yield averages was found to be statistically significant at the $p \leq 0.01$ level. While the highest crude protein yield was obtained from the 25%IR + 75%BC with 234.07 g m^{-2}, the difference between the 50%IR + 50%BC with 166.07 g m^{-2} was not statistically significant (Table 4). The lowest crude protein yield was obtained from IRM N0 with 50.10 g m^{-2}

and it was in the same importance group with IRM N5 and IRM N10. Climatic conditions during the vegetation period in which the study was carried out made berseem clover more productive than Italian ryegrass. For this reason, the crude protein yield of the plots with high berseem clover in the mixture was also high. If Italian ryegrass is thought to be grown in summer in Central Anatolia and similar climatic conditions, we can recommend it to be grown in a mixture with berseem clover instead of giving high nitrogen fertilizer to Italian ryegrass for high protein yield per unit area. However, to make this inference healthier, it would be beneficial to conduct similar experiments with different varieties and for many years.

Researchers who conducted nitrogen fertilizer studies on Italian turf [19] determined that the crude protein ratio changed between 91.6 g m^{-2} (N0) – 172.5 g m^{-2} (25 g m^{-2} N), [18] 36.12 (N0) – 68.18 g m^{-2} (12 g m^{-2} N), [27] 30.83 g m^{-2} (N0) – 79.89 g m^{-2} (20 g m^{-2} N), [20] 49.3 g m^{-2} (N0) – 266.9 g m^{-2} (60 g m^{-2} N) and [21] 57.6 g m^{-2} (N0) – 121.4 g m^{-2} (20 g m^{-2} N). In all the research results mentioned above, the effect of nitrogen fertilizer doses on crude protein yield was found to be statistically significant, in general, the lowest crude protein yields were obtained from control (N0) parcels where nitrogen was not applied, and there was an increase in crude protein yields depending on nitrogen dose.

In studies of Italian grass and berseem clover mixture, crude protein yield ranged from 114.11 g m^{-2} (IRM) to 190.76 g m^{-2} (80%BC+20%IR) [13], from 132.4 g m^{-2} (20%BC + 80%IR) to 234.30 (80%BC + 20%IR) [28] and from 76.3 g m^{-2} (IRM) to 205.5 g m^{-2} (80%BC + 20%IR) from the [29]. All the researchers mentioned above have determined the highest proportion of crude protein in mixtures containing 80% berseem clover. Similarly, in the study we conducted, the highest crude protein yield among the mixtures was obtained from the mixtures with the highest berseem clover ratio (75%BC + 25%IR).

Conclusions

Within the scope of the study, the effects of nitrogen fertilizer application and mixture of Italian ryegrass + berseem clover on grass yield and quality of Italian ryegrass were tried to be determined. The potential of obtaining the same yield and quality in Italian ryegrass by making a mixture with berseem clover instead of nitrogen fertilizer has been investigated.

The results obtained as a result of the study are summarized below.

The effect of increasing nitrogen fertilizer doses on the yield and quality of Italian ryegrass was found to be positive and significant. The yield and quality of Italian ryegrass increased in parallel with the increase in fertilizer dose. In Konya conditions and similar ecologies, nitrogen fertilizer can be applied with the calculation of 15-20 g m^{-2} pure substance in Italian ryegrass.

As the ratio of berseem clover increased in mixtures of Italian ryegrass and berseem clover, the yield and quality of the mixture increased. Dry grass and dry matter yields were obtained from the highest 25%IR + 75%BC and50%IR+50%BC. The reason for this is the low dry matter ratio in the berseem clover. Italian ryegrass increased the dry matter ratios of the mixtures. For Konya and similar ecologies, 25%IR + 75%BC and 50%IR + 50%BC ratios of Italian ryegrass + berseem clover mixtures can be recommended.

When nitrogen fertilization and mixture experiment are evaluated together, green fodder yields generally increased due to the increase in the ratio of berseem clover in the mixtures and the increase in the nitrogen dose in nitrogen fertilization. The issue that should not be ignored while making an evaluation here is that a significant part of the green forage yield in mixtures originates from the berseem clover. In order to get high green grass yield from Italian ryegrass, it would be more appropriate to grow it in a mixture with berseem clover and to increase the ratio of berseem clover in the mixture instead of fertilizing with high doses of nitrogen.

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Conflict of Interest

The authors declare no conflict of interest.

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