

How to cite: Orou Ouennon Assouma, S. M. S. & A. E. Çelen, 2022. Effects of different nitrogen doses and cultivars on some nutritive value of annual ryegrass (*Lolium multiflorum* var. *westerwoldicum*) silage. Ege Univ. Ziraat Fak. Derg., 59 (2): 225-234, <u>https://doi.org/10.20289/zfdergi.970316</u>



Research Article (Araștırma Makalesi)

Sabi Mohamed Sani OROU OUENNON

Ahmet Esen ÇELEN2*

¹ Ege University, Graduate School of Natural and Applied Sciences, 35100, Bornova, İzmir, Türkiye

² Ege University, Faculty of Agriculture, Department of Field Crops, 35100, Bornova, İzmir, Türkiye

*Corresponding author (Sorumlu yazar): <u>esen.celen@ege.edu.tr</u>

Keywords: Cultivar, annual ryegrass, nitrogen dose, nutritive value, silage

Anahtar sözcükler: Azot dozu, besin değerleri, tek yıllık çim, silaj

Ege Üniv. Ziraat Fak. Derg., 2022, 59 (2): 225-234 https://doi.org/10.20289/zfdergi.970316

Effects of different nitrogen doses and cultivars on some nutritive value of annual ryegrass (*Lolium multiflorum* var. *westerwoldicum*) silage*

Farklı azot dozları ve çeşitlerin tek yıllık çim (*Lolium multiflorum* var. *westerwoldicum*) silajının bazı besin değerleri üzerine etkileri

* This article has been summarized from the first author's PhD thesis. This article was supported by the Unit of Scientific Research Projects, Ege University as project number FDK-2020-21529.

Received (Aliniş): 12.07.2021

Accepted (Kabul Tarihi): 04.12.2021

ABSTRACT

Objective: The objective of this study was to determine the effects of different nitrogen doses and cultivars on some nutritive value of annual ryegrass (*Lolium multiflorum* var. *westerwoldicum*) silage.

Material and Methods: The study was conducted as a field experiment at Ege University, Department of Field Crops during the growing season of 2019-2020. Four annual ryegrass cultivars (Elif, Big Boss, Baquend, and Medoacus) and five nitrogen doses (0, 75, 150, 225, and 300 kg ha⁻¹) were investigated. Some characteristics tested in the experiment were silage dry matter, crude ash, crude protein contents, NDF and ADF contents and silage RFV.

Results: The characteristics studied were affected by the applications. While the highest silage dry matter ratio was obtained with 225 kg N ha⁻¹, 300 kg N ha⁻¹ application gave the highest crude protein rate and RFV. The lowest NDF and ADF rates were obtained at a fertilizer rate of 300 kg N ha⁻¹ application. Baquend cv. gave the highest silage dry matter ratio and crude ash ratio.The highest crude protein ratio, lowest NDF and ADF ratios and highest silage RFV were obtained from Elif cv.

Conclusion: Elif cultivar had acceptable silage properties at a nitrogen dose of 225 kg ha⁻¹.

ÖΖ

Amaç: Bu çalışma, farklı azot dozları ve çeşitlerinin tek yıllık çim (*Lolium multiflorum* var. *westerwoldicum*) silajının bazı besin değerleri üzerine etkilerini belirlemek amacıyla yapılmıştır.

Materyal ve Yöntem: Deneme, 2019-2020 yetiştirme sezonunda Ege Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü'nde tarla denemesi olarak yürütülmüştür. Dört tek yıllık çim çeşidi (Elif, Big Boss, Baquend ve Medoacus) ve beş azot dozu (0, 75, 150, 225 ve 300 kg ha⁻¹) incelenmiştir. Çalışmada silaj kuru maddesi, ham kül, ham protein içeriği, NDF ve ADF içeriği ve Nispi Yem Değeri giibi bazı özellikler incelenmiştir.

Araştırma Bulguları: İncelenen özellikler uygulamalardan etkilenmiştir. En yüksek silaj kuru madde oranı 225 kg N ha⁻¹ ile elde edilirken, en yüksek ham protein oranı ve Nispi Yem Değerini 300 kg N ha⁻¹ uygulaması vermiştir. En düşük NDF ve ADF oranları 300 kg N ha⁻¹ uygulamasından alınmıştır. Baquend çeşidi en yüksek silaj kuru madde oranı ve ham kül oranını vermiştir. En yüksek ham protein oranı, en düşük NDF ve ADF oranları ve en yüksek silaj Nispi Yem Değeri Elif çeşidinden elde edilmiştir.

Sonuç: Elif çeşidi 225 kg ha⁻¹ azot dozunda kabul edilebilir silaj özelliklerine sahip olmuştur.

INTRODUCTION

Climate change, which is much more pronounced than in the past, particularly in terms of agricultural products, has a negative impact on not only human nutrition but also animal feed. As a result, scientists have begun to explore a broader range of materials with high resistance to animate and inanimate pressure components (Pimentel et al., 2008). Annual ryegrass is also known as annual ryegrass because it was originally grown in Italy as an annual forage crop and recently attracted the interest in Türkiye. Annual ryegrass (*Lolium multiflorum* Lam.) is one of the grasses with the greatest potential for forage production and fertilizer use efficiency. Herbage yields of 15.000 to 25.000 kg ha⁻¹ and hay yields of 5.000 to 8.000 kg ha⁻¹ can be obtained under natural conditions. It can be harvested 2-3 times in areas with plenty of water, yielding 40.000-60.000 kg ha⁻¹ fresh herbage and 7.500-15.000 kg ha¹ hay (Baytekin et al., 2009). Annual ryegrass production in Türkiye has increased recently due to government subsidies for forage crops. In 2014, although the cultivation area was 483.2 ha and production was 17.023 tons of fresh herbage whereas in 2019, the cultivation area was 10.341 ha and production was 448.086 tons of fresh herbage (TUIK, 2019).

Annual ryegrass should be fertilized with the necessary combination and rate in the required time to achieve the desired yield and quality. Forage consistency, in addition to yield, is critical for animal welfare. The most of dry matter is made up of nitrogen, which is the most essential nutrient for plants. Plant proteins, chlorophyll, enzymes, and vitamins also include nitrogen. The most important fertilizer consumed by grasses is nitrogen. Excessive nitrogen use in plants results in the buildup of nitrates and alkaloids, while adequate nitrogen fertilization increases grass protein content. The use of nitrogen fertilizers generated a strong positive response in annual ryegrass (Ozdemir et al., 2019).

In experiments on fertilizer use on annual ryegrass, different findings have been obtained. In an analysis of fertilizer rate and application time in annual ryegrass in southern America, Linn and Martin (1989) found that applying 55 kg ha⁻¹ in the autumn and 55 kg ha⁻¹ in the spring yielded the most costeffective results. The study provided 13500 kg ha⁻¹ of high-quality forage, with an average of 24% crude protein and 22% ADF over two years. Pavinato et al. (2014) found the highest dry matter and crude protein yield with a dosage of 120 kg ha⁻¹ of N in Brazil. Ozdemir et al. (2019) reported that 500 kg ha⁻¹ of nitrogen was ideal for high yield and high quality annual forage yields. Demiroglu Topcu et al. (2021) indicated that all characteristics were affected from cultivars and harvesting times and Rambo or Vaspolini cultivars may be preferred. Researchers also found that harvesting at heading stage resulted in higher quality feed.

In their studies of the nutritive value of annual ryegrass hay and silage, Ohshima et al (1988) found no difference in nutrient content, but the dry matter digestibility of silage (77%) was higher than that of hay (73%). Bernard (2003) who worked with corn silage and annual ryegrass silages and their mixture found that herd fed annual ryegrass silage yielded more milk than the herd fed corn silage. When the silages were combined, it was discovered that a high energy and protein silage was created. The researchers did point out, however, that using too much silage in the ration decreased digestibility significantly.

Aganga et al. (2004) found that increase the number of cutting increased crude protein decreased, NDF, ADF and ADL increased and In Vitro True Digestibility (IVTD) decreased in annual ryegrass.

Besides studies on nitrogen rates, several other studies such as the use of additives, timing of harvest, inoculation, wilting of the plant before silage have also been conducted on annual ryegrass. However, there is no study on the combination of nitrogen application and silage in annual ryegrass. Hence a study was conducted and the objective of this study was to determine how different nitrogen doses affect the nutritive value of the silage of annual ryegrass cultivars.

MATERIAL and METHODS

Location of experiment

The study was conducted during the 2019-2020 growing season in the experimental area of the Department of Field Crops (38°27'05.93" N, 27°13'29.39" E) of the Agricultural Faculty of Ege University, Izmir, Türkiye, at about 20 m elevation above sea level with typical Mediterranean climate characteristics.

The average monthly air temperature of the research site and the monthly values of total precipitation are shown in Table 1, and the soil properties of the experiment area are tabulated in Table 2. In terms of the climatic and soil characteristics of the research site, there were no limiting factors for growing annual ryegrass plants.

Mantha	Aver	age Temperature	e (°C)	Total Precipitation (mm)			
Months –	2019	2020	LYA*	2019	2020	LYA	
January	9.1	8.6	8.8	412.3	40.0	121.0	
February	10.4	11.1	9.5	109.1	70.1	101.9	
March	13.6	13.6	11.7	41.4	58.6	74.3	
April	16.2	16.4	15.8	49.9	51.0	47.0	
Мау	21.6	21.4	20.8	13.3	79.1	29.3	
June	27.4	25.1	25.6	23.2	38.3	8.3	
July	28.2	29.0	28.0	0.9	0.0	2.0	
August	29.5	28.6	27.6	0.0	0.0	2.2	
September	24.3	26.8	23.6	33.0	0.0	15.7	
October	20.5	21.5	18.8	23.1	48.5	44.3	
November	17.9	14.5	14.1	67.2	4.9	95.0	
December	11.7	12.7	10.5	97.0	290.4	144.1	
Average/Total	19.2	19.1	17.9	870.4	680.9	685.1	

Table 1. Some meteorological data of the experimental area

 Cizelge 1. Deneme alanına ait bazı meteorolojik veriler

* Long Years Average.

Table 2. Some physical and chemical characteristics of the soil of the experimental area

 Çizelge 2. Deneme alanı toprağının bazı fiziksel ve kimyasal özellikleri

Characteristics	0-30 cm	Characteristics	0-30 cm
рН	7.880	Available phosphorus (ppm)	6.75
Total salt (%)	0.091	Available potassium (ppm)	505.70
Lime (%)	14.180	Available calcium (ppm)	5747.80
Sand (%)	42.400	Available magnesium (ppm)	367.10
Silt (%)	20.720	Available sodium (ppm)	72.80
Clay (%)	36.880	Available iron (ppm)	0.18
Structure	Silty-clay	Available zinc (ppm)	1.95
Organic matter (%)	1.990	Available copper (ppm)	1.10
Total nitrogen (%)	0.050	Available manganese (ppm)	1.17

Field applications and experimental design

In the study, the effects of five different nitrogen rates (0, 75, 150, 225 and 300 kg ha⁻¹) on the silage nutritive value of four annual ryegrass cultivars (Elif, Big Boss, Baquend and Medoacus) were investigated. Elif cv. was obtained from Ege Agricultural Research Institute while the others were taken from two commercial companies. The experiment was conducted as a randomized block split plot trial

design with three replications. Nitrogen rates were placed in the main plots and annual ryegrass cultivars in the subplots. Each experimental plot was 6 m^2 in size (2 m x 3 m = 6 m^2) and consisted of 60 plots in total. The total experimental area covered 360 m² ($6 \text{ m}^2 \text{ x } 60 \text{ plots}$). There were 10 rows in each plot, 3 blocks of 20 plots each and 2 m space between blocks and 1 m space between main plots.

Once the soil was prepared, the seeds of annual ryegrass were sown on November 20, 2019. The seeding rate was 30 kg ha⁻¹ (Soya et al. 1997). At the time of sowing, it was applied equally to each plot by calculating 50 kg of pure Triple Super Phosphate (45% TSP) fertilizer per hectare. The nitrogen doses studied were applied with fertilizers containing 20.5% ammonium sulfate and 33 % ammonium nitrate. Half of each nitrogen dose investigated (20.5% ammonium sulfate) was applied to the soil with the seed at sowing, and the other half (33% ammonium nitrate) was applied manually between rows when the plants were 20-25 cm tall. Irrigation was undertaken immediately after sowing to help in the emergence of the plants. Since there was enough rainfall during the study, no irrigation was applied. Hand and hoe were used to control the emerging weeds. No diseases were observed on the annual ryegrass plants during the vegetative growth.

Measurements, silage making and chemical analysis

In order to determine the nutritional value of the annual ryegrass cultivars used in the study, all plots were harvested at different dates between the milky and doughy phase period. The plots were harvested at ground level with a manual sickle. The harvested plants were transported to the laboratory and chopped into 2-3 cm pieces using a silage cutter, 0.5% table salt (NaCl) was added to the prepared feed. Feed samples were ensiled in special nylon bags using a vacuum machine (Johnson et al., 2005). In order to complete the fermentation process, the vacuum silage samples were stored in a cool, dry, light-free environment for 60 days.

Silage dry matter (DM) ratio was determined by drying silage samples in an oven at 105 °C for 24 hours (Bulgurlu and Ergül, 1978). The air-dried silage samples were ground (less than 1 mm) and the silage crude protein (CP) content was determined by the Kheldahl method (Kacar, 1972). Ground silage samples were burned for around 4 hours in a 550°C oven to determine the crude ash content (Bulgurlu and Ergül, 1978). The NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) ratios of the silage cell wall were calculated using the analytical methods described by Goering and Van Soest (1970). The formula (DDM x DMI)/1.29 was used to calculate the relative feed value (RFV) of silage, with Digestible Dry Matter (DDM) = 88.9 - (0.779 x ADF) and Dry Matter Intake (DMI) = 120 / NDF (Ball et al., 1996).

Statistical analysis

Throughout the study, the data from the plots were statistically evaluated using analysis of variance (ANOVA) in the statistical program "R" using the "Agricolae" kit created by De Mendiburu (2020). Statistical significance was defined as a probability level of 0.05 or less. The mean values of each parameter were compared according to LSD test described by Steel and Torrie (1980).

RESULTS and DISCUSSION

Silage dry matter

The results of the statistical analysis in terms of silage dry matter showed that there were statistically significant differences in the effects of nitrogen doses and cultivars as well as in the N x C interaction. In terms of average N rates, the highest silage dry matter was obtained as 47.60% with 225 kg N ha⁻¹ application. Nitrogen rates of 75 kg ha⁻¹ (47.29%) and 150 kg ha⁻¹ (46.68%) revealed similar values and were included in the same statistically group. The lowest silage dry matter was obtained as 45.33% with a nitrogen dose of 300 kg ha⁻¹, followed by the control (45.91%) which was in the same statistically group. In terms of cultivar means, the highest and lowest silage dry matter contents were

found in Baquend and Elif as 57.52 and 40.89%, respectively. The statistically significant N x C interaction indicated that the effect of nitrogen rates on silage dry matter ranged from 37.66% to 58.66% among cultivars. In fact, the highest silage dry matter ratios were found in the Baquend cv. in control and 225 kg N ha⁻¹ applications, while the lowest silage dry matter ratios were obtained in the control and 300 kg N ha⁻¹ applications in the Medoacus cv. (Table 3).

С	Elif	Big Boss	Baquend	Medoacus	Mean	Elif	Big Boss	Baquend	Medoacus	Mean	
N	Silage dry matter (%)						Silage crude ash (%)				
0	40.27h	47.07ef	58.65a	37.66i	45.91bc	8.32f	10.56de	13.18a	9.64ef	10.43	
75	42.31g	48.66d	55.29c	42.88g	47.29a	9.72ef	11.00cde	12.84ab	10.30de	10.97	
150	40.75h	45.66f	56.77bc	43.53g	46.68ab	10.89cde	10.93cde	12.36abc	11.42bcd	11.40	
225	40.55h	43.58g	58.66a	47.62de	47.60a	10.80cde	11.40bcd	11.27bcde	10.54de	11.01	
300	40.59h	43.77g	58.24ab	38.74i	45.33c	11.63abcd	12.76ab	10.74cde	10.22de	11.34	
Mean	40.89d	45.75b	57.52a	42.09c	46.56	10.27c	11.33b	12.08a	10.43c	11.03	
N:** C:** NxC:** CV = 2%					N:ns C:** NxC:** CV = 9.1%						
	Silage crude protein (%)					Silage NDF (%)					
0	4.60k	3.38m	3.53lm	3.991	3.87e	45.34m	65.41a	51.36hi	51.90h	53.50bc	
75	6.05i	5.10j	5.90i	4.70jk	5.44d	49.79k	64.79a	56.15de	53.82g	56.14a	
150	7.96f	7.78fg	7.09h	7.43gh	7.57c	48.76l	61.07b	55.26ef	52.01h	54.27b	
225	8.50e	9.14c	7.46gh	7.66fg	8.19b	48.461	58.26c	56.80d	50.20jk	53.43c	
300	10.49a	9.76b	9.04cd	8.62de	9.48a	47.981	54.34fg	54.86f	50.78ij	51.99d	
Mean	7.52a	7.03b	6.60c	6.48c	6.91	48.07d	60.77a	54.89b	51.74c	53.87	
	N:** C:** NxC:** CV = 4%					N:** C:** NxC:** CV = 1%					
Silage ADF (%)				Silage RFV							
0	27.71i	42.47a	33.30fg	33.12fg	34.15c	138.2a	79.37m	114.0fg	113.1fg	111.2b	
75	29.87h	40.81b	37.12cd	34.08ef	35.47a	122.7d	82.00m	99.40jk	107.8h	103.0d	
150	30.10h	38.02c	37.73c	33.39fg	34.81b	124.9cd	90.30l	100.2j	112.5g	107.0c	
225	29.83h	36.31d	36.12d	33.15fg	33.85c	126.0bc	96.80k	99.53j	116.9e	109.8b	
300	29.15h	32.61g	34.62e	33.12fg	32.38d	128.3b	108.7h	105.1i	115.6ef	114.4a	
Mean	29.33d	38.05a	35.78b	33.37c	34.13	128.0a	91.43d	103.6c	113.2b	109.1	
	N:** C:** NxC:** CV = 1.9%					N:** C:** NxC:** CV = 1.5%					

Table 3. Effects of different nitrogen doses and cultivars on some nutritive value of annual ryegrass silage

 Çizelge 3. Farklı azot dozları ve çeşitlerinin tek yıllık çim silajının bazı besin değerlerine etkileri

According to LSD test (α = 0.05), there is a significant difference between means indicated with different letters in the same row or column; (*): Significant at the 0.05 level; (**): Significant at the 0.01 level; ns: not significant; N: nitrogen rates (kg ha⁻¹); C: cultivars; NxC: nitrogen rates x cultivars interactions; CV: coefficient of variation.

Once the amount of water is removed from the plant structure, the remaining part is dry matter. Other nutrients (proteins, carbohydrates, minerals and vitamins) are included in this part of the plant. Ruminant feeding is generally based on the dry matter of the feed. This calculation allows to determine the amount of feed necessary to give to the animal. In this respect, the dry matter content of roughage is of great importance. Acikgoz (1995) reported that the dry matter content of a quality silage should be 23.50% and above. When Table 3 was examined in terms of silage dry matter ratio, it was found that the dry matter ratio was 46.56% on average. While Demirel et al. (2010) found 29.46 % silage dry matter ratio in white clover+barley mixture whereas Aykan and Saruhan (2018) found 23.70 % silage dry matter ratio in field pea + barley mixture. In another study (Bengisu, 2019) it was found that Hungarian vetch and barley mixture gave silage dry matter ratios ranging from 27.40 to 33.48 %. It is clear that the findings obtained in this study were superior as compared to the results obtained in the studies in the literature (Table 3). This may be attributed to the plant material used in the study, to the maturity at harvest or the ecological conditions.

Silage crude ash

The results of the statistical analysis in terms of silage crude ash showed that in addition to the N x C interaction, the effect of cultivars were also significant. Among nitrogen rates there were no significant differences. As shown in Table 3, the average silage crude ash contents varied between 10.43 and 11.40% depending on the application of different nitrogen rates. In terms of cultivars, the highest average silage crude ash content was obtained from Baquend cv. with 12.08%, while the lowest crude silage ash contents were taken from Medoacus (10.43%) and Elif (10.27%) cultivars, which were in the same statistical group. The statistically significant N x C interaction showed that the effect of nitrogen rates on silage crude ash content varied between 8.32% and 13.18% depending on the cultivars. Indeed, the highest silage crude ash rates were obtained from nitrogen applications of 300 kg ha⁻¹ in Elif and Big Boss cv., control, 75 and 150 kg ha⁻¹ in Baquend cv., while the lowest silage crude ash rates were obtained from nitrogen applications of 300 kg ha⁻¹ in Elif and Big Boss cv., control and nitrogen application of 75 kg ha⁻¹ in Elif cv. and control in Medoacus cv.

Bernard et al. (2002) found that the crude ash rate was 10.18% in Italian grass silage. Renlong et al. (2017) obtained 7.19% crude ash in annual ryegrass silage when they applied 60 kg N ha⁻¹ and cut at heading period. Also, annual ryegrass silage which was cut at same period and was given 120 kg N ha⁻¹ gave 10.10% crude ash. In this study, the crude ash contents of annual ryegrass silage were not statistically significant among nitrogen doses. However, the fact that slightly higher silage crude ash ratios were obtained at high nitrogen rates as compared to control and 75 kg N ha⁻¹ was compatible with the results of these researchers.

Silage crude protein

The results of the statistical analysis showed that nitrogen rates, cultivars and N x C interaction had significant effects on crude protein of annual ryegrass silage. Among nitrogen rates, the maximum crude protein ratio of 9.48% was recorded in the 300 kg ha⁻¹ application rate and the minimum of 3.87% in the control application. Among the cultivars, the maximum crude protein ratio was recorded in Elif cv. with 7.52%. The minimum ratio of crude protein was determined in Medoacus cv. with 6.48%, followed by Baquend cv. (6.60%) in the same group statistically. In terms of N x C interaction, increasing nitrogen applications in all four cultivars increased the silage crude protein ratio. The highest crude silage protein ratio was obtained in Elif cv. with 10.49% at a nitrogen application of 300 kg ha⁻¹. The lowest ratio was determined in the control application with the Big Boss cv. (3.38%) and Baquend cv. (3.53%) in the same statistical group (Table 3).

In this study, silage CP ratio improved with increasing N levels added to Italian rye. In many studies, it has been stated that N fertilizers applied to plants at a certain stage improve the CP ratios of plants (Bolat and Kara, 2017). According to Cinar et al. (2020) found, for example, that the higher the N added to Italian rye, the higher the CP ratio. This argument is in agreement with the findings obtained in this study. In addition, the results were consistent with the findings of many researchers who found similar results (Bernard, 2003; Aganga et al., 2004 and Fonseca et al., 2005). On the other hand, lower CP contents of about 10% have been reported for silage. For example, Bernard et al. (2002) reported CP values of 5.8% to 9.9% for annual ryegrass silage.

Silage NDF

According to the results of statistical analysis, the independent effects of nitrogen rates and cultivars on silage NDF ratio and the effect of N x C interaction were found to be significant. When the results were examined in terms of nitrogen rates, numerically, the highest NDF ratio was determined to be 56.14% at a nitrogen rate of 75 kg ha⁻¹, and the lowest NDF ratio was 51.99% at a nitrogen rate of 300 kg ha⁻¹ (Table 3). When the NFD rates are examined in terms of cultivars, it could be stated that the lowest and highest NDF rates were recorded in Elif (48.07%) and Big Boss (60.77%). In terms of N x C

interaction, the average silage NDF ratio ranged from 45.34% to 65.41%. In fact, the highest silage NDF ratios were found in Big Boss cv. in control and 75 kg N ha⁻¹ applications, while the lowest were obtained in control application in Elif cv.

This study revealed that as N levels applied to annual ryegrass increased, NDF contents in silage feed generally decreased, excluding control application. NDF content in feeds varies largely with plant age, or harvest time. The relative increase in the components that make up the cell wall (cellulose, hemicellulose, and lignin) causes an increase in NDF content as the plant ages (Kavut et al., 2014). However, increase in NDF ratio reduces the feed's digestion level. The composition of cell walls is related to feed intake capability. Colak and Sancak (2017), for example, found that annual ryegrass cultivars had a major impact on NDF rates in various nitrogen dose applications, ranging from 54.14 to 56.01%. According to Van Soest (1985), the NDF composition should be 36% in order to ensure optimal milk yield in dairy cattle. The results for silage NDF content obtained in this study were similar to those of Colak and Sancak (2017) but lower than those of Fonseca et al. (2005), who found 63.8%.

Silage ADF

The results of the statistical analysis revealed that the N x C interaction, as well as the independent effects of nitrogen rates and cultivars on silage ADF ratio were significant. Among nitrogen rates, the highest ADF ratio of 35.47% was determined in 75 kg N ha⁻¹ application and the lowest ratio of 32.38% in 300 kg N ha⁻¹ application. Among the varieties, the highest and the lowest ADF ratios were found as 38.05% and 29.33% in Big Boss and Elif cultivars, respectively. In terms of N x C interaction, the average silage ADF ratio ranged from 27.71 to 42.47%. In fact, the highest and lowest silage ADF ratios were found in the control applications of the Big Boss and Elif cultivars, respectively (Table 3).

Analyzing the findings of this study on the ADF content of annual ryegrass silage in general, as well as the NDF content, we found that, apart from the control, increased nitrogen levels decreased ADF content. This decline is regarded as a good indication for feed consistency. Since ADF includes cellulose, lignin, and silica, it reflects the cell wall portion left over from the feed's processing under acid detergent conditions. This characteristic is also largely related to plant age, but it was positively affected by increasing N levels in our research. The results for silage ADF content were smaller than those of Fonseca et al. (2005) (39.3 %), but higher than those of Amrane and Michalet-Doreau (1993) (21.9% to 27.4%). According to several researchers (Ball et al., 1996), if NDF contents in forages are greater than 40% and ADF contents are greater than 30%, feed value starts to decrease. From this perspective, it was seen that the ADF levels obtained in control applications in Elif cv. ensured a quality feed. Increasing N levels enhanced these properties allowing annual ryegrass to be evaluated as silage.

Silage RFV

The results of the statistical analysis in terms of relative feed value of silage showed that in addition to the N x C interaction, the effects of nitrogen rates and cultivars also showed statistically significant differences. As shown in Table 3, the highest average silage RFV 114.4 was obtained with a nitrogen rate of 300 kg ha⁻¹, whereas the lowest value 103.0 was taken from nitrogen rate of 75 kg ha⁻¹. In terms of cultivars, the highest average silage RFV was observed in Elif with 128, whereas the lowest average silage RFV was obtained in Big Boss with 91.43. In terms of N x C interaction, the average relative feed value of silage ranged from 79.37 to 138.2. In fact, the highest average silage RFV was determined from control application in Elif cv., whereas the lowest values were obtained from control and 75 kg N ha⁻¹ applications in Big Boss cv. (Table 3).

The results, apart from the control, showed that as N levels applied to annual ryegrass increased, RFV in silage feed generally increased. As it is known, the relative value of feed is a measure that is calculated using the NDF and ADF values of feed and numerically shows the quality of feed and is taken

into account not only by researchers but also by feed traders in the quality assessment. In addition, RFV is negatively correlated with ADF and NDF ratios. Consequently, a high relative feed value is expected for low ADF and NDF components. If the RFV of the feed is greater than 151 and it is graded as "high quality," between 151 and 125 as "first class," 124-103 as "second class," 102-87 as "third class," 86-75 as "fourth class," and less than 75 as "fifth class" (Trotter and Johnson, 1992; Ball et al., 1996). RFVs obtained in the present study ranged from 103-114.4 depending on nitrogen rates and 91.43-128 depending on cultivars. The evaluation of the RFV values obtained in the study according to the quality class determined by Trotter and Johnson (1992), whatever the nitrogen rate applied, they were included in the "second class". However, according to the cultivars, Elif cv. was in the "first class", Baquend and Medoacus cultivars were in the "second class" and Big Boss cv. in the "third class".

Colak and Sancak (2017) found RFV of 111.2 in annual ryegrass at a nitrogen rate of 240 kg ha⁻¹ in a study conducted in Ankara. Kusvuran et al. (2014), on the other hand, stated RFV of 94.0 in annual ryegrass. The RFV values obtained in this research were comparable to the values reported by Colak and Sancak (2017) and higher than that of Kusvuran et al (2014).

Linn and Martin (1989) stated that the relative feed value of forage for high yielded dairy cows should be at least 124. The RFV obtained in this study for the Elif cv. was agreed with the value stated above.

CONCLUSION

In this study, in which the effects of different nitrogen doses on some silage nutritional values of annual ryegrass cultivars were investigated, it was found that the nitrogen doses and cultivars had significant effects on silage dry matter and quality. While the increase in nitrogen doses caused increases in the crude protein ratio of annual ryegrass silage, it also made improvements in the NDF and ADF ratios, which have significant effects on feed intake and digestibility. In addition, the RFV value of the feed increased with the increase in nitrogen doses. When the results are evaluated in general, it can be stated that Elif cultivar has acceptable silage properties at a nitrogen rate of 225 kg N ha⁻¹.

ACKNOWLEDGEMENTS

The authors would like to thank the Coordination of Scientific Research Projects of Ege University for its financial support.

REFERENCES

Acikgoz, E., 1995. Yem Bitkileri (II. Baskı), Uludağ Üniversitesi Ziraat Fakültesi Basımevi No:7-025-0210, Bursa, 456 s.

- Aganga, A. A., U. J. Omphile, T. Thema & L. Z. Wilson, 2004. Chemical composition of ryegrass (*Lolium multiflorum*) at different stages of growth and ryegrass silages with additives. Journal of Biological Sciences, 4 (5): 645-649. https://doi.org/10.3923/jbs.2004.645.649
- Amrane, R. & B. Michalet-Doreau, 1993. Effect of maturity stage of annual ryegrass and lucerne on ruminal nitrogen degradability. Annales de Zootechnie, 42 (1): 31-37. https://hal.archives-ouvertes.fr/hal-00888860
- Aykan, Y. & V. Saruhan, 2018. Farklı oranlarda silolanan yem bezelyesi (*Pisum sativum* L.) ve arpa (*Hordeum vulgare* L.) karışımlarının silaj kalite özelliklerinin belirlenmesi. Dicle Üniversitesi Veteriner Fakültesi Dergisi, 11 (2): 64-70.
- Ball, D.M., C.S. Hovelend & G.D. Lacefield, 1996. Forage quality in Southern Forages. Potash & Phosphate Institute, Norcross, Georgia, 124-132.
- Baytekin, H., M. Kızılsimsek & G Demiroglu, 2009. "Çim ve Ayrık Türleri, 561-572". In: Yem Bitkileri Buğdaygil ve Diiğer Familyalardan Yem Bitkileri Cilt III (Eds. R. Avcıoğlu, R. Hatipoğlu & Y. Karadağ), Tarım ve Köy İşleri Bakanlığı, Izmir, 843 s.

- Bengisu, G., 2019. A Study on the silage properties of hungarian vetch (*Vicia pannonica* Crantz.) and barley (*Hordeum vulgare* L.) grass mixtures in different rates. Legume Research, 42 (5): 680-683. https://doi.org/10.18805/LR-494
- Bernard, J. K., 2003. "Feeding ryegrass silage in the South East US, 45-51". Proceedings of the 40th annual Florida Dairy Production Conference (29-30 April, 2003, Gainesville), 149 pp.
- Bernard, J. K., J. W. West & D. S. Trammell, 2002. Effect of replacing corn silage with annual ryegrass silage on nutrient digestibility, intake, and milk yield for lactating dairy cows. Journal of dairy science, 85 (9): 2277-2282. https://doi.org/10.3168/jds.S0022-0302 (02)74307-5
- Bolat, İ. & Ö. Kara, 2017. Bitki Besin Elementleri: Kaynakları, İşlevleri, Eksik ve Fazlalıkları. Bartın Orman Fakültesi Dergisi, 19 (1): 218-228.
- Bulgurlu, Ş. & M. Ergül, 1978. Yemlerin Fiziksel, Kimyasal ve Biyolojik Analiz Metodları. Ege Üniversitesi Ziraat Fakültesi Yayınları No:127, İzmir, 76 s.
- Cinar, S., M. Ozkurt & R. Cetin, 2020. Effects of nitrogen fertilization rates on forage yield and quality of annual ryegrass (*Lolium multiflorum* L.) in central black sea climatic zone in Türkiye. Applied Ecology and Environmental Research, 18 (1): 417-432. https://doi.org/10.15666/aeer/1801417432
- Colak, E. & C. Sancak, 2017. Effect of nitrogen fertilizer doses on grass quality of grass (*Lolium italicum* L.) varieties. Mediterranean Agricultural Sciences, 30 (3): 245-251. https://doi.org/10.29136/mediterranean.359996
- De Mendiburu, F., 2020. Agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-3. (Web page: https://CRAN.R-project.org/package=agricolae) (Date accessed: March 2021).
- Demirel, R., V. Saruhan, M.S. Başaran, N. Andiç & D. Demirel, 2010. Farklı oranlarda ak üçgül (*Trifolium repens*) ve arpa (*Hordeum vulgare* L.) karışımlarının silolanma özelliklerinin belirlenmesi. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 20 (1): 26-31.
- Demiroglu Topcu, G., A.E. Celen & S.S. Ozkan, 2021. The effects of different harvest times on yield and some quality components of annual ryegrass (*Lolium multiflorum* Lam.) varieties. Fresenius Environmental Bulletin, 30 (2A): 1810-1816.
- Fonseca, A. J. M., A. R. J. Cabrita, C. S. S. Nogueira, D. S. P. Melo, Z. M. C. Lopes & J. M. F. Abreu, 2005. Lactation responses of dairy cows to whole-crop wheat or ryegrass silages. Animal feed science and technology, 118 (1-2): 153-160. https://doi.org/10.1016/j.anifeedsci.2004.10.006
- Goering, H. K. & P. J. Van Soest, 1970. Forage Fibre Analyses (apparatus, reagents, procedures, and some applications). Agriculture Handbook No. 379, Agricultural Research Service, U.S. Department of Agriculture, Washington, DC, USA, 20 pp.
- Johnson, H.E., R.J. Merry, D.R. Davies, D.B. Kell, M.K. Theodorou & G.W. Griffith, 2005. Vacuum packing: a model system for laboratory-scale silage fermentations. Journal of Applied Microbiology, 98 (1): 106-113. https://doi.org/10.1111/j.1365-2672.2004.02444.x
- Kacar, B., 1972. Bitki ve Toprağın Kimyasal Analizleri: II. Bitki Analizleri. Ankara Üniversitesi Ziraat Fakültesi Yayın No: 453, Ankara, 464 s.
- Kavut T., H. Geren, H. Soya, R. Avcıoğlu & B. Kır, 2014. Karışım oranı ve hasat zamanlarının bazı yıllık baklagil yembitkileri ile tek yıllık çim karışımlarının kışlık ara ürün performansına etkileri. Ege Üniversitesi Ziraat Fakültesi Dergisi, 51 (3): 279-288. https://doi.org/10.20289/euzfd.08912
- Kusvuran, A., M. Kaplan & R. İ. Nazlı, 2014. Effects of ratio and row spacing in hungarian vetch (*Vicia pannonica* Crantz.) and annual ryegrass (*Lolium multiflorum* Lam.) intercropping system on yield and quality under semiarid climate conditions. Turkish Journal of Field Crops, 19 (1): 118-128. https://doi.org/10.17557/tjfc.97892
- Linn, J. G. & N. P. Martin, 1989. Forage Quality Tests and Interpretation. Minnesota Extension Service, University of Minnesota, USA, 6 pp.
- Ohshima, M., T. Nagatomo, H. Kubota, H. Tano, T. Okajima, & R. Kayama, 1988. Comparison of nutritive values between hays and silages prepared from annual ryegrass (*Lolium multiflorum* Lam.) and its pres cake using goats. Japanese Journal of Grassland Science, 33 (4): 396-401. https://doi.org/10.14941/grass.33.396
- Özdemir, S., E. B. Çarpici & B. B. Aşik, 2019. Farklı azot dozlarının tek yıllık çimnin (*Lolium multiflorum westerwoldicum* Caramba) ot verimi ve kalitesi üzerine etkileri. Tarim ve Doga Dergisi, 22 (1): 131-137. https://doi.org/10.18016/ksutarimdoga.vi.437556

- Pavinato, P. S., R. Restelatto, L. R. Sartor & W. Paris, 2014. Production and nutritive value of ryegrass (cv. Barjumbo) under nitrogen fertilization. Revista Ciência Agronômica, 45 (2): 230-237. https://doi.org/10.1590/S1806-66902014000200002
- Pimentel, D., A. Marklein, M.A. Toth, M. Karpoff, G.S. Paul, R. McCormack, J. Kyriazis & T. Krueger, 2008. Biofuel impacts on world food supply: use of fossil fuel, land and water resources. Energies, 1 (2): 41-78. https://doi.org/10.3390/en1010041
- Renlong, L.V., M. El-Sabagh, T. Obitsu, T. Sugino, Y. Kurokawa & K. Kawamura, 2017. Effects of nitrogen fertilizer and harvesting stage on photosynthetic pigments and phytol contents of annual ryegrass silage. Animal Science Journal, 88 (10): 1513-1522. https://doi.org/10.1111/asj.12810
- Soya, H., R. Avcıoğlu & H. Geren, 1997. Yem Bitkileri. Hasad Yayıncılık Ltd. Şti, İstanbul, 223 s.
- Steel, R.G.D. & J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edition, McGraw-Hill Book Company, New York, USA 633 pp.
- Trotter, D.J. & K.D. Johnson, 1992. Forage-Testing: Why, How, and Where. Purdue University. Cooperative Extension Service Paper, 337 pp.
- TUIK, 2019. Türkiye İstatistik Kurumu, Ankara. (Web sayfası: https://biruni.tuik.gov.tr/bitkiselapp/bitkisel.zul) (Erişim tarihi: Ocak 2021).
- Van Soest, P.J., 1985. "Composition, Fiber Quality, and Nutritive Value of Forages, 412-421". In: Forages: The Science of Grassland Agriculture (Eds. M.E. Heath, R.F. Barnes & D.S. Metcalfe), Iowa State University Press, Iowa, 643 pp.