

FORAGE YIELD AND QUALITY OF PERENNIAL RYEGRASS (*Lolium perenne* L.) LINES IN THE BLACK SEA COASTAL AREA OF TURKEY

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ABSTRACT

In present study, perennial ryegrass (*Lolium perenne* L.) lines were developed by using the half sib family selection breeding method. The aim of this research was to determine the forage yield and quality of perennial ryegrass breeding lines. The experiment of design was a Randomized Complete Block Design with three replications in 2009-2011. In this research, significant differences were determined among dry matter yield (DMY) (420-629 kg/da), crude protein content (CPC) (9.43-12.09%), crude protein yield (CPY) (45.07-75.21 kg/da), acid detergent fiber (ADF) (37.24-40.36%), neutral detergent fiber (NDF) (55.31-58.52%), total digestible nutrient (TDN) (49.24-53.27%), relative feed value (RFV) (92.19-98.57%), potassium (0.44-0.71%), calcium (0.42-0.61%), magnesium (0.22-0.27%) and tetany ratio (0.85-1.38). According to results it was decided that 3, 13, 14 and 17 numbered perennial ryegrass lines were selected to test in Regional Yield Experiments.

Key words: *Lolium perenne*, perennial ryegrass, dry matter yield, crude protein

INTRODUCTION

Breeding of new herbage varieties should be based on livestock responses (Casler and Vogel, 1999; Wilkins and Humphreys, 2003). Grass is a very important source of nutrients for domesticated ruminants as it forms a big portion of their diet for a large part of the year (Taweel et al. 2005). Perennial ryegrass (*Lolium perenne* L.) is one of the most important forages for dairy cows in temperate regions because it has a high forage yield and nutritive value, and, hence, provides a cheaper feed than silage or concentrates (Smit et al. 2005). Perennial ryegrass has the highest nutritive value of the temperate grasses with a metabolizable energy concentration of dry matter under appropriate environmental and management conditions (Turner et al. 2006). Traditionally, perennial ryegrass cultivars were mainly bred for high forage production. However, there were no improved perennial ryegrass cultivars in Turkey for forage production. In present study, perennial ryegrass (*Lolium perenne* L.) seeds were collected from Black Sea Coastal Region in 2002. Then, 18 lines were obtained using half sib family selection breeding method. Yield experiment was established in 2009. The aim of the present research was to determine forage yield and quality of perennial ryegrass lines.

MATERIALS AND METHODS

Perennial ryegrass (*Lolium perenne* L.) seeds were collected in the Black Sea Coastal Region in 2002 and the collected seeds were sown and observed according to growing status. In 2003, perennial ryegrass plants were selected based on the flowering, status of stem (thick or thin), and leaf size and width. In 2004, quintet group were constituted for using half sib family selection breeding method. Total 18 groups were constituted. They were closed by cloth in flowering stage against the cross pollination. This proceeding was applied in four years. After the perennial ryegrass seeds were increased, yield trial was established in 2009. Total selected 18 lines and 2 standard cultivars (Liprinta and Lipresso) were used as experimental material.

Field studies were conducted at Black Sea Agricultural Research Institute (15 km east of Samsun, Turkey) in Çarşamba plain (elevation 4 m). The trials were carried out for two growing seasons (2009-2010 and 2010-2011) on clay-loam soil. Soil pH was 6.9; organic matter 1.98 g kg⁻¹; available P, 21 g kg⁻¹; available K, 94 g kg⁻¹. The monthly total rainfall and mean temperature for November through June was 755 mm and 13.0 °C in 2009-2010 and

785 mm and 11.6 °C in 2010-2011. The 30-yr mean for the same months was 562 mm and 11.8°C.

Table 1. Dry mater yield (DMY), crude protein content (CP) and crude protein yield (CPY) of perennial ryegrass lines.

Lines	DMY (kg/da)			CP (%)			CPY (kg/da)		
	2010	2011	mean	2010	2011	mean	2010	2011	mean
1	482 ac	768 a	625 a	12.56 a	11.62 ab	12.09 a	60.44 a	89.98 a	75.21 a
2	336 de	505 e	420 e	12.08 ac	9.75 ac	10.92 ad	40.71 bc	49.43 ef	45.07 e
3	517 a	74 ac	629 a	10.36 ac	10.14 ac	10.25 bd	53.76 ab	75.17 ad	64.47 ac
4	432 ae	749 ab	591 ab	11.72 ac	11.69 a	11.70 ab	50.41 ac	87.48 ab	68.95 ab
5	404 ae	596 de	500 ce	10.33 ac	9.43 ac	9.88 cd	41.32 bc	55.64 df	48.48 de
6	441 ad	607 de	524 bd	9.88 bc	9.66 ac	9.77 cd	43.23 bc	58.15 df	50.69 de
7	367 ce	640 ad	506 bd	9.76 c	9.09 c	9.43 d	35.80 c	57.68 df	46.74 de
8	429 ae	667 ad	548 ad	10.41 ac	9.79 ac	10.10 bd	45.11 ac	65.34 cf	55.22 ce
9	381 be	717 ae	549 ad	10.33 ac	9.44 ac	9.88 cd	39.45 bc	67.77 bf	53.61 ce
10	385 be	629 be	507 bd	10.70 ac	11.23 ac	10.97 ad	40.94 bc	70.90 ad	52.92 ce
11	478 ac	696 ad	587 ac	10.46 ac	10.04 ac	10.25 bd	49.78 ac	69.57 ae	59.68 bd
12	393 be	683 ad	538 bd	11.84 ac	9.50 ac	10.67 ad	46.92 ac	65.34 cf	56.13 ce
13	500 ab	612 ce	556 ad	11.82 ac	11.43 ac	11.62 ab	59.20 a	69.70 ac	65.45 ac
14	425 ae	511 e	468 de	10.22 ac	9.27 vc	9.75 cd	42.97 bc	47.52 f	45.24 e
15	366 ce	608 de	487 de	11.72 ac	9.35 ac	10.53 ad	42.64 bc	56.96 df	49.80 de
16	408 ae	597 de	503 bd	10.24 ac	9.29 bc	9.77 cd	41.88 bc	55.37 df	48.63 de
17	444 ad	712 ad	578 ac	10.22 ac	11.63 ab	10.93 ad	45.11 ac	82.97 ac	64.04 ac
18	319 e	712 ad	516 bd	12.38 ab	10.09 ac	11.24 ac	38.33 bc	71.45 ad	54.99 ce
St1	406 ae	659 ad	533 bd	9.85 bc	9.95 ac	9.90 cd	39.68 bc	66.27 cf	52.97 ce
St2	388 be	620 ad	534 bd	10.63 ac	9.59 ac	10.11 bd	41.19 bc	65.42 cf	53.30 ce
Mean	415 B	655 A	535	10.88 A	10.09 B	10.49	44.95 B	66.41 A	55.68
CV (%)	14.59	10.21	11.93	12.67	11.83	11.75	17.95	16.24	17.10

Means followed by the same letter and column are not significantly different at $p=0.05$ level.

The field trial was conducted in a Randomized Complete Block Design, with 3 replications. Plots were established on November in 2009. Each plot consisted of 6 rows, 4 m in length. The row spacing was 20 cm. The seeding rate was 2 kg/da. The plots were harvested on 25 May and 22 June of 2010 and on 23 May and 17 June of 2011 at 50% flowering stage. Samples were collected following the harvest, dried at 70°C for 48 h and weighed. The dried samples were reassembled and ground to pass through a 1-mm screen. The crude protein content was calculated by multiplying the Kjeldahl nitrogen concentration by 6.25 (Kacar and İnal 2008). K, Ca and Mg contents of samples were determined using an atomic spectrophotometer after digesting the samples with $\text{HClO}_4:\text{HNO}_3$ (1:4) (AOAC, 1990); Tetany ratios (K / (Ca + Mg)) were calculated on a mill equivalent basis (Cherney and Marten, 1982). The ADF (acid detergent fiber) and NDF (neutral detergent fiber) concentrations were measured according to Ankom Technology. The total digestible nutrient (TDN) and the relative feed value (RFV) were estimated according to the following equations adapted from Albayrak et al. (2011):

$$\text{TDN} = (-1.291 \times \text{ADF}) + 101.35$$

$$\text{RFV} = (120/\text{NDF}) \times ((88.9 - (0.779 \times \text{ADF})) \times (0.775)).$$

The statistical analysis of the yield and quality data was performed using the General Linear Model procedure of SAS (SAS Inst. 1998). The means were compared using the DUNCAN test at the 0.05 probability level (Steel, and Torrie, 1997).

RESULTS AND DISCUSSION

Dry matter yield, crude protein content and yield

Perennial ryegrass (*Lolium perenne* L.) differed ($P<0.01$) consistently in dry matter yield (DMY) (Table 1). There was a line x year interaction ($p<0.01$), 2011 belonged to the highest group and 2010 to lowest group. In 2010, DMY varied from 319 to 517 kg/da in 2010 and 505 to 768 kg/da in 2011. Based on the average of two years, the highest DMY was determined for line 3, 1, 4, 11, 17, 13, 9 and 8 (548-629 kg/da). Albayrak and Güler (2005) obtained 297-425 kg/da DMY from perennial ryegrass in Samsun conditions. Başbağ (2008) reported that DMY in perennial ryegrass was 952-1163 kg/da under Diyarbakır conditions. Gül (2006) found that DMY was 1060-1293 kg/da in Ankara conditions. Çakmakçı et al. (2005) found that DMY of ryegrass was 489 kg/da in Antalya conditions. Our findings did not agree with the other findings. These discrepancies should be from the ecological conditions and cultivar differences.

During the experimental period the crude protein content (CPC) varied with year, line and the line x year interaction was also significant (Table 1). CPC varied from 9.76 to 12.56% in 2010 and from 9.09 to 11.69% in

2011. CPC was higher in the first year than that of in the second year. Based on the average of two years, the highest CPC was obtained from lines 1, 4, 13, 18, 10, 17, 2, 12 and 15 (10.53-12.09%). The CPC from 5.5 to 15.9% have been reported by others (Fisher et al. 2004; Smit et al. 2005; Tawell et al. 2005; Tas et al. 2006). Albayrak

and Güler (2005) reported that the quality of forages may be altered due to differences in temperature and precipitation.

Year, lines and year x lines interactions of crude protein yield (CPY) were significant (Table 1). In the first year, the highest CPY were determined on lines 1, 13, 3, 4, 11, 12, 17 and 8 (45.11-60.44 kg/da), in the second year, on lines 1, 4, 17, 3, 18, 10, 13 and 11 (69.57-89.98 kg/da). Based on the average of two years, the highest CPY was obtained from line 14, 3, 13 and 17 (64.04-75.21 kg/da). Albayrak and Güler (2005) obtained 31-44 kg/da CPY from perennial ryegrass. Başbağ (2008) reported that CPY in perennial ryegrass was 112-125 kg/da. Crude protein yield related to CPC and DMY.

Acid detergent fiber, neutral detergent fiber contents and total digestible nutrient, relative feed value values

Variation was found for several quality parameters of ryegrass lines; ADF, NDF, TDN and RFV differed among lines in two years (Table 2 and 3). The lowest ADF content was obtained from lines 12, St2 and 4 (35.92-36.47%) in the first year, although there were no statistical differences among the ryegrass lines (38.01-41.93%) in the second year. Based on the average of two years, the lowest ADF content was determined for line 7, 3, 1 and 4 (37.24-37.99%). Low NDF contents were found for lines 10, 9, St2 (53.07-54.32%) in 2010, and for lines 12, 15 and 18 (55.11-55.90%) in 2011.

Table 2. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents of perennial ryegrass lines

Lines	ADF (%)			NDF (%)		
	2010	2011	mean	2010	2011	mean
1	37.17 af	38.30	37.74 cd	55.42 bf	59.76	57.59 ad
2	38.38 ae	38.58	38.48 ad	56.00 be	60.24	58.12 ac
3	36.88 bf	38.84	37.86 bd	55.82 be	58.91	57.37 ad
4	36.47 df	38.01	37.24 d	56.32 bd	57.98	57.15 ad
5	39.05 ab	41.00	40.03 ac	57.77 ab	58.75	58.26 ab
6	38.56 ad	40.44	39.50 ad	59.06 a	57.98	58.52 a
7	36.74 cf	39.25	37.99 bd	55.56 bf	57.74	56.65 ad
8	38.42 ae	40.24	39.33 ad	55.57 bf	59.04	57.30 ad
9	39.02 af	40.13	39.08 ad	53.56 ef	58.17	55.86 bd
10	36.99 bf	39.86	38.43 ad	53.07 f	58.50	55.78 cd
11	39.30 a	41.07	40.19 ab	56.35 bd	57.75	57.05 ad
12	35.92 f	40.51	38.22 ad	55.35 bf	55.90	55.62 d
13	37.33 af	40.26	38.80 ad	54.73 cf	56.69	55.71 cd
14	38.94 ac	41.79	40.36 a	56.97 ac	57.13	57.05 ad
15	38.30 ae	41.93	40.12 ab	55.46 bf	55.43	55.44 d
16	37.36 af	40.02	38.69 ad	55.04 cf	57.89	56.47 ad
17	38.67 ad	40.65	39.66 ac	56.15 bf	57.60	56.88 ad
18	38.98 ac	40.84	39.91 ac	57.12 ac	55.11	56.12 ad
St1	38.76 ac	41.27	40.02 ac	57.22 ac	56.99	57.11 ad
St2	36.25 ef	41.80	39.02 ad	54.32 df	56.31	55.31 d
Mean	37.83 B	40.24 A	39.03	55.84 B	57.69 A	56.77
CV (%)	3.04	5.18	4.32	2.40	3.65	3.11

Means followed by the same letter and column are not significantly different at p= 0.05 level.

Average of two years, line 10, 13, 12, 15 and St2 had the lowest NDF content (55.31-55.78%). It was reported that ADF and NDF contents of ryegrass ranged from 26 to 33% and from 40 to 64% (Jackson et al. 1996; Fisher et al. 2004; Smit et al. 2005; Tas et al. 2005; Turner et al. 2006). The ADF and NDF contents of ryegrasses in present study were found to be similar to other researches findings.

The TDN refers to the nutrients available for livestock and are related to the ADF concentration of the forage. Based on the average of two years, the TDN value varied from 49.24 to 53.27% for ryegrass lines (Table 3).

Uzun (2010) stated that forages with an the RFV value over 151, 150-125, 124-103, 102-87, 86-75, and less than 75 are categorized as prime, premium, good, fair, poor and rejected, respectively. Based on the average of the 2 years, the ryegrass lines had relative feed values ranging from

92.19 to 98.97 and, they may be categorized as fair qualities (Table 3).

Mineral contents and tetany ratio

Perennial ryegrass lines K, Ca, Mg contents and tetany ratio are given Tables 4 and 5. The American National Research Council (NRC, 1996) recommended that forage crops should contain 0.31% Ca and 0.65% K concentration for beef cattle. Tajeda et al. (1985) reported that forage crops should contain at least 0.8% of K, 0.3% of Ca and 0.2% of Mg for ruminants. In present study, K content varied from 0.44 to 0.71%, Ca content was between 0.42 and 0.61%, Mg content was varied from 0.22 to 0.27%. According to the results of chemical properties of research material, nutritional quality values of ryegrass lines were similar to the all recommended standard values for ruminant.

Table 3. Total digestible nutrient (TDN) and relative feed value (RFV) of perennial ryegrass lines

Lines	TDN (%)			RFV (%)		
	2010	2011	mean	2010	2011	mean
1	53.36 af	51.91	52.64 ab	100.62 bf	91.94	96.28 ac
2	51.80 bf	51.55	51.67 ad	98.02 di	90.91	94.47 ac
3	53.74 ae	51.21	52.48 ac	100.31 bf	92.63	96.47 ac
4	54.26 ac	52.28	53.27 a	99.92 bg	95.18	97.55 ab
5	50.93 ef	48.42	49.67 bd	94.16 ij	90.21	92.19 c
6	51.57 cf	49.05	50.35 ad	92.69 j	92.08	92.39 c
7	53.91 ad	50.67	52.29 ac	101.00 ae	93.98	97.49 ab
8	51.76 bf	49.41	50.58 ad	98.72 ch	90.80	94.76 ac
9	52.26 af	49.55	50.91 ad	102.96 ac	92.35	97.65 ab
10	53.60 ae	49.89	51.74 ad	105.34	91.95	98.65 ab
11	50.61 f	48.33	49.47 cd	96.20 a fj	91.73	93.96 ac
12	54.97 a	49.05	52.01 ad	102.39 ad	95.54	98.97 ab
13	53.15 af	49.37	51.26 ad	101.72 ae	94.58	98.15 ab
14	51.08 df	47.40	49.24 d	95.59 gj	91.85	93.72 bc
15	51.90 bf	47.22	49.56 cd	99.14 ch	94.54	96.84 ac
16	53.11 af	49.69	51.40 ad	101.07 ae	92.72	96.89 ac
17	51.43 cf	48.88	50.15 bd	97.38 ei	92.52	94.95 ac
18	51.03 df	48.62	49.83 bd	95.32 hj	96.46	95.89 ac
St1	51.32 df	48.07	49.69 bd	95.43 hj	92.81	94.12 ac
St2	54.55 ab	47.39	50.97 ad	103.87 ab	93.05	98.46 ab
Mean	52.52 A	49.40 B	50.96	99.09 A	92.89 B	95.99
CV (%)	2.83	5.45	4.27	2.38	4.83	3.73

Means followed by the same letter and column are not significantly different at $p=0.05$ level.

Table 4. Potassium (K) and calcium (Ca) content of perennial ryegrass lines

Lines	K (%)			Ca (%)		
	2010	2011	mean	2010	2011	mean
1	0.72 ab	0.65 ad	0.69 a	0.58	0.62 ab	0.60 a
2	0.41 d	0.70 ad	0.56 ac	0.56	0.66 a	0.61 a
3	0.43 cd	0.80 ab	0.62 ac	0.54	0.48 ce	0.51 bc
4	0.50 ad	0.81 ab	0.66 ab	0.56	0.51 ce	0.54 bc
5	0.65 ad	0.43 cd	0.54 ac	0.59	0.45 ef	0.52 bc
6	0.40 d	0.75 ac	0.58 ac	0.53	0.45 ef	0.49 cf
7	0.55 ad	0.37 d	0.46 bc	0.56	0.37 gh	0.47 cg
8	0.77 a	0.60 ad	0.69 a	0.53	0.49 ce	0.51 bc
9	0.57 ad	0.67 ad	0.62 ac	0.55	0.39 fh	0.47 cf
10	0.54 ad	0.67 ad	0.61 ac	0.55	0.47 de	0.52 bc
11	0.39 d	0.49 bd	0.44 c	0.47	0.56 bc	0.52 bc
12	0.69 ac	0.60 ad	0.65 ac	0.59	0.54 cd	0.57 ab
13	0.60 ad	0.76 ac	0.68 a	0.59	0.40 fg	0.50 cd
14	0.41 d	0.61 ad	0.51 ac	0.51	0.35 gi	0.43 dg
15	0.42 cd	0.65 ad	0.54 ac	0.51	0.35 gi	0.43 dg
16	0.57 ad	0.65 ad	0.61 ac	0.52	0.32 hj	0.42 fg
17	0.76 a	0.65 ad	0.71 a	0.53	0.46 ef	0.49 ce
18	0.53 ad	0.87 a	0.70 a	0.47	0.55 bc	0.51 bc
St1	0.47 bd	0.63 ad	0.55 ac	0.54	0.30 ij	0.42 g
St2	0.56 ad	0.67 ad	0.61 ac	0.59	0.27 j	0.43 eg
Mean	0.55 B	0.65 a	0.60	0.54 A	0.45 B	0.50
CV (%)	25.11	25.83	25.63	10.94	9.07	10.27

Means followed by the same letter and column are not significantly different at $p=0.05$ level.

Table 5. Magnesium (Mg) content and tetany ratio of perennial ryegrass lines

Lines	Mg (%)			Tetany ratio		
	2010	2011	mean	2010	2011	mean
1	0.23	0.26	0.24	1.47 ac	0.74 bc	1.10 ac
2	0.23	0.26	0.25	0.96 d	0.76 bc	0.85 c
3	0.24	0.22	0.23	1.03 cd	1.13 ac	1.08 ac
4	0.24	0.22	0.23	1.13 cd	1.12 ac	1.13 ac
5	0.24	0.21	0.23	1.36 ad	0.65 c	1.00 ac
6	0.24	0.22	0.23	0.99 d	1.13 ac	1.06 ac
7	0.26	0.21	0.24	1.25 cd	0.65 c	0.95 bc
8	0.24	0.24	0.24	1.70 ab	0.83 bc	1.27 ab
9	0.23	0.24	0.23	1.26 bd	1.07 ac	1.17 ac
10	0.24	0.30	0.27	1.22 cd	0.85 bc	1.04 ac
11	0.28	0.26	0.27	1.10 cd	0.60 c	0.85 c
12	0.24	0.25	0.25	1.42 ad	0.77 bc	1.09 ac
13	0.24	0.21	0.23	1.22 cd	1.27 ab	1.24 ab
14	0.23	0.20	0.22	1.01 cd	1.11 ac	1.06 ac
15	0.24	0.20	0.22	1.10 cd	1.21 ac	1.15 ac
16	0.26	0.18	0.22	1.40 ad	1.31 ab	1.36 a
17	0.27	0.21	0.24	1.72 a	0.98 ac	1.35 a
18	0.25	0.22	0.24	1.38 ad	1.13 ac	1.26 ab
St1	0.28	0.21	0.25	1.14 cd	1.26 ab	1.20 ac
St2	0.26	0.18	0.22	1.22 cd	1.54 a	1.38 a
Mean	0.25 a	0.23 B	0.24	1.25 A	1.01 B	1.13
CV (%)	18.29	23.93	21.09	18.71	24.27	24.10

Means followed by the same letter and column are not significantly different at $p=0.05$ level.

The most important disease for livestock is grass tetany caused by mineral matter imbalance in feeds. Tetany is associated with Mg deficiency in the blood of animals. The threshold for risk of grass tetany increases at $K / (Ca + Mg)$ ratio of 2.2 or higher (Elkins et al., 1977). In present study, tetany ratio changed from 0.85 to 1.38, in this research and was below the critical level.

CONCLUSION

Eighteen perennial ryegrass lines and two standard cultivars were evaluated in a field trial for forage yield and quality parameters in the Black Sea Coastal Area. According to the results, indicated that perennial ryegrasses had higher forage yield and quality parameters than the standard perennial ryegrasses. According to the findings, some of the perennial ryegrass lines tested in this study will have a chance in Region Yield Trials. In order to be on the registered cultivar list for the region will obtain in perennial ryegrass.

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