

Yield and forage quality of red clover (*Trifolium pratense L.*) varieties in Black Sea Coastal Area of Turkey

Mustafa SÜRME¹ Tamer YAVUZ² Sebahattin ALBAYRAK³

ABSTRACT: The aim of the present study was to determine the changes in the forage yield and quality of red clover (*Trifolium pratense L.*) varieties throughout a vegetation period. Total eleven red clover varieties were developed by using to half sib family selection breeding method. Plots were established in 2009 in Samsun, Turkey, in a randomized complete block design with 3 replicates. Three cuttings were done during the 2010 and 2011 vegetative periods. The levels of crude protein content (CP), neutral detergent fiber (NDF) rate, acid detergent fiber (ADF) rate, and relative feed value (RFV) were determined. The highest average dry matter (DM) yields were obtained from the number of 4, 7, 5, 8 and 2 varieties (11.45, 10.68, 10.41, 9.9 and 9.82 t ha⁻¹, respectively). Varieties 2, 4, 7, 8, and 11 exhibited the lowest ADF (295-305 g kg⁻¹) and NDF (398-401 g kg⁻¹) concentrations. Same varieties displayed the highest CP content (183-197 g kg⁻¹). The RFV of the red clover varieties ranged from 127 to 156. The results indicated that 2, 4, 5, 7, 8 and 11 numbered red clover varieties were selected to test in region yield experience.

Key words: Crude protein content, dry matter yield, forage quality, red clover



Orta Karadeniz Bölgesi Sahil Kuşağında Bazı Çayır Üçgülü (*Trifolium pratense L.*) Hatlarının Verim ve Kalite Özelliklerinin Belirlenmesi

ÖZET: Bu çalışmanın amacı, çayır üçgülü (*Trifolium pratense L.*) varyetelerinin bir vejetasyon dönemi boyunca yem üretimi ve kalitesindeki değişiklikleri saptamaktır. Toplam onbir çayır üçgülü varyetesi, yarı kardeş aile seleksiyon ıslahı yöntemi kullanılarak geliştirilmiştir. Deneme 3 tekerrürlü tesadüf blokları deneme desenine göre 2009 yılında Samsun'da kurulmuş, 2010 ve 2011 yıllarında da bitkilerin uygun vejetatif gelişme dönemlerinde üç biçim yapılmıştır. Kuru madde (KM) verimi, ham protein (HP), nötral deterjan lif (NDF), asit deterjan lif (ADF) ve nispi yem değeri (RFV) düzeyleri ölçülmüştür. En yüksek ortalama kuru madde verimi 4 nolu hattın (11.45 t ha⁻¹) elde edilmiş ve bunu sırasıyla 7, 5, 8 ve 2 nolu hatlar (10.68, 10.41, 9.90 ve 9.82 t ha⁻¹) takip etmiştir. En düşük ADF (295-305 g kg⁻¹) ve NDF (398-401 g kg⁻¹) oranı 2, 4, 7, 8 ve 11 nolu hatlardan elde edilmiştir. Aynı hatların yüksek ham protein oranı (183-197 g kg⁻¹) ihtiva ettiği belirlenmiştir. Çayır üçgülü hatlarının nispi yem değerleri 127 ile 156 arasında değişmiştir. Sonuçta, 2, 4, 5, 7, 8 ve 11 nolu çayır üçgülü hatları bölge verim şartlarında test edilmek için seçilmiştir.

Anahtar Kelimeler: Ham protein, kuru madde verimi, yem kalitesi, çayır üçgülü

¹ Iğdır Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri, Iğdır, Türkiye

² Ahi Evran Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri, Kırşehir, Türkiye

³ Süleyman Demirel Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri, Isparta, Türkiye

Sorumlu yazar/Corresponding Author: Mustafa SÜRME, mustafa.surmen@igdir.edu.tr

INTRODUCTION

Red clover (*Trifolium pratense* L.) is an important forage legume grown in temperate regions throughout the world (Mihovski et al., 2011). Red clover is adapted to a wide range of climatic conditions, soil types, fertility levels, use patterns and management. It is easy to establish, has high seedling vigour, is an excellent nitrogen fixer, and is suitable for use in crop rotations. Red clover is also of very good quality in the light of its nutritive value and ensiling (Drobna, 2009). The cause of yield variation and quality differences of red clover cultivars in various environmental conditions are different climatic characteristics of each individual area, primarily precipitation and temperatures, different ploidy degree of individual cultivars and a different growth rhythm (Leto et al., 2004). Red clover quality depends primarily on the stage of development at cutting, height of cutting and environmental conditions (Vasiljevic et al., 2011). In present study, red clover seeds were collected from Black Sea Coastal Region in 2002. Then, 11 varieties were obtained by using to 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 red clover varieties.

MATERIALS AND METHODS

Red clover (*Trifolium pratense* L.) seeds were collected from Black Sea Coastal Region in 2002. Same year, collected seeds were sown and observation according to growing status. In 2003, red clover plants were selected according to flowering, status of stem thick or thin, and leaf size and width. In 2004, quintet group were constituted by using to half sib family se-

lection breeding method. Total 11 groups were constituted. They were closed by cloth in flowering stage due to the cross pollination. This proceeding was applied from 2009. After the red clover seeds were increased, yield experiment was established in 2009. Total 11 varieties and 1 standard cultivar (Raja) were used as experimental material.

Field studies were conducted at Black Sea Agricultural Research Institute (15 km east of Samsun, Turkey) in an area of the Çarşamba plain (elevation 4 m). The experiments were carried out during 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 (Rowell, 1996). The monthly total rainfall and mean temperature for November through October was 885 mm and 12.4 °C in 2009-2010 and 892 mm and 11.7 °C in 2010-2011. The 30-yr mean for the same months was 702 mm and 11.5°C.

Plots were established on November in 2009. Each plot consisted of 6 rows, each 4 m in length. The between-row spacing was 20 cm. The seeding rate was 20 kg ha⁻¹. The plots were harvested on 5 June, 2 August, and 28 September of 2010 and on 4 June, 3 August, and 3 October 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). The ADF (acid detergent fiber) and NDF (neutral detergent fiber) concentrations were measured according to Ankom Technology. The relative feed value (RFV) were estimated according to the following equations adapted from Albayrak et al. (2011):

Table 1: Mean squares for red clover forage yield and quality parameters

Source of variation	df	DMY	CP	ADF	NDF	RFV
Block (B)	2	233.67ns	7.29ns	2.11ns	12.28ns	9.47ns
Variety (V)	11	529.20**	128.45**	906.43**	906.98**	2439.17**
Error 1	22	100.17	32.11	47.30	56.75	131.55
Cutting (C)	2	22985.09**	2445.26**	5435.26**	8035.26**	19561.44**
V × C	22	98.40**	4.61ns	23.73ns	30.13ns	102.65**
Error 2	48	30.32	7.35	18.48	24.41	34.95
Year (Y)	1	2760.32**	442.09**	212.44**	295.55**	598.77**
V × Y	11	134.31**	59.55**	297.07**	275.57**	587.43**
C × Y	2	634.99**	4.53ns	10.13ns	14.14ns	75.51ns
V × C × Y	22	25.59ns	9.45ns	8.84ns	21.81ns	44.07ns
Error 3	72	28.91	16.04	24.61	30.94	60.87
CV (%)		17.73	6.91	4.84	4.16	5.50

df = degrees of freedom, CV = coefficient of variation, ns = not significant. *P < 0.05 and **P < 0.01.

Table 2: Dry matter (DM) yields of red clover varieties (t ha⁻¹)

Variety	2010 cuttings				2011 cuttings				Average of 2 years
	1	2	3	total	1	2	3	total	
1	2.39d	1.49	0.75d	4.63d	4.05de	1.42e	1.16e	6.63d	5.63e
2	5.24ab	2.48	1.64ac	9.36ab	5.64bc	2.66ac	1.98ac	10.29bc	9.82ac
3	3.41bd	2.18	1.61ac	7.21bd	3.87e	1.73de	1.34de	6.94d	7.07de
4	5.67a	2.52	1.87a	10.01a	7.56a	2.95ab	2.37a	12.88a	11.45a
5	4.55ac	2.60	1.62ac	8.77ac	6.73ab	3.25a	2.07ab	12.06ab	10.41ac
6	4.58ac	1.85	1.33bc	7.76ac	4.92ce	2.29bd	1.37de	8.57cd	8.16cd
7	5.28ab	2.48	1.76ab	9.51ab	6.61ab	3.18a	2.05ab	11.85ab	10.68ab
8	4.71ac	2.24	1.54ac	8.49ac	6.44ab	3.10a	1.82bd	11.36ab	9.93ac
9	2.88cd	2.13	1.27bc	6.28cd	5.71bc	2.81ab	1.86bc	10.38bc	8.33bd
10	3.87ad	1.93	1.16cd	6.96bd	5.65bc	3.12a	1.93ac	10.70ac	8.83bd
11	5.44a	2.38	1.74ab	9.56ab	5.32bd	1.90ce	1.54ce	8.75cd	9.15ad
12-Raja	4.15ad	2.29	1.32bc	7.76ac	6.44ab	3.18a	2.05ab	11.67ab	9.71ac
Mean	4.35a	2.21b	1.47c	8.03B	5.74a	2.63b	1.80c	10.17A	9.10
CV %	25.53	18.99	20.13	19.96	14.60	17.89	15.86	13.51	13.47

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

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

The experiment was conducted in a randomized complete block design with 3 replications. A split-split plot design was used for combined analysis of the 2 years (Table 1). The statistical analysis of the yield and quality data was performed using the SAS general linear model procedure (SAS Institute, 1998). The means were compared using Duncan's test at the 0.05 probability level.

RESULTS AND DISCUSSION

The results of the variance analysis showed that the effects of the varieties, cutting, year, variety x cutting, year x variety and year x cutting interactions on the dry matter (DM) yield were significant (Table 1). The yields from the first cutting were the largest of the 2 and 3 for both years (Table 2). The DM yield decreased after the first cutting in both years. In both years, the highest yields were recorded in the first cut, followed by the second cut and the lowest from the third cut. In the first year, the DM yield varied from 5.67 to 2.39 t ha⁻¹ and all variety were same statistically group except variety 1, 3 and 9 in first cut. There were not statistically differences among the varieties in second cut. In third cut, the DM yield varied from 1.87 to 1.54 t ha⁻¹ and varieties 1, 6, 12, 9 and 10 had lower DM yields than other varieties. The highest total DM yield was determined in variety 4 (10.01 t ha⁻¹) (Table 2). In the second year, the highest DM yield was determined on

variety 4, 5, 7, 8 and 12 (7.56-6.44 t ha⁻¹) in first cut. Varieties 1, 3, 11 had lower DM yields than other varieties in second cut. In third cut, Varieties 4, 2, 5, 7, 10 and 12 had higher DM yields (2.37-1.93 t ha⁻¹) than other varieties DM yields (1.16-1.86 t ha⁻¹). The highest total DM yield was determined in variety 4 (12.88 t ha⁻¹) (Table 2). The highest DM yield over both years was obtained from the number of 4, 7, 5, 8 and 2 varieties (22.89, 21.36, 20.83, 19.85 and 19.65 t ha⁻¹, respectively) (Table 2). Leto et al. (2004) obtained 8.92 and 9.86 t ha⁻¹ DM yields from red clover varieties. Hejdug (2011) found that red clover DM yield varied from 17.10 to 90.50 t ha⁻¹ at 3 cuts. Cupina et al. (2004) reported that the highest DM yields from the 3 cuts were obtained by 14.5-12.2 t ha⁻¹ in red clover. These results are consistent with our results. Red clover DM yields were found between 4.35 to 15.8 t ha⁻¹ from some other researchers (Mihovski et al., 2011; Vasiljevic et al., 2011; Golaszewska et al., 2010; Tavlas et al., 2009).

Varieties, cutting, year and year x variety interactions were significant for the CP content (Table 1). In general, CP content increased after the first cutting (Table 3) for the red clover varieties. Crude protein contents in our experiment were the lowest in the first cut and were increasing depending on varieties, in the second or the third. This confirmed the results of Sheaffer et al. (1998) who also found the highest content of crude protein in the third cut. In the first year, the CP content varied from 182 to 155 g kg⁻¹ and all variety were same statistically group except variety 3, 9 and 10 in first cut. The highest CP content was determined on variety 4 (203 g kg⁻¹) and variety 9 the lowest (173 g kg⁻¹).

Table 3: Crude protein (CP) contents of red clover varieties (g kg⁻¹)

Variety	2010 cuttings				2011 cuttings				Average of 2 years
	1	2	3	mean	1	2	3	mean	
1	171ac	190b	209b	190b	144e	163cd	195cf	167e	179ce
2	180ab	194b	216a	196ab	162bd	169bd	185eg	172d	184bd
3	158bc	178d	204b	180c	145e	162d	180fg	162f	171e
4	176ac	203a	223a	201a	179a	193a	210ac	194a	197a
5	168ac	183c	198c	183c	160bd	173b	202bd	179c	181ce
6	182a	194b	214a	183c	170ab	188a	217ab	192a	194ab
7	174ac	190b	214a	197ab	156ce	172bc	189dg	172d	183be
8	176ac	186c	204b	193b	168ac	184a	197ce	183b	186ad
9	155c	173e	189c	172c	158bd	170bd	206ad	178c	175de
10	158bc	180d	202b	179c	151de	163cd	174g	163ef	171e
11	170ac	194b	212b	192b	176a	189a	205ad	190a	191ac
12-Raja	168ac	178d	195c	180c	171ab	185a	220a	192a	186ad
Mean	170c	187b	207a	188A	162b	176ab	198a	179B	183
CV %	8.18	7.59	9.47	7.90	4.92	3.23	4.99	1.43	6.91

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

Table 4: Acid detergent fiber (ADF) contents of red clover varieties (g kg⁻¹)

Variety	2010 cuttings				2011 cuttings				Average of 2 years
	1	2	3	mean	1	2	3	mean	
1	399a	366a	320ab	362a	378a	351a	316a	348a	355a
2	344de	329cd	283cd	319d	303c	293b	277b	291b	305d
3	382ab	363a	339a	361a	361ab	345a	325a	344a	352a
4	332ef	319df	279cd	310e	313c	297b	273b	294b	302d
5	323fg	307fh	281cd	303fg	358ab	342a	323a	341a	322c
6	357cd	338bc	311b	336b	373a	343a	320a	345a	340ab
7	326eg	312fh	271de	303fg	320c	307b	272b	299b	301d
8	332ef	315eg	268de	305ef	329bc	307b	258b	298b	301d
9	368bc	345b	311b	341b	385a	354a	314a	351a	346a
10	355cd	326ce	303bc	328c	388a	354a	321a	355a	341ab
11	310g	299h	252e	287h	334bc	313b	265b	304b	295d
12-Raja	324eg	305gh	265de	298g	383a	364a	326a	358a	328bc
Mean	346a	327b	290c	321B	352a	331b	299c	327A	324
CV %	3.48	2.58	4.98	1.17	6.28	4.62	7.23	5.39	4.84

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

¹) in the second cut. In the third cut, CP contents of red clover varieties were varied from 223 to 189 g kg⁻¹. The highest mean CP content was determined in variety 4, 7 and 2 (201, 197 and 196 g kg⁻¹, respectively) (Table 3). In the second year, variety 4, 6, 8, 11 and 12 had higher CP content both first cut (179-168 g kg⁻¹) and second cut (193-184 g kg⁻¹). Varieties 4, 6, 9, 11 and 12 had higher CP content (220-205 g kg⁻¹) other varieties in third cut. The highest mean CP content was determined in variety 4, 6, 11 and 12 (194-190 g kg⁻¹) (Table 3). In present study, following the mean values of crude protein content for years and cuts number of 4, 6, 11, 12 and 8 varieties featured the highest CP content (197, 194, 191, 186 and 186 g kg⁻¹, respectively). Markovic et al, (2011) reported that CP content of red clover varieties varied from 174 to 260 g kg⁻¹. Red clover had 138 to 158 g kg⁻¹ CP content (Golaszewska et al., 2010).

While red clover had 156 g kg⁻¹ CP content in the first cut, CP content was increased at 187 g kg⁻¹ in second cut (Vasiljevic et al., 2011). These results are consistent with our results.

Differences in ADF and NDF concentrations occurred among the variety, cutting and years (Table 1). The year × variety interaction also was observed. In the first year, the least ADF concentration was determined on variety 11 at all cuts and means. In the second year, variety 2 had the least ADF concentration except third cut. Similarly, variety 11 and 2 had the least NDF concentration all cuts both first year and second year, respectively. (Table 4, 5). The red clover varieties (2, 4, 7, 8 and 11) demonstrated the lowest ADF (295-305 g kg⁻¹) and NDF (398-401 g kg⁻¹) concentrations. On average, the last 2 cuttings of all varieties exhibited lower NDF and ADF concentrations than the first cutting, and

Table 5: Neutral detergent fiber (NDF) contents of red clover varieties (g kg⁻¹)

Variety	2010 cuttings				2011 cuttings				Average of 2 years
	1	2	3	mean	1	2	3	mean	
1	479ab	449a	414ab	448b	488a	455ab	430ab	458a	453a
2	440ce	417ce	383cd	413e	405d	395d	358d	386d	400c
3	486a	440ab	429a	452a	470ab	444ab	405ac	440ab	446a
4	433de	407ef	461e	400f	434cd	406cd	465cd	402cd	401c
5	476ab	434ab	398bc	436c	438bd	399d	465cd	401cd	418b
6	462bc	440ab	398bc	433cd	493a	449ab	405ac	449ab	441a
7	444ce	404ef	333f	394g	437bd	405d	364cd	402cd	398c
8	426e	401fg	370de	399f	434cd	400d	364cd	399cd	399c
9	461bc	426bd	408ab	431d	476a	437ac	414ab	442ab	437a
10	460bc	431bc	402bc	431d	486a	460a	446a	464a	447a
11	422e	388g	325f	378g	440bc	427bd	393bd	420bc	399c
12-Raja	451cd	412df	383cd	415e	493a	464a	419ab	459a	437a
Mean	453a	421ab	384b	419B	458a	428ab	394b	427A	423
CV %	3.01	2.14	3.35	0.50	4.50	4.42	7.10	4.60	4.16

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

Table 6: Relative feed value (RFV) of red clover varieties (g kg⁻¹)

Variety	2010 cuttings				2011 cuttings				Average of 2 years
	1	2	3	mean	1	2	3	mean	
1	112e	125g	143fg	126i	114d	126d	139cd	126c	127d
2	131bc	141cd	162cd	144e	150a	156a	176a	160a	153a
3	113e	128fg	136g	125i	120cd	130cd	146bd	132bc	129cd
4	135ab	146bc	173b	150c	138ab	151ab	173a	153a	153a
5	125cd	139d	157de	139f	130bc	145ab	163ab	145ab	143b
6	123d	133ef	151ef	135h	113d	129cd	147bd	129c	133cd
7	133b	149b	189a	154b	136b	150ab	174a	152a	155a
8	138ab	149b	171bc	152c	136b	151ab	177a	153a	154a
9	122d	136de	146f	134h	116d	131cd	145bd	130bc	133cd
10	124cd	137de	151ef	137g	112d	124d	133d	123c	130cd
11	143a	157a	199a	164a	133bc	141bc	163ac	145ab	156a
12-Raja	131bc	147b	166bd	147d	111d	121d	141bd	124c	136bc
Mean	128c	141b	162a	142A	126c	138b	157a	139B	142
CV %	3.53	2.50	3.39	0.74	6.39	5.73	9.01	6.45	5.50

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

the ADF and NDF concentrations in 2010 were lower than those in 2011. Some researchers reported that ADF concentration varied from 280 to 384 g kg⁻¹ and NDF concentration varied from 296 to 506 g kg⁻¹ (Markovic et al., 2011; Vasiljevic et al., 2011; Golaszevska et al., 2010; Tavlas et al., 2009).

RFV was affected by variety, cutting, year, variety x cutting and year x variety interactions. The red clover varieties (2, 4, 7, 8 and 11) had higher RFV (153-156) than the other red clover varieties (Table 6). All of the red clover varieties displayed higher RFV at cutting 3 in both years. Albayrak and Türk (2013) stated that forages with an RFV of 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. Van Soest (1996) reported that the RFV is not a direct measure of the nutritional content of forage, but

that it is important for estimating the value of the forage. Based on the average of the 2 years, the red clover varieties had relative feed values ranging from 127 to 156 and, thus, may be categorized as prime and premium quality.

CONCLUSION

Eleven red clover varieties and one standard cultivar were evaluated for forage yield and quality parameters in Black Sea Coastal Area of Turkey. According to present study results, red clover varieties had higher forage yield and quality parameters than standard red clover. End of the present research, Variety of 2, 4, 5, 7, 8 and 11 will take region yield trials. Thus, registered cultivars will obtain in red clover.

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