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Chemical Contents of Red Clover Genotypes on Crop Improvement Systems as Livestock Feed

[1]Halil Yolcu, [2]Ayten Tavlas, [3]Mustafa Tan and [4]Adem Gunes
[1]Kelkit Aydin Dogan Vocational Training School,
Gümüshane University, 29600 Kelkit, Gumushane, Turkey
[2]East Anatolia Agricultural Research Institute, 25240 Erzurum, Turkey
[3]Department of Agronomy, [4]Department of Soil Sciences,
Faculty of Agriculture, Ataturk University, 25240 Erzurum, Turkey

Abstract: A field experiment was conducted in 2000-2001 at the Research Station of East Anatolia Agricultural Research Institute in Pasinler of Erzurum province in Turkey. In this study, 25 red clovers (*Trifolium pratense* L.) genotypes were evaluated in terms of chemical contents for animal feeding in the 2 years results. The red clover genotypes were sown in the spring of 2000. The experiment was designed a randomized complete block with replicated 4 times. The genotypes were harvested in 50% flowering once in 2000 and 3 times in 2001. There were significant differences in N ($p<0.05$), Mg ($p<0.05$), Zn ($p<0.05$), Fe ($p<0.05$), Mn ($p<0.01$) and Cu ($p<0.01$) contents but no in Na content among the red clover genotypes. According to 2 years results, especially L-818 line and Xiashao cultivar in N; L-1881 and L-812 line in Mg; Redwent cultivar, L-77, L-1922 lines and Lucrun cultivars in Zn; L-77 line, Redwent cultivar, L-812, L-1922 lines and Lucrun cultivar in Fe; L-79 line, Redwent cultivar and L-77 line in Mn; L-79 line, Redwent cultivar and L-77 line in Cu had higher chemical contents than the others for animal feeding. The genotypes rich in terms of chemical content can be successfully used to meet the requirement the forage quality of animal and to gain new quality red clover genotypes for animal nutrition.

Key words: Genotypes, feed, livestock, red clover, chemical content, mineral content

INTRODUCTION

Red clover (*Trifolium pratense* L.) is commonly grown throughout the world. It is an important perennial forage legume because it is used in hay or as pasture in crop rotations (Farnham and George, 1993) and have high productivity and protein content (Murray *et al*., 2007). It is especially, suitable for cattle breeding, because red clover has quality and nutritive hay (Acikgoz, 2001).

Mineral contents of forages are very important for animal feeding. A number of inorganic elements are essential for normal growth and reproduction of animals (NRC, 2001). Grazing livestock have to depend largely upon forage to fulfill their mineral requirements (Espinoza *et al*., 1991). But acute and chronic dietary deficiencies in macro and micro minerals have significant impacts on production efficiency of rangelands throughout the world (Pinchak *et al*., 1989). Mineral elements most likely to be lacking under grazing conditions for ruminants are Ca, P, Na, Co, Cu, I, Se and Zn (McDowell, 1996). Therefore, it is important that rich forages in terms of mineral content are used in animal feeding. Among the forage plants, red clover is one of the best forage plants to meet the mineral demands of livestock.

Mineral contents of forage species may show significant variables in a species. A large number of studies have shown important differences in terms of mineral contents in a species (Jones *et al*., 1995; Lema *et al*., 2000; Yolcu *et al*., 2008; Sengul and Haliloglu, 2008). The aim of this study, was to choose superior genotypes in terms of mineral contents for animal feeding among 25 red clover genotypes in field conditions in 2 years results.

MATERIALS AND METHODS

The study was conducted at the East Anatolia Agricultural Research Institute in Pasinler of Erzurum province (39°55'N, 41°61'E, elevation 1705 m) of Turkey in 2000 and 2001.

Corresponding Author: Halil Yolcu, Kelkit Aydin Dogan Vocational Training School, Erzincan University, 29600 Kelkit, Gumushane, Turkey

The soil had loam, clay-loam character with a pH of 7.1. The organic matter content of the soil is low (1.79%) and P and K levels were 69.3 kg P_2O_5 ha^{-1} and 2066 kg K_2O ha^{-1} in research area, respectively. Some chemical and physical properties of experiment soil (0-20 and 20-40 cm) are presented in Table 1.

Total precipitations, the mean temperatures and mean relative humidity were 291.1, 423.9 mm, 6.0, 5.9°C and 60.6, 61.4% in 2000 and 2001, respectively. Generally, While July has the highest mean temperature (17.5°C), February has the lowest mean temperature (-9.6°C) in long-term averages. May has the highest total precipitation (71.9 mm) and also, August has the lowest precipitation (17.5 mm) in this location. The winters are snowy and long. Snow remains 4-5 months in this region. Climatic dates of the research location are presented in Table 2.

The experiment was designed as a randomized complete block design with 25 red clover genotypes (Rotra, Pawera cultivars (tetraploid), Orbit, Rajah, Hamua, Redwent, Xiashao, Piemontese, Colenso, Lakeland, Lucrun cultivars (diploid), Tohum Islah ecotype, L-68, L-77, L-79, L-81, L-509, L-584, L-777, L-812, L-818, L-832, L-1881, L-1920 and L-1922 lines) and four replications. The lines were selected from native flora of eastern Anatolia (Tavlas and Tan, 2005). Differences in terms of evident physical properties of red clover genotypes accounted for in this study are presented in Table 3. Red clover (*Trifolium pratense* L.) genotypes were planted in the

Table 1: Some chemical and physical characteristics of the experiment area soils

Soil depth	Texture class	pH	Lime ($CaCO_3$ %)	Available for plant (kg ha^{-1}) P_2O_5	K_2O	Organic matter (%)
20 cm	Clay-Loam	7.1	2.43	59.5	1687	1.67
40 cm	Loam	7.0	2.24	79.1	2445	1.91
Ort.		7.1	2.34	69.3	2066	1.79

Table 2: Monthly rainfall, temperature and relative humidity of Erzurum in 2000, 2001 years and long-term averages (1929-2000)

Years	Jan.	Feb.	March	Apr.	May	June	July	August	Sep.	Oct.	Nov.	Dec.	Total/mean
Total precipitation (mm)													
1929-2000	18.7	24.3	26.3	50.8	71.9	38.3	25.5	17.5	18.5	47.4	36.7	25.0	400.9
2000	18.8	7.2	61.3	35.0	42.0	9.7	4.0	4.7	40.7	42.3	1.6	23.8	291.1
2001	4.9	11.9	51.1	104.9	68.7	7.3	36.6	9.0	3.8	51.2	33.2	35.1	423.9
Mean temparature (°C)													
1929-2000	-9.4	-9.6	-3.3	4.4	9.3	13.0	17.5	17.1	12.8	6.6	-1.3	-7.0	4.2
2000	-9.7	-11.3	-7.6	7.4	9.8	15.5	22.2	19.3	14.4	7.0	1.2	-5.9	6.0
2001	-12.2	-5.7	-4.4	7.2	9.3	15.4	20.6	19.9	14.9	6.2	-3.6	-5.1	5.9
Mean relative humidity (%)													
1929-2000	76.7	76.0	74.8	65.4	61.4	57.3	51.1	47.7	50.2	61.8	72.5	76.5	64.3
2000	71.2	73.6	73.4	64.8	57.9	47.8	36.7	43.4	47.4	67.2	64.2	79.5	60.6
2001	80.6	71.9	65.0	65.4	61.3	48.1	46.2	44.1	42.0	60.1	71.5	80.4	61.4

Table 3: Some important plant properties of the red clover genotypes in this study

Genotype	Origin	Ploidy levels	Some important plant properties
Tohum Islah (Common ecotype)	Turkey	2n = 14	Little branches
Rotra (Tetraploid)	Germany	2n = 28	Thick stems, abundant leaves, big seeds
Piemontese	Italy	2n = 14	Middle length stems
Hamua	New Zealand	2n = 14	Thick stems, abundant leaves
Xiaoshao	China	2n = 14	Little branches
Lucrun	Germany	2n = 14	Middle length stem
Pawera (Tetraploid)	New Zealand	2n = 28	Lately, long and thick stem
Colenso	New Zealand	2n = 14	Early maturity type
Redwent	Australia	2n = 14	Little branches, thin stems
Lakeland	USA	2n = 14	Middle early maturity type
Orbit	USA	2n = 14	Short stems, little auxiliary branches
Rajah	Denmark	2n = 14	Lately, long and thick stems
L-584	FAO	2n = 14	Thick stems
L-68	Selected from native flora	2n = 14	Little leaves
L-77	Selected from native flora	2n = 14	Middle early maturity type
L-79	Selected from native flora	2n = 14	Short and thin stem, small seeds
L-81	Selected from native flora	2n = 14	Plentiful clusters
L-509	Selected from native flora	2n = 14	Small seeds
L-777	Selected from native flora	2n = 14	Early maturity type
L-812	Selected from native flora	2n = 14	Early maturity type
L-818	Selected from native flora	2n = 14	Middle early maturity type
L-832	Selected from native flora	2n = 14	Thin stems, abundant leaves, small seeds
L-1881	Selected from native flora	2n = 14	Small seeds
L-1920	Selected from native flora	2n = 14	High seed yield
L-1922	Selected from native flora	2n = 14	Middle length stems

April of 2000 and 2 years (2000 and 2001) results were evaluated. The plots were harvested in 50% flowering once in 2000 and three times in 2001. Each plot was 6.0 m long by 1.5 m wide, with 30 cm row spacing. Each plot was 1.5×6.0 = 9.0 m^2 in size.

Nitrogen fertilizer was applied 40 kg N ha^{-1} as ammonium sulphate $(NH_4)_2$ SO_4 only the 1st year then phosphorus fertilizer was applied as triple superphosphate until the phosphorus quantity of the soil was 150 kg ha^{-1} P_2O_5 every year (Comakli, 1991; Serin and Tan, 2001). After rainy season, irrigation made when usable water of the soil decreases to 50%, with intervals 8-10 days as practical (Comakli, 1991).

Samples of 25 red clover genotypes were washed to remove soil using deionised water. The samples were oven-dried at 70°C for 48 h and ground to pass 1 mm. The Kjeldahl method (Bremner, 1996) and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N of the red clover genotypes. Mg, Na, Fe, Mn, Zn and Cu contents of red clover genotypes were determined by atomic absorption spectrometry (AOAC, 2005).

Data were subjected to the analysis of variance using the MSTAT-C procedure and means were separated with Duncan's test for differences among genotypes.

RESULTS AND DISCUSSION

Significant differences were determined in terms of chemical contents among red clover genotypes in 2 years results (Table 4). The highest N content was found in the L-818 line (2.55 g 100 g^{-1} DW) ($p<0.05$). This value was followed by the Xiashao (2.33 g 100 g^{-1} Dw), the Lucrun cultivar (2.25 g 100 g^{-1} Dw), the L-1881 (2.23 g 100 g^{-1} Dw), the L-79 (2.21 g 100 g^{-1} Dw) and the L-584 lines (2.18 g 100 g^{-1} Dw), respectively. The lowest N content value was determined in the L-777 line (1.92 g 100 g^{-1} Dw) among red clover genotypes (Table 4). Red clover genotypes showed significant differences in Mg in 2 years results ($p<0.05$). The highest value was gained the L-1881 line (2038 mg kg^{-1} Dw). The other high values were determined in L-812 line (2015 mg kg^{-1} Dw) and in Hamua cultivar (2007 mg kg^{-1} Dw), respectively (Table 4). The lowest Mg content was found in Rajah cultivar (1883 mg kg^{-1} Dw). There was no significant difference in terms of Na content among genotypes of red clover (Table 4). But significant differences were found in Zn content among red clover genotypes ($p<0.05$). The highest Zn content was determined Redwent cultivar (60.55 mg kg^{-1} Dw). This value was followed by L-77 (58.08 mg kg^{-1} Dw), L-1922 lines (58.00 mg kg^{-1}Dw), Lucrun cultivar (57.95 mg kg^{-1} Dw) and L-777 line (55.50 mg kg^{-1} Dw), respectively (Table 4). Significant differences was also determined in terms of Fe contents

Table 4: Some mineral contents of red clover genotypes in 2 year results (2000-2001)

Cultivars/ lines	N (g 100 g^{-1} Dw)	Mg (mg kg^{-1} Dw)	Na (mg kg^{-1} Dw)	Zn (mg kg^{-1} Dw)	Fe (mg kg^{-1} Dw)	Mn (mg kg^{-1} Dw)	Cu (mg kg^{-1} Dw)
T. Islah	2.09bc	1922a-d	924	40.53a-f	80.47a-d	16.20b-d	30.82a-d
Orbit	2.15bc	1899b-d	861	45.25a-e	90.50a-d	18.12b-d	27.24b-d
Rajah	2.03bc	1883d	966	27.75d-f	85.80a-d	17.16b-d	27.26b-d
Rotra	1.99bc	1953a-d	924	40.43a-f	90.41a-d	18.18b-d	29.29b-d
Hamua	2.03bc	2007a-c	945	37.98a-f	75.50b-d	15.190cd	24.310cd
Redwent	2.09bc	1922a-d	1050	60.550a	120.3800a	24.22ab	40.350ab
Xiashao	2.33ab	1953a-d	987	45.50a-e	100.46a-d	20.20a-d	32.32a-d
Piemontese	2.12bc	1968a-d	1071	20.25ef	75.80b-d	15.16cd	24.260cd
Colenso	2.14bc	1961a-d	966	50.58a-d	100.43a-d	20.23a-d	32.37a-d
Lakeland	2.15a-c	1922a-d	882	32.68b-f	65.70cd	16.14b-d	29.23b-d
Lucrun	2.25a-c	1930a-d	1008	57.95ab	112.98ab	23.23a-c	36.87a-c
Pawera	1.98bc	1907b-d	903	52.83a-d	105.51a-c	21.20a-d	33.82a-d
L-68	2.15a-c	1976a-d	966	40.33a-f	80.07a-d	16.13b-d	25.71cd
L-77	2.07bc	2000a-d	1155	58.08ab	120.50a	24.19ab	40.31ab
L-79	2.21a-c	1961a-d	1155	53.03a-d	105.08a-c	27.21a	43.900a
L-81	1.94bc	1992a-d	1178	30.60c-f	80.37a-d	21.22ad	33.86a-d
L-509	2.12bc	1907b-d	924	50.60a-d	100.12a-d	20.24a-d	32.38a-d
L-584	2.18a-c	1992a-d	966	30.35c-f	60.70d	13.17d	22.78d
L-777	1.92c	1891cd	966	55.50a-c	110.46ab	22.20a-c	35.82a-d
L-812	2.04bc	2015ab	1071	37.83a-f	115.46ab	23.22a-c	37.36a-c
L-818	2.55a	1891cd	1008	15.28f	90.80a-d	18.16b-d	29.32b-d
L-832	2.11bc	1969a-d	1092	40.10a-f	90.45a-d	20.32a-d	32.51a-d
L-1881	2.23a-c	2038a	1050	45.55a-e	75.36b-d	20.18a-d	24.32cd
L-1920	2.10bc	1922a-d	924	32.80b-f	85.46a-d	17.20b-d	27.82b-d
L-1922	2.00bc	2000a-d	945	58.00ab	115.10ab	23.20a-c	37.32a-c
Mean	2.120	1951	996	42.4100	93.3600	19.6800	31.6600
Importance	0.050	0.050	ns	0.05000	0.0500	0.010	0.01
LSD value	0.400	121	-	25.48000	42.0300	8.530	13.63

ns: not significant

among red clover genotypes ($p<0.05$). The highest values were found in L-77 line (120.50 mg kg^{-1} Dw) and Redwent cultivar (120.38 mg kg^{-1} Dw). These values were followed by L-812 (115.46 mg kg^{-1} Dw), L-1922 lines (115.10 mg kg^{-1} Dw), Lucrun cultivar (112.98 mg kg^{-1} Dw) and L-777 line (110.46 mg kg^{-1} Dw). The lowest Fe content was gained L-584 line (60.70 mg kg^{-1} Dw) among red clover genotypes (Table 4).

Red clover genotypes in terms of Mn content showed significant differences in 2 years results ($p<0.01$). The highest Mn value was determined in L-79 line (27.21 mg kg^{-1} Dw). The other high values were found in Redwent cultivar (24.22 mg kg^{-1} Dw), L-77 line (24.19 mg kg^{-1} Dw), Lucrun cultivar (23.23 mg kg^{-1} Dw), L-812 (23.22 mg kg^{-1} Dw), L-1922 (23.20 mg kg^{-1} Dw) and L-777 lines (23.20 mg kg^{-1} Dw), respectively. The lowest Mn content was found in L-584 line (13.17 mg kg^{-1} Dw) (Table 4). There were also significant differences in Cu contents of red clover genotypes in 2 years results ($p<0.01$). The highest Cu content was determined in the L-79 line (43.90 mg kg^{-1} Dw). This value was follo wed in Redwent cultivar (40.35 mg kg^{-1} Dw), L-77 (40.31 mg kg^{-1} Dw), L-812 (37.36 mg kg^{-1} Dw), L-1922 lines (37.32 mg kg^{-1} Dw) and Lucrun cultivar (36.87 mg kg^{-1} Dw), respectively (Table 4). The lowest Cu content was found L-584 line (22.78 mg kg^{-1} Dw).

Red clover genotypes showed significant differences in chemical contents for animal feeding. Similar findings were determined by Sengul and Yolcu, (2002) in N, Mg among 13 lucerne ecotypes, Broderick and Cwintal (2004) in total N among 133 red clover entries, Cwintal and Wilczek (2004) in Mn among diploid and tetraploid red clover, Sengul and Haliloglu (2008) in Mg, Fe, Cu, Mn and Zn among 21 lucerne cultivars and ecotypes, Hopkins *et al.* (2002) in Mg, Na, Zn, Mn among 2 Italian rye grass genotypes, Warda (2001) in N, Mg among 3 Trifolium repens cultivars and Lema *et al.* (2001) in CP among grain sorghum varieties silages.

N is needed mainly for the synthesis of proteins and the dietary requirement is therefore the greatest during periods of rapid growth and during pregnancy and lactation (Whitehead, 2000). Only L-818 line (2.55%) in terms of N content was higher than those of the red clover used in beef cattle diets (2.4 N%, NRC, 2000) among 25 red clover genotypes. Copper is essential for growth and for the prevention of a wide range of clinical and pathological disorders in all types of farm animals (Underwood and Suttle, 1999). Cu contents of the all genotypes were higher than those of red clover used in beef cattle diets (11 Cu mg kg^{-1}, NRC, 2000) and those of legume forages commonly fed to dairy cattle (9 Cu mg kg^{-1}, NRC, 2001). The most important functions of zinc are those, which become limiting to health and production when livestock is deprived of zinc and 2 functions stand out: gene expression and appetite (Underwood and Suttle, 1999). All of the genotypes, except L-818 line (15.28 mg kg^{-1}) with respect to Zn content were higher than those of red clover used in beef cattle diets (17 mg kg^{-1} Zn, NRC, 2000). Zn content of the all genotypes, except for Piemontese cultivar (20.25 mg kg^{-1}) and L-818 line (15.28 mg kg^{-1}) was also, higher than those of legume forages commonly fed to dairy cattle (NRC, 2001). Mg has a part in nerve system, enzyme function and growth of skeleton in animal metabolism (Altin *et al.*, 2005). Fe is mainly present as heamoglobin in a body (Underwood and Suttle, 1999). Also, function of Mn is mainly as constituents of enzymes, or as enzyme activators (Whitehead, 2000). But all of the red clover genotypes in Mg, Fe and Mn were lower than those of red clover used in beef cattle diets (0.38 Mg%, 238 Fe mg kg^{-1}, 108 Mn mg kg^{-1}, NRC, 2000) and those of legume forages (hay, mid maturity) commonly fed to dairy cattle (0.30 Mg%, 207 Fe mg kg^{-1}, 46 Mn mg kg^{-1}, NRC, 2001).

Besides, the mineral contents of all the genotypes were very lower than maximum tolerable concentration of growing cattle, finishing cattle, gestating cow, early lactation cow (0.40 Mg%, 500 Zn, 1000 Fe, 1000 Mn, 100 Cu mg kg^{-1} (NRC, 2000).

CONCLUSION

The chemical composition of red clover showed significant differences among the genotypes as livestock feed. According to 2 years results, especially L-818 line and Xiashao cultivar in N; L-1881 and L-812 line in Mg; Redwent cultivar, L-77, L-1922 lines and Lucrun cultivars in Zn; L-77 line, Redwent cultivar, L-812, L-1922 and Lucrun cultivar in Fe; L-79 line, Redwent cultivar and L-77 line in Mn; L-79 line, Redwent cultivar and L-77 line in Cu had higher chemical contents than the others. These genotypes can be successfully used to meet a part of mineral requirement of livestock and to improve new quality red clover genotypes for animal feeding.

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