Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences 5 (2): 201-206, 2006
ISSN 1682-3974

Effects of Different Levels of Phosphorus on Forage Yield and Quality of White Clover (*Trifolium repens* L.)

[1]Özgür Töngel and [2]Sebahattin Albayrak
[1]Black Sea Agricultural Research Institute, Samsun, Turkey
[2]Department of Field Crops, Faculty of Agriculture,
Süleyman Demirel University, Isparta, Turkey

Abstract: The effects of four different phosphorus rates (0, 5, 10 and 15 kg da^{-1}) on dry matter yield and some yield components of three white clover cultivars (Huita, Klondike and Nanuk) were evaluated under the Black Sea Coastal Area Conditions in Turkey in 2002-2003 and 2003-2004 growing seasons. Phosphorus treatments significantly affected dry matter and crude protein yields in white clover cultivars. According to the results of average two years, the highest dry matter yield (965.8 kg da^{-1}) was obtained from the parcels having 10 kg da^{-1} phosphorus application. In similarity, the highest crude protein ratio (19.23%) and crude protein yield (185.3 kg da^{-1}) were determined in the parcels having 10 kg da^{-1} phosphorus application. But there were no difference between 5 kg da^{-1} and 10 kg da^{-1} phosphorus applications. Consequently, it is possible to obtain high forage yield of good quality with 5 kg da^{-1} phosphorus application in similar ecological conditions. In addition, Huita and Klondike cultivars should be preferred to Nanuk cultivar concerning high quality and yield.

Key words: White clover, phosphorus, forage yield, forage quality

INTRODUCTION

White clover (*Trifolium repens* L.) is the most widely grown clover in the world. It is a short-lived perennial that can reseed itself under favorable conditions. White clover grows rapidly and its branches grow horizontally as stolons. White clover has a shallow root system, which makes it susceptible to drought. It grows best during cool, moist weather on well-drained, fertile soils with a pH between 6 and 7 (Anonymous, 2005). White clover is commonly grown for grazing as it is resistant to grazing, its forage contains high crude protein and it is able to recover rapidly after grazing. Besides this, it has gradually been used for green area as it can supply nitrogen and it forms dark green grass (Altın, 1991). Phosphorus plays an important role in the development of roots of young plants and is vitally important for the formation of root nodules which have a very high P requirement. When a deficiency in this element occurs, therefore, proportionately more carbon and nutrient resources are partitioned to root growth (thereby facilitating better exploration of the soil volume for P), than to shoot production, causing an increase in the root to shoot ratio (Marschner, 1995). This could account for the significant adverse effects of P deficiency on clover leaf development and stolon branching (Rangeley and Bolton, 1986; Hart and Collier, 1994). Many researchers reported that phosphorus fertilization increases forage yield of white clover (Castilhos and Jacques, 2000; Caradus *et al*., 1996; Ortega *et al*., 1994; Robinson and Savoy 1989; Hern *et al*., 1988; Hume and August, 1988; Macedo *et al*., 1985; Miles *et al*., 1984).

The aim of the study was to determine the effects of different rates of phosphorus fertilizers on yield and nutritional value of three white clover cultivars (*Trifolium repens* L.).

MATERIALS AND METHODS

Field studies were conducted at Black Sea Agricultural Research Institute (15 km east of Samsun, Turkey) in an area of Çarşamba plain (elevation 4 m). The experiments were carried out throughout two growing seasons (2002-2003 and 2003-2004) on clay-loam soil. Soil pH was 7.02; organic matter 2.11%; available P, 7 kg da^{-1}; available K, 75 k da^{-1}. White clover (*Trifolium repens* L.) cultivars were obtained from Field Crops Department, Agricultural Faculty of Ankara University. Climatic data for the research area are given in Table 1.

Factorial arrangements of three white clover cultivars (Huita, Klondike and Nanuk) and four phosphorus levels (0, 5, 10 and 15 kg per decare) were evaluated in a randomized complete block design with three replications. Sowing was done by broadcasting

Corresponding Author: Sebahattin Albayrak, Department of Field Crops, Faculty of Agriculture, Süleyman Demirel University, Isparta, Turkey Tel: +90 246 211 46 30 Fax: +90 246 237 16 93

Table 1: Monthly precipitation, mean temperature and relative humidity in the experimental area

	Precipitation (mm)			Temperature (°C)			Relative humidity (%)		
Months	Long term	2002-2003	2003-2004	Long term	2002-2003	2003-2004	Long term	2002-2003	2003-2004
January	58.4	28.1	84.2	6.9	9.3	8.1	68.0	72.2	61.3
February	48.8	77.8	43.9	6.6	4.8	7.5	70.4	74.0	66.3
March	52.7	73.5	66.2	7.8	5.0	8.5	75.8	75.4	75.4
April	58.3	45.0	101.1	11.1	8.7	11.4	79.5	79.6	77.5
May	50.6	54.7	68.6	15.3	16.2	14.9	80.6	78.4	82.4
June	47.9	3.3	53.4	20.0	20.7	19.8	76.3	68.8	81.5
July	31.3	37.2	68.1	23.1	23.7	21.7	73.4	72.5	80.4
August	50.9	94.0	14.6	23.2	24.1	22.9	73.7	72.9	76.5
September	87.4	194.7	66.2	19.8	19.5	18.9	74.7	75.5	78.8
October	78.6	64.0	83.4	15.9	17.5	15.6	75.8	69.3	81.2
November	73.3	104.0	233.4	11.9	11.5	11.1	70.4	79.7	71.3
December	55.7	61.2	109.8	8.9	9.3	7.6	66.8	64.6	68.8
Total	694.0	838.0	993.0	-	-	-	-	-	-
Mean	-	-	-	14.21	14.19	14.0	73.8	73.6	75.1

on 5 November in 2002. Seeding rates were 1 kg da^{-1}. Plot sizes were 2×4 m = 8 m^2. In the first year, Phosphorus fertilizer was applied before seeding, while there was no phosphorus fertilizer application in the second year. Triple super phosphate (42-44%) was used as fertilizer. Plots were harvested on 3 June and 2 July 2003 and 24 May, 29 June and 10 September 2004, respectively.

After all plots had been harvested, all samples were dried at 70°C for 48 h and weighed. Crude protein content was calculated by multiplying the Kjeldahl nitrogen concentration by 6.25 (Nelson and Sommers, 1973). Crude protein yield was calculated by multiplying dry matter yield by crude protein content. Data were analyzed by analysis of variance (SAS, 1998) at the $p \leq 0.05$ and 0.01 levels of significance and means were compared using the least significant difference test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Dry matter yield: In the first year of the study, means of dry matter yield of white clover belonging to 2 harvests were presented in Table 2. There were no statistically differences among the cultivars, whereas effect of the phosphorus fertilizer and cultivar×phosphorus interaction were significant. Klondike and Huita cultivars had higher dry matter yield than Nanuk cultivar, even though there was no statistically difference among the cultivars. The highest dry matter yield (607.6 kg da^{-1}) was obtained from phosphorus application (5 kg da^{-1}). 10 kg da^{-1}, 15 kg da^{-1} phosphorus application and control parcels (605.8, 597.4 and 549.3 kg da^{-1}, respectively) followed the highest yield (Table 2).

Comparing means of average dry matter yields of phosphorus fertilizer applications, the parcels having 5 kg da^{-1} 10 kg da^{-1}, 15 kg da^{-1} doses applications were statistically in the same group, but there was statistically difference between these phosphorus fertilizer applications and control. In the first year of study, the highest dry matter yield (683.9 kg da^{-1}) was obtained from Huita cultivar with 5 kg da^{-1} phosphorus dose. As comparison to control parcels (no phosphorus fertilizer application), the increase of dry matter yield was 25.6% in Huita cultivar with 5 kg da^{-1} phosphorus dose; while it was 12.7% in Klondike cultivar with 10 kg da^{-1} phosphorus dose and 4.9% in Nanuk cultivar with 15 kg da^{-1} phosphorus dose. In the second year of the experiment, means of dry matter yield of white clover belonging to 3 harvests were presented in Table 3. While effects of the phosphorus fertilizer and cultivars were statistically significant, cultivar×phosphorus interaction was insignificant. As dry matter yield means of cultivars, Klondike and Huita cultivars had higher dry matter yield (1354.3 and 1290.5 kg da^{-1}, respectively) than that of Nanuk cultivar (1160.3 kg da^{-1}). Dry matter yields of the second year of the study are higher than that of the first year. As white clover is perennial forage crop, it is evident that its forage yield in second year is higher than forage in first year (Açıkgöz, 1995). Furthermore, plants in the second year growth better and the number of harvest was more than those of the first year of the study.

Comparing means of average dry matter yields of phosphorus fertilizer applications, the highest dry matter yield (1325.7 kg da^{-1}) was obtained from 10 kg da^{-1} phosphorus dose application. The second highest dry matter yield (1302.9 kg da^{-1}) was obtained from 5 kg da^{-1} phosphorus dose application and there was no statistically significant difference between both applications. Dry matter yield of control and 15 kg da^{-1} phosphorus dose applications (1234.9 and 1209.8 kg da^{-1}, respectively) followed these results (Table 3). Concerning average dry matter yields and comparisons between two years, 10 kg da^{-1} phosphorus dose application gave higher dry matter yield more than 5 kg da^{-1} phosphorus dose application in the second year. Although 5 kg da^{-1} phosphorus dose application is adequate to increase the yield, 10 kg da^{-1} phosphorus dose application is more effective in the second year because of low phosphorus mobilization.

Table 2: Effects of the different phosphorus fertilizer doses on green and dry forage yield, crude protein content and yield of white clover cultivars (total of two harvests in the period of 2002-2003)

Cultivars	Phosphorus levels (kg da^{-1}) 0	5	10	15	Mean
Dry matter yield (kg da^{-1})					
Huita	554.4	683.9	632.3	610.6	620.4
Klondike	567.1	602.6	638.9	628.9	609.3
Nanuk	526.4	536.1	546.1	552.7	540.3
Mean	549.3b	607.6a	605.8a	597.4a	590.0
LSD	C×P: 55.9*				
Crude protein content (%)					
Huita	19.61	19.08	19.84	19.33	19.47
Klondike	19.21	19.57	19.02	18.72	19.13
Nanuk	18.84	19.27	19.39	18.95	19.11
Mean	19.22	19.31	19.41	19.00	19.24
LSD	C×P: 0.81				
Crude protein yield (kg da^{-1})					
Huita	108.2	130.9	125.9	117.7	120.7
Klondike	109.1	118.5	122.0	117.7	116.8
Nanuk	99.6	103.5	105.8	105.1	103.5
Mean	105.6b	117.7a	117.9a	113.5a	113.7
LSD	C×P: 10.5				

Means with same letter are not significantly different (p>0.05)

Average dry matter yields belonging to 3 white clover cultivars in different phosphorus fertilizer doses were presented in Table 4. Two cuttings were made in the first year of the study while 3 cuttings were in the second. Effect of the years, cultivars and phosphorus fertilizer applications on dry matter yields of white clovers was statistically significant. Furthermore, phosphorus fertilizer application×year interaction and cultivar×phosphorus× year interactions were significant but cultivar× phosphorus fertilizer application and cultivar×year interaction were statistically insignificant. According to average results of two years, the highest dry matter yield (981.8 kg da^{-1}) was determined in Klondike cultivar, Huita (955.4 kg da^{-1}) and Nanuk (850.3 kg da^{-1}) cultivars followed dry forage yield of Klondike cultivar.

In terms of average of phosphorus fertilizer application in different doses, the highest dry matter yield of white clover cultivars (965.8 kg da^{-1}) was determined in 10 kg da^{-1} phosphorus fertilizer dose application. But there were no statistically differences between dry matter yield of 10 kg da^{-1} phosphorus fertilizer dose application and that of 5 kg da^{-1} phosphorus fertilizer dose application (955.3 kg da^{-1}). While the parcels having 15 kg da^{-1} phosphorus fertilizer gave an average 903.6 kg da^{-1} dry matter yield, the lowest dry matter yields (892.1 kg da^{-1}) were obtained from the control parcels (Table 4). As comparison to control parcels (no phosphorus fertilizer application), the increase of dry matter yield was 6.71% in Klondike cultivar with 10 kg da^{-1} phosphorus dose; while it was 6.37% in Klondike cultivar with 5 kg da^{-1} phosphorus dose and 12.91% in Nanuk cultivar with 10 kg da^{-1} phosphorus dose. Significant decreases of dry forage yield were observed in all 3 white clover cultivars with 15 kg da^{-1} phosphorus dose.

Reuther and Crawford (1946); Loneragеren (1951); Langin *et al.* (1962) reported that excessive phosphorus fertilizer application might reduce the minerals intake such as Zinc. Aktaş (1991) also reported that there was a correlation between Nitrogen and phosphorus, the increasing effects of phosphorus on production quantity was depending on Nitrogen quantity. Many researchers reported that yield increases with phosphorus fertilizers (Castilhos and Jacques, 2000; Caradus *et al.*, 1996; Ortega *et al.,* 1994; Robinson and Savoy, 1989; Hern *et al.*, 1988; Hume and August, 1988; Macedo *et al.*, 1985; Miles *et al.*, 1984). These results are similar to our findings, whereas Schils and Snijders (2004); Sincik *et al.* (2002) reported that phosphorus fertilizer did not effect the dry forage yield of white clover as precipitation and climate conditions were effective.

Crude protein content: In the first year of the experiment, average crude protein contents of dry forage yield of white clover belonging to 2 harvests were presented in Table 2. There was no statistically difference among the white clover cultivars, effect of the phosphorus fertilization and cultivar×phosphorus interaction were statically insignificant, either. In terms of average crude protein contents of white clover cultivars, even though all cultivars were statistically in the same group, Huita cultivar had higher crude protein content than Klondike and Nanuk cultivars did (19.47, 19.13 and 19.11%, respectively).

Comparing means of average crude protein contents of phosphorus fertilizer applications, the highest crude protein content (19.41%) was obtained from 10 kg da^{-1} doses application, there was no difference among the applications, though. 5 kg da^{-1}, control, 15 kg da^{-1} doses

Table 3: Effects of the different phosphorus fertilizer doses on green and dry forage yield, crude protein content and yield of white clover cultivars (total of three harvests in the period of 2003-2004)

	Phosphorus levels (kg da^{-1})				
Cultivars	0	5	10	15	Mean
Dry matter yield (kg da^{-1})					
Huita	1312.3	1301.9	1344.2	1203.5	1290.5a
Klondike	1339.1	1378.1	1395.4	1304.6	1354.3a
Nanuk	1053.3	1228.9	1237.5	1121.5	1160.3b
Mean	1234.9b	1302.9a	1325.7a	1209.8b	1268.4
LSD	C×P: 87.8				
Crude protein content (%)					
Huita	19.40	18.92	19.14	18.87	19.08
Klondike	18.73	19.20	18.76	18.62	18.83
Nanuk	18.68	19.02	19.24	18.68	18.91
Mean	18.95	19.05	19.04	18.72	18.94
LSD	C×P: 0.82				
Crude protein yield (kg da^{-1})					
Huita	255.6	246.6	257.3	229.3	247.2a
Klondike	252.1	264.6	262.1	244.9	255.9a
Nanuk	197.1	232.8	238.9	208.7	219.4b
Mean	234.9b	247.9a	252.8a	227.7b	240.8
LSD	C×P: 18.02*				

Means with same letter are not significantly different (p>0.05)

applications followed the highest crude protein content (19.31, 19.22 and 19.00%, respectively) (Table 2). In the first year, the highest crude protein content (19.84%) was determined in Huita cultivar with 10 kg da^{-1} phosphorus dose application, while Klondike cultivar with 15 kg da^{-1} phosphorus dose application had the lowest crude protein content (18.72%).

In the second year of the experiment, average crude protein contents of dry forage yield of white clover belonging to 3 harvests were presented in Table 3. There was no statistically difference among the white clover cultivars, effect of the phosphorus fertilization and cultivar×phosphorus interaction were statically insignificant as it was in the first year. As average crude protein contents of white clover cultivars, Nanuk cultivar had the highest crude protein content (19.08%), while the lowest crude protein was determined in Klondike cultivar (18.83%).

Comparing means of average crude protein contents of phosphorus fertilizer applications, the highest crude protein content (19.05%) was obtained from 5 kg da^{-1} doses application. 10 kg da^{-1}, control, 15 kg da^{-1}doses applications followed the highest crude protein content (19.04, 18.95 and 18.72%, respectively) (Table 3). In the second year, the highest crude protein content (19.40%) was determined in Huita cultivar without phosphorus dose application, while Klondike cultivar with 15 kg da^{-1} phosphorus dose application had the lowest crude protein content (18.62%).

Average crude protein contents belonging to 3 white clover cultivars in different phosphorus fertilizer doses were presented in Table 4. Only the effect of the phosphorus fertilizer applications on crude protein content was statistically significant; whereas cultivar×phosphorus fertilizer application, cultivar×year, phosphorus fertilizer application× year and cultivar× phosphorus×year interactions were statistically insignificant.

According to average results of two years, the highest crude protein content of white clover cultivars (19.27%) was determined in Huita cultivar. Crude protein contents of Nanuk (19.01%) and Klondike (18.98%) cultivars followed that of Huita cultivar.

As average of phosphorus fertilizer application in different doses, the highest crude protein content of white clover cultivars 19.23% was determined in 10 kg da^{-1} phosphorus fertilizer dose application. But there was no statistically difference among the applications of 5 kg da^{-1}, control, 10 kg da^{-1} phosphorus fertilizer doses and they were in the same group, statistically (19.18, 19.08 and 19.23%). The lowest crude protein content of the white clover cultivars was 18.86% in the application of 15 kg da^{-1} phosphorus fertilizer dose (Table 4).

Partly a decrease or a slight increase of crude protein content was observed in all 3 white clover cultivars with 15 kg da^{-1} phosphorous dose comparing with control parcels. The reason of this, plants could not find sufficient nitrogen in the soil with excessive phosphorus; therefore it might cause that plant tissues contained low quantity nitrogen (Özyazıcı and Manga, 1996).

Crude protein yield: In the first year of the experiment, Average crude protein yields of dry forage yield of white clover belonging to 2 harvests were presented in Table 2. There was no statistically difference among the white clover cultivars; effect of the phosphorus fertilization was

Table 4: Effects of the different phosphorus fertilizer doses on green and dry forage yield, crude protein content and yield of white clover cultivars (averages of 2002-2003 and 2003-2004)

	Phosphorus levels (kg da^{-1})				
Cultivars	0	5	10	15	Mean
Dry matter yield (kg da^{-1})					
Huita	933.4	992.9	988.4	907.0	955.4a
Klondike	953.1	990.3	1017.1	966.7	981.8a
Nanuk	789.8	882.5	891.8	837.1	850.3b
Mean	892.1b	955.3a	965.8a	903.6b	929.2
LSD	Y: 42.8** C×P: 50 C×Y:74 P×Y:41** C×P×Y: 71**				
Crude protein content (%)					
Huita	19.51	19.00	19.49	19.09	19.27
Klondike	18.97	19.39	18.89	18.67	18.98
Nanuk	18.76	19.15	19.31	18.82	19.01
Mean	19.08ab	19.18ab	19.23a	18.86b	19.09
LSD	Y: 0.34 C×P: 0.55 C×Y: 0.39 P×Y: 0.45 C×P×Y: 0.78				
Crude protein yield (kg da^{-1})					
Huita	181.9	188.8	191.6	173.5	186.4a
Klondike	180.6	191.5	192.1	181.3	183.9a
Nanuk	148.3	168.1	172.3	156.9	161.4b
Mean	170.3b	182.8a	185.3a	170.6b	177.3
LSD	Y: 9.8** C×P: 10.1 C×Y:17 P×Y:8.22** C×P×Y:14.2**				

Means with same letter are not significantly different (p>0.05)

significant; whereas cultivar×phosphorus interaction were statistically insignificant. As average crude protein yields of white clover cultivars, even though all cultivars were statistically in the same group, Huita cultivar had higher crude protein content than Klondike and Nanuk cultivars did (120.7, 116.8 and 103.5 kg da^{-1}, respectively).

Comparing means of average crude protein yields of phosphorus fertilizer applications, 5 kg da^{-1}, 10 kg da^{-1} and 15 kg da^{-1} phosphorus fertilizer doses applications were in the same group, statistically, but there was significant difference between all phosphorus fertilizer doses applications and control (no phosphorus). In the first year, the highest crude protein yield (130.9 kg da^{-1}) was obtained from Huita cultivar with 5 kg da^{-1} doses application and 25.6% forage yield increase was supplied according to the same cultivar without phosphorus. The highest crude protein yields of Klondike and Nanuk cultivar were determined in 10 kg da^{-1} phosphorus dose application (122.0 and 105.8 kg da^{-1}).

In the second year of the experiment, average crude protein yields of dry forage yield of white clover belonging to 3 harvests were presented in Table 3. There was statistically significant difference among the white clover cultivars and effect of the phosphorus fertilization applications. Cultivar×phosphorus interaction was also statistically significant. As average crude protein yields of white clover cultivars, Klondike and Huita cultivars had higher average crude protein yields (255.9 and 247.2 kg da^{-1}, respectively) than Nanuk cultivar (219 kg da^{-1}).

Comparing means of average crude protein yields of phosphorus fertilizer applications, the highest crude protein yield (252.8 kg da^{-1}) was obtained from 10 kg da^{-1} dose application. 5 kg da^{-1} dose application had the second high crude protein yield (247.9 kg da^{-1}) and there was no difference between 10 kg da^{-1} and 5 kg da^{-1} dose application. Control and 15 kg da^{-1} dose application followed the h ighest crude protein yield (234.9 and 227.7 kg da^{-1}, respectively) (Table 3). Campillo (1990) reported that 6.6 kg da^{-1} phosphorus fertilizer dose supplied significant increases and especially crude protein yield in the second year was higher than crude protein yield in the fist year. These findings are similar with our study.

Average crude protein yields belonging to 3 white clover cultivars in different phosphorus fertilizer doses were presented in The effects of the phosphorus fertilizer applications, cultivars and years on crude protein yield were statistically significant; furthermore phosphorus fertilizer application×year and cultivar×phosphorus fertilizer application×year interactions were statistically significant but cultivar×phosphorus fertilizer application and cultivar×year interactions were statistically insignificant. According to average results of two years, the highest crude protein yield of white clover cultivars (186.4 kg da^{-1}) was determined in Huita cultivar. Crude protein yields of Klondike (183.9 kg da^{-1}) and Nanuk (161.4 kg da^{-1}) cultivars followed that of Huita cultivar.

As average of phosphorus fertilizer application in different doses, the highest crude protein yield of white clover cultivars was determined in 10 kg da^{-1} phosphorus fertilizer dose application. But there was no statistically difference between 5 kg da^{-1} and 10 kg da^{-1} phosphorus fertilizer applications and they were in the same group, statistically (182.8 and 185.3 kg da^{-1}, respectively). Average crude protein yield of the white clover cultivars was 170 kg da^{-1} in 15 kg da^{-1} phosphorus fertilizer application (Table 4). The lowest crude protein yield (170.3 kg da^{-1}) was obtained from the control (no phosphorus fertilizer application) (Table 4). The reason of crude protein yield decrease in high phosphorus dose application (15 kg da^{-1}) was due to low dry forage yield in the same phosphorus fertilizer dose (Özyazıcı and Manga, 1996).

According to results of the study which was conducted 2 years in Blacksea coastal conditions and examined the effects of the different phosphorus fertilizer doses on forage yield and quality of white clover cultivars. The highest dry matter and crude protein yield were obtained from the parcels having 10 kg da^{-1} phosphorus fertilizer application. However there was no statistically difference between 5 and 10 kg da^{-1} phosphorus fertilizer applications in terms of dry matter and crude protein yields. Therefore, it is feasible to obtain high quality crop from white clover (*Trifolium repens* L.) with 5 kg da^{-1} phosphorus fertilizer dose in similar ecologies. In addition, Huita and Klondike cultivars should be preferred to Nanuk cultivar concerning yield and quality.

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