



Some chemical and morphological properties of five clover species (*Trifolium* sp.) at different aspect of pasture in Belovets village (Razgrad), Bulgaria

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Abstract

The aim of investigation was to determine the effects of aspect (north and south) on some morphological [plant height (cm), number of stems per plant, main stem diameter (mm), number of leaves per main stem, petiole length (cm), leaflet length (cm), leaflet width (cm), leaf: stem ratio] and chemical [crude protein (%), crude cellulose (%), calcium (%), phosphorus (%), potassium (%) and magnesium (%) ratios] properties of five clover species [hare's-food clover (*Trifolium arvense* L.), field clover (*T. campestre* Schreb.), suckling clover (*T. dubium* Sibth.), alsike clover (*T. hybridum* L.) and zigzag clover (*T. medium* L.)] in pasture vegetation. The plant height, main stem diameter, number of leaves per main stem, petiole length, leaflet length, leaflet width, leaf: stem, crude protein, crude cellulose, calcium and potassium ratios were affected by aspect. The maximum plant height (65.53 cm), leaf: stem ratio (1.17) and crude protein ratio (20.00%) were found in zigzag clover on north-facing of pasture. The highest magnesium ratio (0.41%) and lowest number of stems per plant (4.85) were determined from the zigzag clover. The hare's-food clover showed lower values ($P < 0.01$) than the other clovers for phosphorus content (0.275%). The main stem diameter ranged from 3.53 to 5.27 mm. The north-facing of pasture had highest number of leaves per main stem (17.07) and petiole length (1.83 cm). The maximum leaflet length (3.50 cm) and width (2.55 cm) were measured for alsike clover on north-facing of pasture. The highest crude cellulose (28.23%) and calcium (3.13%) ratios were determined in hare's-food clover on south-facing of pasture, whereas the lowest potassium ratio (2.37-2.39) was found in same clover species on each aspect of pasture.

Keywords: Aspect; Clover species; Forage quality; Morphological characters; Pasture.

Introduction

Over 3 billion hectares of the land area of the world is grazing land and another 4 billion hectares of forest and woodland have some grazing potential. World grasslands support approximately 1.5 billion cattle equivalents (cattle, *Bos* sp.; buffalo, sheep, *Ovis aries*; goat, *Capra* sp.; and camel, *Camelus* sp.) and forages provide over 90% of the feed energy

consumed by these herbivorous animals (Givens et al., 2007). Grasslands can furnish high-quality, low-cost feed for cattle, horses and sheep. The efficient use of grasslands, however, requires very careful planning and good management of both animals and forage crops. The use of some type of rotational grazing is essential to control plant growth and furnish animals with the amount and quality of forage necessary to meet their nutritional requirements, but the careless planning and the bad management of the grasslands in the world has resulted in a great deficiency in forage production. However, permanent grasslands decreased. For example, permanent grasslands decreased in 15 EU countries, progressively, in 64, 62, 59 and 56 million hectares in 1970, 1980, 1990 and 2000, respectively (Cherney and Fick, 2001; Porqueddu et al., 2003).

Topography is the principal controlling factor in vegetation growth and that the amount of rainfalls and the type of soil play secondary roles at the scale of hill slopes (Dawes and Short, 1994; Jin et al., 2008). Aspect, slope, and altitude are three base topographic factors that control the distribution and botanic composition of pasture and other plant vegetations in areas. Besides, grazing of animals affects aspect and slope. Holechek et al. (1982) observed that cattle on south exposure slopes tended to consume grasses throughout the year, whereas cattle grazing north-facing slopes had a greater diversity of grasses, forbs, and shrubs available.

Clovers are in the tribe *Trifolieae* of the subfamily *Papilionoideae*, family *Fabaceae*, *Trifolium* L. The genus contains approximately 230-250 species. Clovers are used for forage, pasture, soil improvement and silage (Tekeli and Ates, 2003). Besides, clovers are an important source of nutrients for livestock and are grown throughout grasslands in the world. Ruminant animals have the capacity to convert forage into meat, milk, and wool, which are products desired by livestock breeders. Because clovers are basic to ruminant livestock production, it is necessary to produce clovers cultivars that continue to be high in quality and possess a minimum of anti-quality components (Ates and Servet, 2004). These components exhibit a continuous range of expression and are quantitatively inherited. The expression of this quantitative inheritance is probably also influenced by the environment (climatic, topographic and etc. conditions) (Ates and Tekeli, 2004).

The aim of this study was to effects of aspect (north and south) on some morphological [plant height (cm), number of stem per plant, stem diameter (mm), number of leaf per main stem, petiole length (cm), leaflet length (cm), leaflet width (cm), leaf: stem ratio] and chemical [crude protein ratio (%), crude cellulose (%), calcium (Ca) (%), phosphorus (P) (%), potassium (K) (%) and magnesium (Mg) (%) ratios] properties of five clover species [hare's-foot clover (*Trifolium arvense* L.), field clover (*T. campestre* Schreb.), suckling clover (*T. dubium* Sibth.), alsike clover (*T. hybridum* L.) and zigzag clover (*T. medium* L.)] in pasture vegetation.

Materials and Methods

This research was conducted in the pasture of Belovets village in Razgrad (Bulgaria), for three years, between 2006 and 2008. The experimental pasture (43° 48' N, 26° 39' E) had an altitude of 641-650 m, with a total precipitation of 525 mm on average and an annual overall temperature of 10.8 °C. The precipitation was 499 mm, 522 mm and 514 mm, respectively, in the three experimental years, with the temperatures (2006, 10.5 °C; 2007,

11.2 °C and 2008, 11.1 °C) being similar to the long-term average. The pasture soil (north and south) where the study was conducted was good in organic matter (4.6%), rich in P content (average of 130.5 kg ha⁻¹), but moderate in K content (72.0 kg ha⁻¹) and with pH 5.9. This pasture replaced the original oak (*Quercus* sp.) forests and has been maintained since one hundred fifty years by the grazing activity of wild (deer, *Cervus* sp.) and domestic (sheep, cattle, goat, and horse, *Equus* sp.) animals.

The north (0.2 ha) and south (0.2 ha) aspects (slope: 33%) of pasture were divided for three blocks. The experimental design was a randomized complete block with three replicates. Plots (50 × 50 m) with in randomly selected areas were defined. Five clover species (hare's-foot clover, field clover, suckling clover, alsike clover and zigzag clover) were used in the experiments. Sixty samples of species were collected each year (May to September) at full-bloom stage of species. The plant height (cm), number of stems per plant, main stem diameter (mm), number of leaves per main stem, petiole length (cm), leaflet length (cm) and width (cm) were determined in these samples, which were chosen not damaged by biotic and abiotic factors (Pederson et al., 1999). Petiole and leaflet lengths, leaflet width were measured on the leaf at the third node of the plants. The main stem diameter was measured between the third and fourth node. Measurements of width and length of leaflet were concluded at the terminal leaflet (Ates and Servet, 2004). Whole plant samples were sterilized with 2% sodium hypochlorite for 15 min. Samples were hand-separated into leaf (including leaf sheath and inflorescence) and stem components. After extensive rinses with running tap water and dematerialized water and immediately dried at 55 °C for 48 h and stored room temperature (Ates and Tekeli, 2007). The components were weighed, and the leaf dry weight was divided by the stem dry weight to calculate leaf: stem ratio.

All dried samples were ground to small (0.5 mm) pieces and used for the analyses. The crude protein (CP) content (%) was determined by the micro-Kjeldahl method. Analysis of the samples for crude cellulose (CC) (%), P (%), Ca (%), Mg (%) and K (%) contents were carried out by the procedures of AOAC (2007). All samples were analyzed in triplicate. The results were analyzed using the MSTAT-C statistical computer package program. There were no significant differences between years ($P > 0.05$ and 0.01). Same program was used for the comparison test (Fisher's Least Significant Difference, LSD) of the means from the three years.

Results and Discussion

The results of the analyses of the properties investigated are given Tables 1 to 4. Values of plant height, leaf: stem and CP ratios varied depending on aspects and species ($P < 0.01$). Meanwhile aspect x species was also significant in term of plant height, leaf: stem and CP ratios ($P < 0.01$). The north-facing of pasture showed the highest plant height (46.70 cm), leaf: stem ratio (0.98) and CP ratio (18.50%). The maximum plant height (65.53 cm), leaf: stem ratio (1.17) and CP ratio (20.00%) were found in zigzag clover on north-facing of pasture (Tables 1, 2 and 3). Awan et al. (1999), Walburger et al. (2000) mentioned that the morphology and CP ratios of plant species changed depending on aspects. The plant height, number of stems per plant, main stem diameter, number of leaves per plant, petiole length, leaflet width and length are important traits used to determine green fodder and dry matter

yields; besides, leaf: stem ratio, number of stems per plant, main stem diameter, number of leaves per plant, petiole length, leaflet width and length, crude protein and cellulose ratios, mineral contents are important characters for forage quality (Ates and Servet, 2004). Agricultural and botanical characters of some annual clover species were studied by Tekeli and Ates (2003), those obtained that the plant height, leaf: stem ratio and CP ratio ranging from 15.33-70.67 cm, 0.64-1.95 and 16.23-22.23%, respectively. The results were similar to those reported by these researchers. The lowest plant height (50.00 cm) of zigzag clover was measured by Stace et al. (2010).

Table 1. The effects of different aspects on plant height, number of stems per plant, main stem diameter, number of leaves per main stem of five clover species.

Traits	Species						Average of aspects	SE±
	Aspects	Hare's-food clover	Field clover	Suckling clover	Zigzag clover	Alsike clover		
Plant height, cm	North	45.03 ^c	34.73 ^e	32.67 ^f	65.53 ^a	55.53 ^b	46.70 ^a	Aspect: 3.00E-02** Species: 4.83E-02**
	South	36.23 ^d	24.83 ^g	21.17 ^h	55.80 ^b	46.03 ^c	36.81 ^b	
Average of species		40.63 ^c	29.78 ^d	26.92 ^e	60.67 ^a	50.78 ^b	41.76	Aspect x Species: 8.83E-02**
Number of stems per plant	North	5.10	7.60	9.90	4.83	33.60	12.21	Aspect: NS Species: 7.72E-02**
	South	4.73	7.53	9.90	4.87	33.40	12.09	
Average of species		4.92 ^d	7.57 ^c	9.90 ^b	4.85 ^d	33.50 ^a	12.15	Aspect x Species: NS
Main stem diameter, mm	North	4.43	3.70	3.53	4.80	5.00	4.29 ^b	Aspect: 3.62E-03** Species: 1.36E-02**
	South	4.90	4.07	4.13	5.27	4.73	4.62 ^a	
Average of species		4.67 ^c	3.89 ^d	3.83 ^d	5.04 ^a	4.87 ^b	4.46	Aspect x Species: NS
Number of leaves per main stem	North	16.00	16.33	18.33	20.00	14.67	17.07 ^a	Aspect: 4.55E-02** Species: 8.845E-02**
	South	12.00	13.00	17.00	18.67	11.00	14.33 ^b	
Average of species		14.00 ^{cd}	14.50 ^c	17.67 ^b	19.34 ^a	12.84 ^d	15.70	Aspect x Species: NS

**P<0.01, ^{NS}Not Significant.

The effects of aspect and aspect x species interactions on number of stems per plant, P and Mg contents were found to be not significant (P>0.01, 0.05) (Tables 1, 3 and 4). The highest Mg ratio (0.41%) and lowest number of stems per plant (4.85) were determined from the zigzag clover (P<0.01). The hare's-food clover showed lower values (P<0.01) than the other clovers for P content (0.275%). The data for main stem diameter, number of leaves per main stem and petiole length were analyzed and analysis are shown Tables 1 to 2. According to the analysis of variance, the effect of aspect and species on main stem diameter, number of leaves per main stem and petiole length were found to be significant (P<0.01). The main stem diameter ranged from 3.53 to 5.27 mm. The north-facing of

pasture had highest number of leaves per main stem (17.07) and petiole length (1.83 cm). The influence of aspect on the pattern of seasonal growth in the *Parmelia glabratula* ssp. *fuliginosa* (Fr. Ex Duby) laund were studied by Armstrong (1975), who reported that the some morphological characters of its not changed depending on aspects; whereas Andic (1993) mentioned that the number of main stem of plant species changed depending on aspect. The negative effect of slope on P content in soil, as well as that of aspect on pH, could be due to nutrient loss through surface runoff. Northern aspects usually have higher levels of precipitation, thus facilitating loss of nutrients through surface runoff, a parameter also greater on steep slope sites (Chase et al., 2000). Acar et al. (2001) reported 2-5 stem per plant from hare's-food clover. A lower main stem diameter of 2.27-3.69 mm was measured by Tekeli and Ates (2003, 2006a). Tekeli et al. (2003) found a P content of 0.47% and an Mg content of 0.45% for Persian clover (*T. resupinatum* L.). The results of Mg content were lower to those reported by these researchers. Environmental factors such as aspect and slope affected plant community structure, nutritional contents of plant and soil, most likely due to their effect on precipitation, surface runoff, and grazers' behavior (Amezaga et al., 2004). Number of leaves per plant increases on north aspect has been reported by Scheidel and Bruelheide (2004).

Table 2. The effects of different aspects on petiole length, leaflet length, leaflet width and leaf:stem ratio of five clover species.

Traits	Species						Average of aspects	SE±
	Aspects	Hare's-food clover	Field clover	Suckling clover	Zigzag clover	Alsike clover		
Petiole length, cm	North	1.53	1.17	0.90	2.57	3.00	1.83 ^a	Aspect: 5.66E-03** Species: 1.31E-02** Aspect x Species: NS
	South	1.27	0.67	0.78	1.90	2.27	1.38 ^b	
Average of species		1.40 ^c	0.92 ^d	0.84 ^d	2.24 ^b	2.64 ^a	1.61	1.31E-02**
Leaflet length, cm	North	1.57 ^e	1.27 ^g	1.00 ^h	2.70 ^c	3.50 ^a	2.01 ^a	Aspect: 2.57E-03** Species: 1.22E-02** Aspect x Species: 1.73E-02*
	South	1.27 ^f	0.67 ⁱ	0.67 ⁱ	2.07 ^d	2.97 ^b	1.53 ^b	
Average of species		1.42 ^c	0.97 ^d	0.84 ^d	2.39 ^b	3.24 ^a	1.77	1.73E-02*
Leaflet width, cm	North	0.40 ^h	1.90 ^c	0.62 ^e	1.64 ^d	2.55 ^a	1.42 ^a	Aspect: 7.16E-03** Species: 5.66E-03** Aspect x Species: 9.11E-02**
	South	0.27 ⁱ	1.30 ^e	0.48 ^h	1.28 ^e	2.07 ^b	1.08 ^b	
Average of species		0.34 ^e	1.60 ^b	0.55 ^d	1.46 ^c	2.31 ^a	1.25	9.11E-02**
Leaf:stem ratio	North	0.88 ^d	0.83 ^e	0.95 ^c	1.17 ^a	1.06 ^b	0.98 ^a	Aspect: 1.15E-03** Species: 2.56E-03** Aspect x Species: 4.33E-03**
	South	0.82 ^e	0.78 ^e	0.87 ^d	0.98 ^c	0.95 ^c	0.88 ^b	
Average of species		0.85 ^d	0.81 ^e	0.91 ^c	1.08 ^a	1.01 ^b	0.93	4.33E-03**

* P<0.05, ** P<0.01, ^{NS} Not Significant.

Table 3. The effects of different aspects on CP, CC, Ca and P ratios of five clover species.

Traits	Aspects	Species					Average of aspects	SE±
		Hare's-food clover	Field clover	Suckling clover	Zigzag clover	Alsike clover		
CP, %	North	19.27 ^c	17.90 ⁱ	15.77 ^h	20.00 ^a	19.57 ^b	18.50 ^a	Aspect: 1.61E-02** Species: 9.63E-03** Aspect x Species: 1.35E-02**
	South	18.40 ^e	17.07 ^g	15.00 ⁱ	18.90 ^d	18.97 ^d	17.67 ^b	
Average of species		18.84 ^c	17.49 ^d	15.39 ^e	19.45 ^a	19.27 ^b	18.09	
CC, %	North	27.37 ^b	24.20 ^f	23.50 ^h	26.03 ^c	23.83 ^g	24.99 ^b	Aspect: 1.81E-02** Species: 1.59E-02** Aspect x Species: 2.25E-02**
	South	28.23 ^a	25.07 ^d	24.33 ^f	27.33 ^b	24.67 ^e	25.93 ^a	
Average of species		27.80 ^a	24.64 ^c	23.92 ^e	26.68 ^b	24.25 ^d	25.46	
Ca, %	North	2.87 ^b	2.10 ^d	1.60 ^g	1.67 ^f	1.35 ⁱ	1.92 ^b	Aspect: 4.51E-03** Species: 5.83E-03** Aspect x Species: 9.10E-02**
	South	3.13 ^a	2.63 ^c	1.72 ^e	1.73 ^e	1.45 ^h	2.13 ^a	
Average of species		3.00 ^a	2.37 ^b	1.66 ^d	1.70 ^c	1.40 ^e	2.03	
P, %	North	0.280	0.360	0.350	0.440	0.490	0.390	Aspect: NS Species: 1.50E-03** Aspect x Species: NS
	South	0.270	0.370	0.350	0.430	0.490	0.380	
Average of species		0.275 ^e	0.365 ^c	0.350 ^d	0.435 ^b	0.490 ^a	0.385	

** P<0.01, ^{NS} Not Significant.

Table 4. The effects of different aspects on K and Mg ratios of five clover species.

Traits	Aspects	Species					Average of aspects	SE±
		Hare's-food clover	Field clover	Suckling clover	Zigzag clover	Alsike clover		
K, %	North	2.37 ^f	2.45 ^e	2.57 ^d	2.75 ^c	3.33 ^b	2.69 ^b	Aspect: 3.54E-03* Species: 2.65E-03** Aspect x Species: 3.71E-02**
	South	2.39 ^f	2.47 ^e	2.58 ^d	2.79 ^c	3.55 ^a	2.76 ^a	
Average of species		2.38 ^e	2.46 ^d	2.58 ^c	2.77 ^b	3.44 ^a	2.73	
Mg, %	North	0.26	0.30	0.20	0.41	0.33	0.30	Aspect: NS Species: 2.65E-04** Aspect x Species: NS
	South	0.26	0.31	0.20	0.41	0.33	0.30	
Average of species		0.26 ^d	0.31 ^c	0.20 ^e	0.41 ^a	0.33 ^b	0.30	

* P<0.05, ** P<0.01, ^{NS} Not Significant.

The aspect, species and aspect x species interactions significantly affect the leaflet length and width (Table 2). The maximum leaflet length (3.50 cm) and width (2.55 cm) were measured for alsike clover on north-facing of pasture. Awan et al. (1999), Hovenden and Vander Schoor (2003) mentioned that the morphological properties of leaf in plant

species changed depending on aspect. Tekeli and Ates (2006a) stated that the leaflet width varied 0.5 to 3.0 cm in alsike clover. The lowest leaflet length (1.0-3.0 cm) was found by Edinçliler et al. (2004).

The means of the CC, Ca and K ratios from the aspect are significantly different by a LSD test at the P=0.01 level of probability. These results suggests that the CC (25.93%), Ca (2.13%) and K (2.76%) ratios of clovers on the south-facing and north-facing of pasture are significantly different (Tables 3 and 4). The highest CC (28.23%) and Ca (3.13%) ratios were determined in hare's-food clover on south-facing of pasture, whereas the lowest K ratio (2.37-2.39) was found in same clover species on each aspect of pasture. Monogastric animals lack the necessary enzymes to cleave the linkages of glucose molecules in cellulose. Hence, they are poor users of fibrous forage crops. The microorganisms in the rumen contain the enzymes cellulases; hence, forage that is high in fiber can be utilized effectively by ruminants. On the other hand, the mineral elements play a very important role in the growth of plants and animals. Eighteen mineral elements are known to be required by at least some animal species. They can be divided into two groups (macro and microelements) based upon the quantity required in the forage. Macro elements are required in amounts ranging from a few tenth of a gram, to one or more grams per day. Microelements are required in minute quantities, ranging from a microgram to a milligram per day. The two groups, based on the amounts needed in the forage, are the following: macro elements: sodium (Na), chlorine (Cl), Ca, P, Mg, K, and sulphur (S); micro elements: chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), molybdenum (Mo), selenium (Se), silicon (Si) and zinc (Zn) (Tekeli and Ates, 2006b). NRC (2001) reported that the requirement for major mineral nutrients for gestating beef cows or lactating beef cows is 0.6-0.8% (w/w) for K, 0.18-0.44% for Ca, 0.18-0.39% for P and 0.04-0.10% for Mg. The K, Ca and Mg levels in plants are usually in the range 1.39-2.50%, 0.77-3.00% and 0.20-1.20%, respectively, which is adequate for plant growth (Acikgoz, 1994; Tekeli et al., 2003). The results were similar to those reported by these researchers.

In conclusion, the some morphological (plant height, main stem diameter, number of leaves per main stem, petiole length, leaflet length, leaflet width, leaf: stem) and chemical (crude protein, crude cellulose, calcium and potassium ratios) traits were affected by aspect. The variability in the morphological and chemical properties of clover species were related to different photoperiod, light intensity, temperature and moisture, possibly due to their effect on aspects.

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