NUTRITIVE VALUES OF SOME ANNUAL CLOVERS (Trifolium sp.) AT DIFFERENT GROWTH STAGES

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Manuscript received: April 9, 2005; Reviewed: April 12, 2005; Accepted for publication: May 16, 2005

ABSTRACT

This study was conducted between the years of 2001-2002 in the experimental area and laboratory of Field Crops Department of Agriculture Faculty in Trakya University, Tekirdağ (Turkey). Five different clovers [Persian (Trifolium resupinatum L. var. majus Boiss.), Mediterranean (T. spumosum L.), narrow-leaved (T. angustifolium L.), hedgehog (T. echinatum M. Bieb.) and lappa (T. lappaceum L.) clovers] were used. Each plot consisted of 8 rows with a length of 5 m. Row spacing of 30 cm and sowing rate of 10 kg ha^{-1} were used. Sowing times were on 2.25.2001 and on 2.28.2002. Plots were not irrigated and fertilized after sown and harvest. One cut was taken in both years at 4 growing stages such as pre-bud, pre-bloom, 50% bloom and full-bloom. The central 1 m^{-2} sections was cut at ground level for dry matter. Approximately 500g samples were dried at 55 °C for 24 hours and stored for one day at room temperature then found dry matter. Crude protein (%) was determined by Kjeldahl method.

KEYWORDS: calcium, crude cellulose, crude protein, magnesium, phosphorus, potassium ratio

INTRODUCTION

The clovers are in the tribe Trifolieae of the subfamily Papilionoideae, family Fabaceae, Trifolium L. The genus contains approximately 230-250 species. The clovers are used for forage, pasture, soil improvement and silage [22]. The clovers are an important source of nutrients for livestock and are grown throughout the world. Animals have the capacity to convert forage into meat, milk and wool, which are products desired by livestock breeders. Because clovers are basic to livestock production, it is necessary to produce clovers that continue to be high in quality and possess a minimum of anti-quality components. Clovers quality might be considered as the characteristic nutritive value for animals. Nutritive value can be determined in terms of production milk, meat or etc. It's, may supply from 15-100% of the protein requirement and 20-100% of the energy requirement for animals, depending on the type of animal and season of the year [11]. Although the levels of cell-wall components in clovers are lower than those in grasses, the cell walls of clovers are highly lignified and less available than those of grasses. Many factors determine the effect of clovers nutritive values and mineral composition on forage digestibility and intake. Macro factors that affect the nutritive values and mineral composition of clovers during growth and development include; a) climatic factors, b) growth stage, c) cutting time, d) leaf ratio, e) stem ratio, f) disease damage, g) insect damage, h) weeds ratio and i) soil traits.

Mineral nutrients play a very important role in the growth of plant and animals. Consisted ratio at mineral nutrients are approximately 1.5-5% of animal body; of this 1.33% calcium (Ca), 0.74% phosphorus (P), 0.19% potassium (K) and 0.041% magnesium (Mg) [25]. NRC [17] 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 0.04-0.1% for Mg. Voisin [28] mentioned that when concentrations of K and nitrogen (N) are high, 0.25% Mg in the forage may be required to prevent grass tetany. Nitrogen, K, Ca and Mg levels in plants are usually in the range 3.0-6.0%, 2.3-2.5%; 0.77-3.0%, 0.20-1.20% respectively, which is adequate for plant growth [2, 18]. Essig [11] stated that the 2.36% K, 1.41% Ca, 0.31% P and 0.30% Mg in crimson clover (T. incarnatum L.) at full-bloom stage. Anonymous [6] stated that the protein, P and K concentrations of red clover (T. pratense L.) declined from pre-bloom to the late-bloom stage. Frame et al. [12] determined that the N content (35-40 g kg^{-1}) in white clover (T. repens L.). They emphasized the N, P, K content of red clover and alfalfa declined with maturity. Mediterranean clover (T. spumosum L.) produced 5.30 t ha^{-1} dry matter yields [15]. Persian clover (T. resupinatum L.) is provides a high-quality forage (6.8-26.9% dry matter, 16.8-24.4% crude protein, 11.2-21.2% crude cellulose, 0.24-0.51% P, 1.39-2.08% K, 1.50-1.20% Ca and 0.40-0.80% Mg) for animals throughout the growing season [25]. Recommended ratio at Ca:P is approximately 1.5 or above 1.5 [14]. It is very important to keep this balance; even though one element may be at the minimum, the other element may be in excess of the balance, consequently creating an imbalance within the animal's body [16]. Rodriguez Julià [19] determined that 6.25 K: P, 2.64 Ca:P and 0.45 Ca:K ratios from the white clover/grass mixtures.

The aim of this study was to determine of some chemical traits and nutritive values [hedgehog clover (T. echinatum M.Bieb.), lappa clover (T. lappaceum L.), Mediterranean clover, narrow-leaved clover (T. angustifolium L.), and Persian clover (T. resupinatum L. var. majus Boiss.)] in some annual clovers at different growth stages.

MATERIALS AND METHODS

A randomized complete block design experiment with three replications [26] was initiated in the spring of 2001 and 2002 at Tekirdağ Agricultural Faculty (41.0º N, 27.5º E) in dry condition. The climatic conditions during the growing season are given table 1. The analysis of soil samples taken from the experimental area showed that organic matter content was low and that soil was clay (Table 2.).

Five annual clover species were used in the experiments. Narrow-leaved clover collected from grasslands of the Trakya region, Turkey. Persian clover (cultivar Demet-82) obtained from Department of Field Crops, Agriculture Faculty of Tekirdağ, Turkey. Other species (Mediterranean, lappa and hedgehog clovers) were obtained from Israel Gene Bank. Each plot consisted of 8 rows with a length of 5 m. Row spacing of 30 cm and sowing rate of 10 kg ha^{-1} were used [7, 9]. Sowing times were on 2.25.2001 and on 2.28.2002. Plots were not irrigated and fertilized after sown and harvest. One cut was taken in both years at 4 growing stages such as pre-bud, pre-bloom, 50% bloom and full-bloom. The central 1 m^{-2} sections was cut at ground level for dry matter. Approximately 500g samples were dried at 55 °C for 24 hours and stored for one day at room temperature then found dry matter. The crude protein (%) was determined by Kjeldahl method [13, 20]. Analysis of the samples for crude cellulose (%), P (%), K (%), Ca (%), and Mg (%) contents were carried out by the procedure of Açıkgöz et al. [1], Akyıldız [4], Altinok et al. [5], Tekeli et al. [20] and calculated the K:P, Ca:P, Ca:K ratio [8]. The results

Table 1. The climatic conditions during the growing season and long years' mean (LYM= 1930-1990)

	Total Rainfall (mm)			Mean Temperature (ºC)			Relative Humidity (%)		
Month	2001	2002	LYM	2001	2002	LYM	2001	2002	LYM
February	86.6	35.9	52.4	7.2	8.2	5.2	79.3	78.7	80.0
March	22.8	55.0	54.0	12.3	9.4	6.9	74.5	76.2	79.0
April	68.6	37.9	43.1	12.4	10.9	11.6	76.5	74.0	76.0
May	57.2	5.6	37.3	16.9	17.1	16.5	67.0	68.6	76.0
June	9.2	43.8	38.0	21.3	22.3	20.9	61.5	66.7	71.0
July	20.8	42.9	28.0	25.7	26.0	23.7	65.3	66.6	71.1
August	8.6	12.7	18.7	25.2	24.8	23.9	67.0	67.4	70.3

Table 2. The soil characteristics of the experimental area

Year	Depth (cm)	Texture	pH	Organic Matter (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})
2001	0-20	Clay	6.9	0.89	58.0	777.0
	20-40	Clay	6.9	0.55	22.0	689.0
2002	0-20	Clay	7.5	1.34	70.1	677.0
	20-40	Clay	7.5	1.31	22.6	643.0

were analyzed using the TARİST software [3].

RESULTS AND DISCUSSION

The clovers are used more widely for grazing than for harvested forage, but are also important as hay, silage, and green-chop. Although they are annuals or short-lived perennials, stands can be maintained for long periods of time because they can generally be re-established easily or allowed to seed naturally [27]. Several winter annuals (Persian clover, Mediterranean clover, crimson clover, T. incarnatum L. and arrowleaf clover, T. vesiculosum Savi.) have become important for winter grazing in the Mediterranean climatic conditions. Besides, the clovers are usually grown with a grass, providing nitrogen to the grass and increasing the protein of the forage. In the subtropical regions, Persian clover (usually T. resupinatum L. var. typicum Fiori et Paol.) is typically grown in meadows and pasture with a cool-season perennial grass such as perennial ryegrass (Lolium perenne L.), or tall fescue (Festuca arundinacea Schreb.). The dry matter, crude protein, crude cellulose, P, Ca, K, Mg, Ca:P and K:P ratios were all affected by the different species and growth stages. The differences between growth stages for Ca:K were found to be not significant; but, differences in Ca:K ratios of the clover species were significant. The dry matter, crude protein, crude cellulose, mineral composition and mineral balance are the most important traits for forage yield and quality [23]. The highest dry matter were obtained from hedgehog (11.728%) and Mediterranean (11.513%) clover ($P \leq 0.01$); besides, maximum dry matter (13.149%) was determined at the full-bloom stage ($P \leq 0.01$) (Table 3). These results were in agreement with those of Loi et al. [15] and Tekeli et al. [25]. Ates and Tekeli [7] pointed out that Persian clover provides 6.40-12.74 t ha^{-1} of dry matter yield under dry conditions. Tekeli and Ates [22] reported 2.8 t ha^{-1} of dry matter yield in hedgehog clover.

After plant cell growth stops, cell walls thicken and the secondary wall is formed. In contrast to primary

Table 3. Nutritive values and mineral composition of some annual clovers at different growth stages.

G. S.	Pre-bud	Pre-bloom	50% Bloom	Full-bloom		Pre-bud	Pre-bloom	50% Bloom	Full-bloom	
S.P.		Dry Matter (%)			Average		Crude Protein (%)			Average
Persian clover	6.766	10.903	11.926	12.233	10.457**b**	20.733	19.166	18.233	17.900	19.008**d**
Mediterranean clover	7.536	11.473	12.840	14.203	11.513**a**	25.500	24.233	22.600	22.100	23.608**b**
Narrow-leaved clover	6.823	10.160	11.466	12.653	10.276**b**	26.266	25.633	24.066	22.056	24.505**a**
Lappa clover	6.733	10.403	12.017	12.433	10.397**b**	24.283	24.433	22.467	21.933	23.279**b**
Hedgehog clover	8.637	11.027	13.023	14.223	11.728**a**	24.300	23.200	22.367	22.160	23.090**c**
Average	7.299**d**	10.793**c**	12.254**b**	13.149**a**		24.217**a**	23.200**b**	21.947**c**	21.230**d**	
LSD		S.P.: 0.264**	G.S.: 0.236**				S.P.: 0.448**	G.S.: 0.401**		
		Crude Cellulose (%)					P (%)			
Persian clover	14.470	15.106	17.200	17.860	16.159**a**	0.410	0.450	0.510	0.520	0.473**a**
Mediterranean clover	12.957	13.267	15.297	16.620	14.535**c**	0.397	0.443	0.490	0.500	0.458**b**
Narrow-leaved clover	11.893	12.390	14.680	16.353	13.829**d**	0.410	0.440	0.480	0.513	0.461**b**
Lappa clover	12.287	14.070	15.270	16.563	14.548**c**	0.380	0.413	0.473	0.417	0.420**c**
Hedgehog clover	14.180	15.137	16.720	17.263	15.825**b**	0.410	0.433	0.493	0.523	0.465**ab**
Average	13.157**d**	13.994**c**	15.833**b**	16.932**a**		0.401**c**	0.436**b**	0.489**a**	0.495**a**	
LSD		S.P.: 0.261**	G.S.: 0.233**				S.P.: 0.010**	G.S.: 0.009**		
		Ca (%)					K (%)			
Persian clover	1.160	1.220	1.240	1.260	1.220**a**	1.380	1.470	1.503	1.517	1.468**a**
Mediterranean clover	1.123	1.193	1.223	1.253	1.198**b**	1.374	1.460	1.487	1.514	1.459**b**
Narrow-leaved clover	1.124	1.194	1.220	1.257	1.199**b**	1.360	1.434	1.487	1.530	1.453**b**
Lappa clover	1.117	1.184	1.230	1.270	1.200**b**	1.424	1.480	1.510	1.524	1.485**a**
Hedgehog clover	1.144	1.194	1.250	1.264	1.213**a**	1.390	1.437	1.490	1.547	1.466**a**
Average	1.134**d**	1.197**c**	1.233**b**	1.261**a**		1.386**d**	1.456**c**	1.495**b**	1.526**a**	
LSD		S.P.: 0.007**	G.S.: 0.007**				S.P.: 0.019**	G.S.: 0.017**		

G.S.: Growth stage, S.P.: Species, **P≤0.01

Table 4. Magnesium content and K:P, Ca:P, Ca:K balance of some annual clovers at different growth stages (dry matter basis)

G. S.	Pre-bud	Pre-bloom	50% Bloom	Full-bloom		Pre-bud	Pre-bloom	50% Bloom	Full-bloom	
S.P.		Mg (%)			Average		Ca:P			Average
Persian clover	0.440	0.420	0.450	0.493	0.451**a**	2.850	2.690	2.420	2.400	2.590**b**
Mediterranean clover	0.427	0.417	0.450	0.477	0.443**b**	2.834	2.690	2.497	2.507	2.632**b**
Narrow-leaved clover	0.457	0.407	0.454	0.497	0.454**a**	2.740	2.710	2.544	2.447	2.610**b**
Lappa clover	0.420	0.387	0.506	0.494	0.452**a**	3.080	2.864	2.600	2.427	2.743**a**
Hedgehog clover	0.430	0.400	0.470	0.517	0.454**a**	2.790	2.754	2.534	2.414	2.623**b**
Average	0.435**c**	0.406**d**	0.466**b**	0.496**a**		2.859**a**	2.742**b**	2.519**c**	2.439**d**	
LSD		S.P.: 0.008*	G.S.: 0.009**				S.P.: 0.062**	G.S.: 0.055**		
		K:P					Ca:K			
Persian clover	3.377	3.240	2.927	2.894	3.110**d**	0.837	0.830	0.834	0.830	0.833**a**
Mediterranean clover	3.464	3.294	3.020	3.027	3.201**b**	0.817	0.820	0.824	0.827	0.822**b**
Narrow-leaved clover	3.317	3.260	3.097	2.980	3.164**c**	0.827	0.834	0.820	0.827	0.827**a**
Lappa clover	3.747	3.577	3.197	2.930	3.363**a**	0.820	0.800	0.810	0.827	0.814**b**
Hedgehog clover	3.394	3.314	3.024	2.957	3.172**c**	0.824	0.834	0.840	0.814	0.828**a**
Average	3.460**a**	3.337**b**	3.053**c**	2.958**d**		0.825	0.824	0.826	0.825	
LSD		S.P.: 0.008**	G.S.: 0.072**				S.P.: 0.010*	G.S.: NS		

G.S.: Growth stage, S.P.: Species, *P≤0.05, **P≤0.01, NS.: P≥0.05 and 0.01

walls, secondary walls do not contain protein and may vary significantly in composition and structure among cell types. Secondary walls consist of a network of cellulose fibrils embedded in an amorphous matrix of hemicelluloses, pectin and lignin. Generally, young plant cell walls are richer in pectin and lower in cellulose than older plant cell walls. The crude cellulose content usually correlates with the digestibility of the dry matter only to the extent that its availability is determined by lignifications or other limiting factors [21]. The mean values for crude cellulose and P of clover species and growth stages are given table 3. There were significant differences between clover species and growth stages ($P \leq 0.01$). Persian clover were produced more crude cellulose (16.159%) and P (0.473%) than the all clover species. First growth stage had lowest crude cellulose (13.157%) and P (0.401%). The content of P in the rumen is also important, with higher levels of P favoring Mg absorption. Cows grazing P-deficient pastures may have low concentrations of P in the rumen, and Mg absorption may be further impaired [4, 8, 10]. The highest crude protein were found from plants at pre-bud stage (24.217%) ($F=161.600^{**}$). The maximum crude protein (24.505%) was obtained from the narrow-leaved clover while the lowest crude protein (19.008%) from the Persian clover ($P \leq 0.01$). The crude protein, cellulose, and P values were similar to those reported by Tekeli et al. [25]. The lowest P was found 0.31% by Essig [11].

The Ca content in the blood also plays a role in the development of grass tetany in some cows. If it decreases, the concentration of Mg in the cerebrospinal fluid falls more rapidly when Mg in the blood decreases, as absorption is insufficient. The ability of cows to absorb Ca from pasture usually decreases after the autumn break and increases again when the pastures mature in spring. Feeding high quality legume hay to cows is one way of ensuring that they absorb sufficient Ca to maintain the Ca level in their blood. On many farms, it is an essential step in the prevention of grass tetany [4, 8, 10]. Differences in Ca, K and Mg of the growth stages were significant ($P \leq 0.01$). Maximum Ca (1.261%), K (1.526%) and Mg (0.496%) ratio were designated from plants at the full-bloom stage (Table 3 and 4). Anonymous [6] emphasized that the protein, P and K concentrations of red clover declined from pre-bloom to the late-bloom stage. Frame et al. [12] states that the N, P, K content of red clover and alfalfa declined with maturity. The highest Ca value (1.213-1.220%) was found from hedgehog and Persian clovers ($P \leq 0.01$). Hedgehog, lappa and Persian clovers have given higher values than clovers for the K ratio (1.466-1.485%) ($F=6.829^{**}$). The lowest Mg ratio (0.443%) was determined from the Mediterranean clover ($P<0.05$) (Table 4). These values about Ca, K and Mg were found like Açıkgöz [2], Essig [11], Plank [18] and Tekeli et al. [25].

Mineral elements are very important to keep this balance; even though one element may be at the minimum, the other element may be in excess of the balance, consequently creating an imbalance within the animal's body [16]. Lappa clover produced the highest Ca:P (2.743) and K:P (3.363). Pre-bud growth stage determined the maximum Ca:P (2.859) and K:P (3.460). According to Ca:K ratio there were no significant differences between growth stages ($P>0.05$; 0.01). Ca:K ratio changed 0.814 to 0.833 from clovers($P \leq 0.05$) (Table 4). Tekeli and Ateş [24] reported a 3.41 Ca:P and 0.64 Ca:K ratios in white clover. Rodriguez Julià [19] and Hill Lab [14] determined similar results.

CONCLUSIONS

The rate of growth in a growing animal and the milk yield of a lactating animal depend first upon the intake of nutrients, and second upon the efficiency of conversion of ingested nutrients into body tissue or milk. The concentrations of the mineral contents also reflect the mineral status of the soil and the supply of the fertilizer nutrients, and are influenced by the species of the forage crops. The high-quality forage may be obtained from these clovers cut at all the growing stages. According to forage quality components, these clovers can be sown in Turkey as well as in subtropical climate conditions.

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