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Chemical composition of a *Trifolium repens* L. population in a grazed mountainous grassland in Central Greece

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Abstract. The purpose of this study was to evaluate the effect of grazing on the herbage production and on the nutritive value of a natural population of *Trifolium repens* L. in mountainous grassland.The research was conducted in Pindos mountain, in Central Greece in July 2013. Three plots of 9 m² each in the study area were fenced on 2012, in order to be protected from sheep grazing. Herbage production was measured in the grazed and fenced plots. Hand-plucked samples of *Trifolium repens* from the grazed plots and the protected ones were analyzed for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL). Also, phenols (TPH), tannins (TT) and condensed tannins (CT) concentration were measured. As expected, the herbage production was significant higher in the protected plots (2350 kg DM/ha) compared to the grazed ones (957 kg DM/ha). The CP content of *Trifolium repens* was increased significantly in the grazed plots (183 g/kg DM) compared to the protected ones (142 g/kg DM). The NDF, ADF, ADL content and TPH and TT concentrations decreased significantly in the grazed areas compared to the protected ones. On the other hand, CT concentration was significantly lower in the protected plots (2 mg/g DM QE) compared to the grazed (5.7 mg/g DM QE). It seems that grazing ameliorates the nutritive value of *Trifolium repens*.

Keywords. Forage quality – Tannins – Small ruminants – Legumes.

La composition chimique d'une population de* Trifolium repens *L. dans une prairie montagneuse pâturée en Grèce Centrale

Résumé. *Le but de cette étude était d'évaluer l'effet du pâturage sur la production d'herbe et sur la valeur nutritive d'une population naturelle de* Trifolium repens *L. dans une prairie montagneuse. L'essai a été menée dans la montagne du Pinde, en Grèce centrale, en Juillet 2013. Trois parcelles de 9 m² chacune dans la zone d'étude ont été clôturées en 2012, afin d'être protégées contre le pâturage des moutons. La production de plantes fourragères a été mesurée dans les parcelles pâturées et dans les surfaces clôturées. Des échantillons de* Trifolium repens *ont été rammassés à main dans les parcelles pâturées aussi que dans celles clôturées, et puis ont été analysés pour la protéine brute (CP), la fibre à détergent neutre (NDF), la fibre au détergent acide (ADF) et la lignine (ADL). En outre, la concentration en phénols (TPH), tanins (TT) et tanins condensés (CT) ont été mesurées. Comme prévu, la production d'herbe était significativement plus élevé dans les parcelles protégées (2350 kg MS / ha) par rapport à celles pâturées (957 kg MS / ha). Le contenu de CP de* Trifolium repens *a été augmenté de façon significative dans les parcelles pâturées (183 g / kg MS) par rapport à celles qui sont protégées (142 g / kg MS). Le contenu en NDF, ADF, ADL et les concentrations de TPH et TT était diminué significativement aux énchantillons des surfaces pâturées. Au contraire, la concentration CT était significativement plus faible dans les parcelles protégées (2 mg / g MS QE) par rapport à la pâturée (5,7 mg / g MS QE). Il semble que le pâturage améliore la valeur nutritive de* Trifolium repens*.*

Mots-clés. *La qualité du fourrage – Des tanins – Petits ruminants – Les légumineuses.*

I – Introduction

In mountainous countries such as Greece, transhumance system integrates the different environments and the mobility of shepherds and animals (Sarno, 2014). This system is a traditional livestock movement between fixed points from the lowlands in winter to the highlands in summer, in order to utilize their herbage production availability (Nyssen *et al*., 2009).

Grasslands are a crucial component for extensive livestock feeding in the Mediterranean region and are often characterized by the abundance of species, which contribute to the variability of herbage composition and production (Maranon, 1985). Moreover, legume species contribute to the higher forage quality of these ecosystems. It is well known that *Trifolium repens* has a high feeding value for lamb and milk production (Ulyatt *et al.*, 1977; Thomson *et al.*, 1985). Furthermore, it is an essential forage legume of the natural grasslands, although it is a spontaneous component of them (Dewhurst *et al*., 2009), due to its ability to fix nitrogen and to withstand grazing (Burggraaf *et al*., 2003).

The aim of this study was to evaluate the effect of grazing on the herbage production and on the nutritive value of a natural population of *Trifolium repens* in a mountainous grassland.

II – Material and methods

The study was conducted in a grassland at the region of Stournareika on the mountains of central Pindus in Greece (39º29' N, 21º 29' E) at 1216 m a.s.l. The climate of the study area is classified as sub – Mediterranean (Mavromatis, 1978) with a mean air temperature of 10.5 ºC and mean annual precipitation of 1542 mm. The area is grazed continuously mainly by transhumant sheep from May to October. Three plots of 9 m^2 each were fenced in the spring of 2012, in order to protect the vegetation from grazing. The grassland of the study area was composed by 36.4% grasses, 15.6% legumes, 46% forbs and 2% shrubs in the grazed area and 9.4% grasses, 43.4% legumes and 47% forbs in the protected plots. *Trifolium repens* occupied 4% of the grazed area and 34% of the protected ones (unpublished data).The species composition was measured during the grazing period.

The herbage production was measured by harvesting the above ground biomass of the vegetation. Three samplings quadrats of 0.5 m x 0.5 m were used in each of the grazed and in the protected plots as well more than a year after fencing, in July of 2013. After that, above ground biomass of *Trifolium repens* L. was separated from the herbage production and oven-dried at 60º C for 48 h, ground through a 1 mm screen and analyzed for Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) (Van Soest *et al*., 1991) using the ANKOM fibre analyzer. Nitrogen was determined using the Kjeldahl procedure (AOAC, 1990), and crude protein was calculated as N content X 6.25. Samples also were analysed for total phenols (TPH), total tannins (TT) and condensed tannins (CT) assays according to Makkar (2003) in three replicates. Total phenols (TPH) and total tannins (TT) in the extract were determined by a modification of the Folin-Ciocalteu method using polyvinylpolypyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols. Both total phenols and total tannins were expressed as tannic acid equivalent (mg/g TAE). The (CT) were determined according to the method of Porter *et al*. (1986), using purified Quebracho CT as the reference standard. The CT contents are therefore expressed as Quebracho equivalent.

For all measured parameters differences between the grazed and protected plots were calculated using one-way ANOVA (Steel and Torrie, 1980). All statistical analyses were performed using the SPSS® statistical software v. 18.0 (SPSS Inc., Chicago, IL, USA). The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

III – Results and discussion

Herbage production was significantly lower in grazed area (957 kg DM/ha), compared to protect one (2350 kg DM/ha) (Fig. 1). According to Ali-Shtayeh and Salahat (2010), there is a direct effect of grazing on the vegetation growth through the foraging behaviour and trampling of animals. Despite the short time of animal exclusion in the protected plots, the herbage production was double compared to the grazed ones. According to Harrison *et al*., (2003) the species composition tends to alter in grazing areas. This is in agreement with our study, however in the grazed area, the percent of *Trifolium repens* in the species composition was significantly decreased as it is a desirable and palatable species.

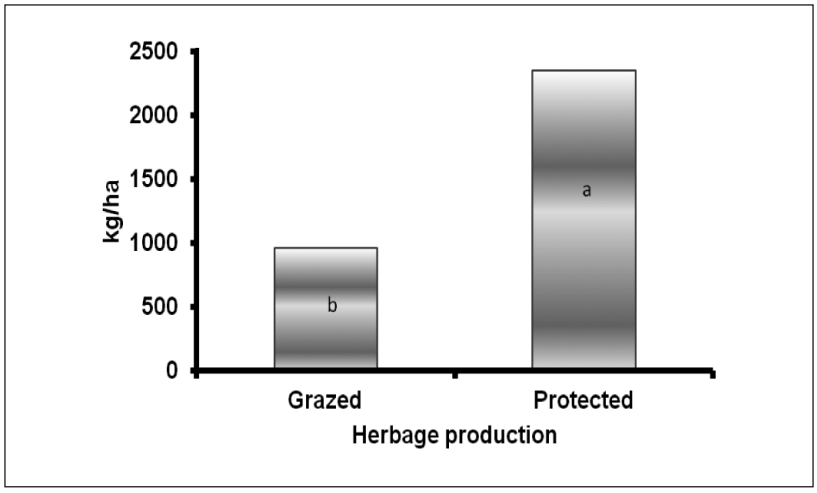

Fig. 1. Herbage production (kg DM/ha) of the grazed and protected areas.
* Different letters in each column indicate significant differences (P ≤ 0.05).

Trifolium repens' CP content from grazing areas was significantly higher compared to the CP from the protected plots (Table 1). The CP increase is probably occurred as a consequence of the *Trifolium* regrowing after grazing. On the other hand, the decrease of CP in the protected plants could be related to the stage of maturity. According to Kilcher (1981), CP content of herbaceous plants decreases as they reach maturity. Our findings indicated that CP content of *Trifolium repens* met the requirements of sheep for maintenance and lactation (Table 1) (NRC, 1985) in the mountainous area.

The NDF, ADF, and ADL from grazing areas were significantly lower compared to the same chemical parameters of *Trifolium repens* from the protect plots (Table 1). Plant phenological stage has a substantial impact on the chemical composition of forage species (Arzani *et al*., 2004). As the plants reach maturity the proportions of structural carbohydrates increase and the plant cell contents decrease.

Similarly TPH and TT concentrations were significantly lower in the grazed plants (Table 1). On the other hand, CT concentration was significantly higher in the grazed plants compared to ungrazed ones. Grazing led to the development of defense mechanisms of plants against herbivores such as condensed tannins (Barroso *et al*., 2001). The CT concentration was less than 50 g kg^{-1} DM, indicating a positive effect on plants' nutritive value (Piluzza *et al*., 2014). As *Trifolium repens* is a legume with a small concentration of tannins, so this increase of CT in the grazed *Trifolium repens* will help to overcome of rapid degradation of protein to ammonia in the rumen (Burggraaf *et al*., 2003).

Table 1. Chemical composition (g/kg DM) of TPH (mg/g DM TAE), TT (mg/g DM TAE), CT (mg/g DM QE) of *T. repens* at the grazed and protected areas

	Grazed	Protected
CP	183[a]	142[b]
NDF	469[a]	524[b]
ADF	293[a]	345[b]
ADL	27[a]	33[b]
TPH	3.9[b]	6.5[a]
TT	1.9[b]	3.9[a]
CT	5.7[a]	2[b]

* Different letters in each row indicate significant differences (P ≤ 0.05).

IV – Conclusions

Generally, grazing reduced the herbage production compared to the protected area. However, it ameliorated the nutritive value of *Trifolium repens*.

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