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Nutritive value of *Trifolium subterraneum* as affected by fertilization and seeding rate under rainfed conditions

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Abstract. Legumes are important feed sources for livestock due to their high nutritive value. The objective of this study was to assess the effects of phosphorous fertilization (40 kg ha^{-1} and control) and seeding rate (20, 17 and 13 kg ha^{-1}) on forage production and the nutritive value of *Trifolium subterraneum* collected at two phenological stages (early inflorescence – May, fruiting – June). The experiment was conducted in Orestiada, northern Greece under rainfed conditions. Seeds were sowed in February 2014. Nutritive value of whole cut plant was evaluated based on chemical composition: crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL). Forage yield was higher at the fruiting (1442 kg ha^{-1}) than in inflorescence stage (909 kg ha^{-1}) and at the 20 kg ha^{-1} seeding rate (1424 kg ha^{-1}) than at 17 and 13 kg ha^{-1}, while it was not affected by the fertilization. According to the results, crude protein content was significantly ($p<0.05$) higher at the flowering stage (209 g kg^{-1} DM) compared to fruiting (126 g kg^{-1} DM) and at 17 kg ha^{-1} seeding rate (176 g kg^{-1} DM) compared to the other seeding rates (20: 170 g kg^{-1} DM, 13: 158 g kg^{-1} DM), while it was not affected by the fertilization. The NDF content was significantly lower, while ADF and ADL were higher at 17 kg ha^{-1} in comparison to the other seeding rates but only at the fruiting stage. Moreover, the fertilization decreased the ADL content in 13 kg ha^{-1} at the fruiting stage.

Keywords. Forage quality – Subterranean clover – Inflorescence – Fruiting – Phosphorus.

Valeur nutritive de trèfle souterrain comme affectée par la fertilisation et le taux de semis en conditions pluviales

Résumé. *Les légumineuses sont une source d'alimentation importante pour les petits ruminants en raison de leur haute valeur nutritive. L'objectif de cette étude était d'évaluer les effets de la fertilisation en phosphore (40 kg ha^{-1} et de contrôle) et taux de semis (20, 17 et 13 kg ha^{-1}) sur la production de fourrage et la valeur nutritive du trèfle souterrain recueillies à deux phénologique étapes (inflorescence début – mai, la fructification –Juin). L'expérience a été menée dans Orestiada, nord de la Grèce en conditions pluviales. Les graines ont été semées en Février 2014. Valeur nutritive de la plante entière de coupe a été évaluée en fonction de la composition chimique: protéines brutes (CP), fibre au détergent neutre (NDF), en fibres au détergent acide (FDA), la lignine au détergent acide (ADL). Le rendement fourrager était supérieur à la fructification (1442 kg ha^{-1}) que dans l'étape de l'inflorescence (909 kg ha^{-1}) et les 20 kg^{-1} ha taux de semis (1424 kg ha^{-1}), alors qu'il n'a pas été affectée par la fertilisation . Selon les résultats, la teneur en protéines brutes était significativement ($p <0,05$) au stade de la floraison (209 g kg^{-1} DM) par rapport à la fructification (126 g kg^{-1} DM) et à 17 kg ha^{-1} taux de semis (176 g kg^{-1} DM) par rapport aux autres taux de semis (20: 170 g kg^{-1} DM, 13: DM 158 g kg^{-1}), alors qu'il n'a pas été affectée par la fertilisation. Le contenu NDF était significativement plus faible, tandis que l'ADF et ADL étaient supérieurs à 17 kg ha^{-1} en comparaison avec les autres taux de semis, mais seulement au stade de la fructification. En outre, la fertilisation a diminué la teneur en ADL 13 kg ha^{-1} au stade de la fructification.*

Mots-clés. *Qualité du fourrage – Trèfle souterrain – Inflorescence – Fructification – Phosphore.*

I – Introduction

Pasture lands provide feed for livestock directly or indirectly in various forms (eg. forage, hay, silage) (Gibson, 2009). The challenge for managers is the appropriate choice of the forage species, as it will have a significant impact on the success of the pastureland, especially under rainfed conditions in the Mediterranean region.

Trifolium subterraneum L. (*T. subterraneum*) is an annual legume species that grows in all types of soils, with a preference to those of moderate texture, lightly acidic to alkaline and tolerates higher pH values Rossiter (1978). The relatively high nutritive value and production of *T. subterraneum* and its resistance to drought make it a possible alternative crop for permanent dry grassland farming systems in order to maintain forage production under the predicted warmer and drier conditions due to the climate change (Stefanou, 2015). Phosphorus fertilization promotes faster growth rates of the plants, while the absence of P fertilizer reduces crop yields (Gaxiola *et al.*, 2011; Richardson *et al.*, 2011). The absence of phosphorus in the soil is a key factor that limits the growth of crops and forage production (Grimoldi *et al*., 2005). The seeding rate is an important factor that it affects the density, the morphological and productive characteristics of plants, as well as the cost of the seeding (Stefanou, 2015, McGuire, 1985). Thus, the objective of the present study was to study the nutritive value of *T. subterraneum* as affected by fertilization and seeding rate under rainfed conditions.

II – Material and methods

The research was conducted at the farm of Democritus University of Thrace in Orestiada, northeastern Greece (41°33´N latitude, 26°31´E, 33 m a.s.l.) from February to June 2014. The soil is silty clay with pH 7.5 and P (Olsen) 13.2 mg kg^{-1}. The climate of study area is classified as Mediterranean type, with a mean air temperature of 14ºC and an average annual rainfall of 506.9 mm (Koutroubas *et al*., 2012). However, during the experimental period mean air temperature was 14,2°C and the rainfall was 65.7 mm (Stefanou, 2015).

T. subterraneum cv Geraldton was seeded in 36 of 4 m^2 each. Three seeding rates were tested: 20 kg ha^{-1}, 17 kg ha^{-1} and 13 kg ha^{-1} with six replications per seeding rate. Two levels of fertilization (40 kg ha^{-1} phosphorus and control) were applied before the seeding.

T. subterraneum samples were collected at two phenological stages, early inflorescence in May and fruiting in June. To determine the above-ground biomass production in each plot, two 25 x 25 cm quadrats were used. Plant material was clipped at ground level and placed in individual paper bags. All samples were oven dried at 60°C for 48 h, weighed and then ground in a mill to pass through 1 mm screen prior to analyses.

Nitrogen content was measured by the Kjeldahl method (AOAC 1990) and crude protein (CP) was calculated by multiplying N by 6.25. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were measured using the procedure described by Van Soest *et al*. (1991) with the ANKOM fibre analyzer (ANKOM Technology Corporation, Macedon, NY, USA), using sodium sulphite, but not α-amylase to the solution for the NDF determination. All analyses were carried out on duplicate samples and results reported on DM basis.

General linear models procedure (SPSS® 18 for Windows) was used for ANOVA. The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

III – Results and discussion

Dry matter production was higher at fruiting stage by 533 kg ha^{-1} compared to the inflorescence stage (Table 1). It has to be noted that dry matter production was higher than that previously reported in Greece (Merou *et al*., 2007). This difference is related to the start of inflorescence stage in different varieties, the climatic conditions and the availability of nutrients in the soil (Smetham 2003, Lemaire *et al*., 2000). NDF and ADF contents were higher at the fruiting than at the inflorescence stage (Table 1). ADL content was also higher at the fruiting stage (83 g ha^{-1}), in comparison to the inflorescence stage (42 g ha^{-1}). In contrast, CP content was higher at the inflorescence stage. The higher contents of NDF, ADF and ADL and the lower content of CP is the result of plants maturity. As plants reach maturity the NDF, ADF and lignin contents increase, while CP decreases (Parissi *et al*. 2005, Kökten *et al.* 2012). However, the nutritive value of *T. subterraneum* obtained in the current study would meet the requirements of small ruminant for CP (NRC, 1981) at both phenological stages. The fertilization significantly affected only the ADL content. Specifically, lower ADL content was obtained in the plots under phosphorus fertilization in comparison to the control (data not shown). The highest DM production was observed in the seeding rate of 20 kg ha^{-1}. CP and ADL contents were significantly higher at the seeding rates of 20 kg ha^{-1} and 17 kg ha^{-1} compared to 13 kg ha^{-1}. NDF content was higher at the seeding rates of 17 kg ha^{-1} and 13 kg ha^{-1}, while ADF was higher at seeding rates 20 kg ha^{-1} and 13 kg ha^{-1}. The seeding rates effect on DM production, CP, NDF and ADF contents was significant only at the fruiting stage.

Table 1. Dry matter and CP, NDF and ADF content (g kg^{-1}) of *Trifolium subterraneum* at different seeding rates and different phenological stages

S.R (kg ha^{-1})	DM (kg* ha^{-1})			CP (g* kg^{-1})			NDF (g* kg^{-1})			ADF (g* kg^{-1})		
	I	F	M	I	F	M	I	F	M	I	F	M
20	902^{a}	1964^{a}	1424^{a}	210^{a}	130^{a}	170^{a}	436^{a}	455^{b}	446^{b}	253^{a}	361^{a}	307^{a}
17	916^{a}	1249^{b}	1085^{b}	218^{a}	133^{a}	176^{a}	439^{a}	502^{a}	471^{a}	251^{a}	328^{b}	290^{b}
13	910^{a}	1114^{b}	1012^{b}	200^{a}	115^{b}	158^{b}	430^{a}	514^{a}	472^{a}	268^{a}	349^{a}	309^{a}
M	909^{B}	1442^{A}		209^{A}	126^{B}		435^{B}	490^{A}		257^{B}	346^{A}	

S.R: Seeding rate, P.S: Phenological stage, I: Inflorescence, F: Fruiting, M: Mean. Means followed by the same small letter in the same column are not significantly different (P ≥ 0.05). Means of each parameter followed by the same capital letter in the same row are not significantly different (P ≥ 0.05).

The interaction between seeding ratios and fertilization for ADL content produced significant results only at the fruiting stage. Specifically, the content of ADL was lower at the seeding rate of 13 kg ha^{-1} under phosphorus fertilization in comparison to all the other treatments (Fig. 1). This indicates that fertilization decreased ADL only at the low seeding rate.

IV – Conclusions

Dry matter production was significantly higher at the fruiting stage, but the nutritive value of *T. subterraneum* was higher at the flowering stage. Moderate grazing can be applied in both phenological stages, as animal requirements for CP were met, while mowing should be done at the fruiting stage when the production is higher, and no reseeding is needed. Phosphorus fertilization did not affect DM production of *T. subterraneum*, while slightly improved its nutritive value. Therefore fertilization is not necessary in soils with moderate to high available phosphorus. The DM production decreased at lower seeding rates. However, the effect of seeding rates on the nutritive value of *T. subterraneum* was not clear.

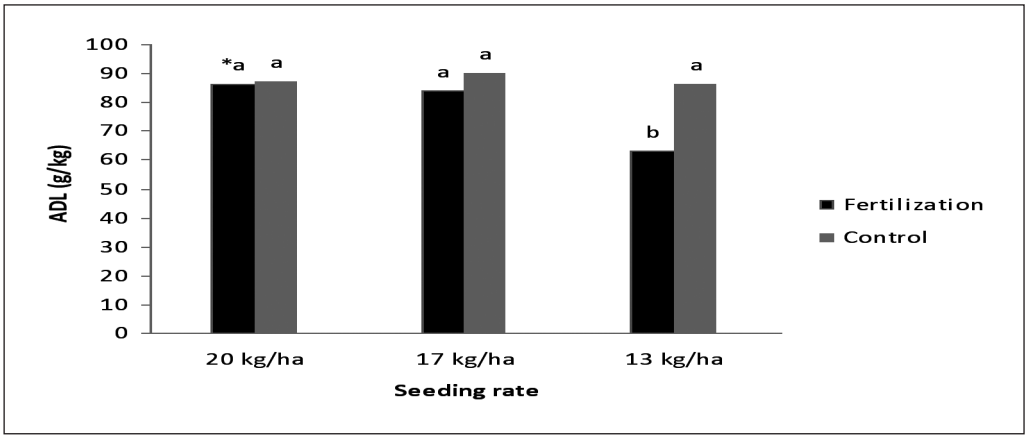

Fig. 1. ADL content (g ha^{-1}) of *Trifolium subterraneum* at the fruiting stage as affected by phosphorus fertilization and seeding rate. *Means followed by the same letter are not significantly different (P ≥ 0.05).

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References

A.O.A.C., 1990. *Official Methods of Analysis*. Washington DC, USA: 15th edn. AOAC, p. 746.

Gaxiola R.A., Edwards M. and Elser J.J., 2011. A transgenic approach to enhance phosphorus use efficiency in crops as part of a comprehensive strategy for sustainable agriculture. *Chemosphere,* Vol. 84, Issue 6, p. 840-845.

Gibson D.J., 2009. *Grasses and grassland ecology*. Oxford University Press Inc., New York, p. 315.

Grimoldi A.A., Kavanová M., Lattanzi F.A. and Schnyder H., 2005. Phosphorus nutrition-mediated effects of arbuscular mycorrhiza on leaf morphology and carbon allocation in perennial ryegrass. *New Phytologist*., Vol. 168, Issue 2, p. 435-444.

Koutroubas S.D., Fotiadis S. and Damalas C.A., 2012. Biomass and nitrogen accumulation and translocation in spelt (*Triticum spelta*) grow in a Mediterranean area. *Field Crops Research*, Vol. 127, p. 1-8.

Kökten K., Kaplan M., Hatipoğlu R., Saruhan, V. and Çinar S., 2012. Nutritive value of Mediterranean shrubs. *The Journal of Animal and Plant Sciences*, Vol. 22, Issue 1, p. 188-194.

Lemaire G., J. Hodgson, A. de Moraes, C. Nabinger and P.C. de F. Carvalho, 2000. *Grassland Ecophysiology and Grazing Ecology*. CAB International, p. 432.

Merou T., Fotiadis G., Tsiftsis S., Vidakis K., Vrahnakis M., Tsiripidis I. and Panastasis, V., 2007. Legumes of Northern Greece - Grasses, Bushes. Photo/Graphs Studio, p. 224 (In Greek).

McGuire W.S., 1985. Subterranean clover. In: Taylor, N.L. (ed.) *Clover Science and Technology*. ASA/CSSA/SSSA, Madison, Wisconsin, p. 515-534.

N.R.C., 1981. *Nutrient Requirements of Goats: Angora, Dairy and Meat Goats in Temperate and Tropical Countries*. Nutrient Requirements of Domestic Animals, No. 15. National Academy Press, Washington, DC, p. 91.

Parissi Z.M., Papachristou T.G. and Nastis A.S., 2005. Effect of drying method on estimated nutritive value of browse species using an in vitro gas production technique. *Animal Feed Science and Technology*, Vol. 123-124, part 1, p. 119-128.

Richardson A.E., Lynch J.P., Ryan P.R., Delhaize E., Smith A., Smith S.E., Harvey P.R, Ryan M.H., Veneklaas E.J., Lambers H., Oberson A., Culvenor R.A. and Simpson R.J., 2011. Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil*, Vol. 349, Issue, 1-2, p. 121-156.

Rossiter R.C., 1978. The ecology of subterranean clover-based pastures. In: *Plant Relations in Pastures,* J. R. Wilson (ed.), p. 325-339. CSIRO, Melbourne.

Smetham M.L., 2003. A review of Subterranean Clover (*Trifolium subterraneum* L.): Its ecology, and use as a pasture legume in Australasia. *Advances in Agronomy*, Vol. 79, p. 303-350.

Stefanou P., 2015. Effect of fertilization in different amounts of seeds of *Trifolium subterraneum* L., Dissertation. Democritus University of Thrace. Orestiada, Greece, (In Greek, English summary).

Steel R.G.D. and Torrie J.H., 1980. Analysis of covariance. In: *Principles and Procedures of Statistics: A Biometrical Approach*. New York: McGraw-Hill, p. 401-437.

S.P.S.S., 2009. *SPSS for Windows*, version 18. SPSS Inc., Chicago, IL, USA.

Van Soest P.J., Robertson J.B. and Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. In: *Journal of Dairy Science*, 74, p. 3583-3597.