EFFECTS OF MANURE AND MINERAL FERTILIZER APPLICATION ON CRUDE PROTEIN CONTENT OF LUCERNE (*MEDICAGO SATIVA* L.)

VILIANA VASILEVA¹ AND MOHAMMAD ATHAR^{2*}

¹Institute of Forage Crops, 89 General Vladimir Vazov Street, 5800 Pleven, Bulgaria ²California Department of Food and Agriculture, 3288 Meadowview Road, Sacramento, CA 95832, USA ^{*}Corresponding author e-mail: atariq@cdfa.ca.gov

Abstract

Effects of mineral and manure fertilization on crude protein content of lucerne (*Medicago sativa* L.) was studied in field trial carried out at the Institute of Forage Crops, Pleven, Bulgaria. The doses of 70, 140 and 210 kg N ha⁻¹ were tested. Ammonium nitrate and well rotted cattle manure were used. It was found that the both types of fertilizers had strong influence on the crude protein yield in the first year of development of lucerne – for mineral fertilization from 15.6 to 31.6%, and for manure – from 33.4 to 47.0% compared with unfertilized control. With increasing age of sward the effect of fertilization significantly decreased, for mineral one. Manure had positive effect on crude protein yield during the whole experimental period. An average mineral nitrogen fertilization at the doses of 70 and 140 kg N ha⁻¹ increased crude protein yield by 4.4 and 6.6%, while manure fertilization at the doses of three levels (70, 140 and 210 kg N ha⁻¹) enhanced protein amount from 13.6 to 16.3%. Regardless their nitrogen fixing ability, lucerne utilizes soil or fertilizer nitrogen in the initial development, since nitrogen assimilation required lower rates of CO₂ and energy, than for nitrogen fixation. This gives an impact on the crude protein yield from plants. In the following years the role of biological nitrogen increases gradually, enhancing the process of demineralization thereby the nitrogen fertilization has less influence on the synthesis of nitrogen compounds.

Introduction

Interest in legumes has increased significantly in recent years due to their importance for sustainable and ecologically friendly agriculture (Frame, 2005; Frame and Laidlaw, 2005; Nyfeler *et al.*, 2006; Porqueddu *et al.*, 2003; Vasileva *et al.*, 2006). Lucerne (*Medicago sativa* L.) is an important forage legume in the temperate climate (Barnes *et al.*, 1995; Michaud *et al.*, 1988; Vasilev, 2003, 2004; Vasilev, 2004). The question about lucerne as a nitrogen-fixing crop is debatable (Jarvis, 1998; Oliveira *et al.*, 2004; Vasileva, 2004). The present work aimed to investigate the effect of mineral and manure fertilizers on crude protein of lucerne.

Materials and Methods

Filed trials were conducted at the Institute of Forage Crops, Pleven, Bulgaria, under no irrigation in the plots measuring 10 m². Soil subtype was leached chernozem. The treatments tested included T-1 (Control N₀, P_{300} , K_{150}), T-2.(MN_{70} , P_{300} , K_{150}), T-3. (MN_{140} , P_{300} , K_{150}), T-4.(MN_{210} , P_{300} , K_{150}), T-5. (ON_{70} , P_{300} , K_{150}), T-6. (ON_{140} , P_{300} , K_{150}), T-7. (ON_{210} , P_{300} , K_{150}) where mineral nitrogen (MN) was applied as mineral fertilizer (ammonium nitrate). Organic nitrogen (ON) was applied as rotted cattle manure, P – as double superphosphate and K – as potassium chloride. The experiment was conducted in randomized block design (RBD) with 4 replicate.

Mineral nitrogen concentrations were calculated on the basis of the quantity of ammonium and nitrate nitrogen both into the soil and mineral fertilizer. Recommended nitrogen mineralization coefficient calculated after the US Compositing Council (www.compositingcouncil.org), the Ohio State University extension and Purdue University (www.ohioline.osw.edu) were used.

The trial was conducted with husbandry machine "Saxonia 200 A" with sowing rate of 30 kg seeds ha⁻¹ and interspaces of 11.5 cm. Lucerne forage was harvested once during the first year, four times during the second and third years, and three times during the fourth year. Dried plants were ground and total nitrogen content was determined by Kjeldahl method (Nelson and Sommers, 1980). Crude protein yield (kg ha⁻¹) was calculated as the product of dry matter yield and crude protein content (Kjeldahl method). Experimental data were statistically processed using SPSS 10.0.

Results and Discussion

Chemical characteristics of soil and manure are provided in Table 1 and 2, while nitrogen mineralization coefficients for application of manure are presented in Table 3. The data for total mineralization of nitrogen for 5 years was 46% of the initial nitrogen in the manure (Table 3). Nitrogen fertilization is essential to obtain

optimal conditions for intensive fixation of nitrogen in plants (Vessey et al., 2004). An important characteristic of legumes, particular for lucerne is crude protein yield in the forage. Results of our field trials showed that nitrogen fertilization affects the crude protein yield depending on the doses applied (Table 4). The most significant increases in crude protein content compared with control were found in the first year. Mineral fertilizer at a dose of 140 kg N ha⁻¹ increased crude protein content by 31.6% while at the doses of 70 and 210 kg N ha⁻¹ the increase in crude protein content were 17.9% and 15.6% respectively. However, application of three doses of manure the increase of the protein content varied from 33.4 to 47.0%.

	Total C%	Total N%	NH_4^+ -N	NO ₃ ⁻ -N	P_2O_5	K ₂ O	pН			
Soil	2.8	0.23	11.2	8.8	5.30	48.4	7.05			

Table 1. Chemical characteristics of leached chernozem soil (mg. 1000g⁻¹).

Table 2. Chemical characteristics of manure used (mg. 1000g ⁻¹).										
	Total C%	Total N%	C/N	NH4 ⁺ -N	NO ₃ ⁻ N	Organic N	P_2O_5	K ₂ O	pH (H ₂ O)	
Manure	15.0	0.72	20.8	42.8	541.4	6616.0	45.3	78.3	6.73	

Table 3. Recommended	nitrogen mineralization	n coefficients for manure.

Year of	Mineraliz	zed N	Mineralized N				
introduction into	based on t	total N	based on organic	based on organic N			
the soil	after incorporation	n into the soil ^a	after incorporation into	after incorporation into the soil ^b			
	% of mineralization	Kg N.t⁻¹	% of mineralization of organic	Kg N.t⁻¹			
	of total N	dry manure	Ν	dry manure			
1	25.0	1.8	30% of organic N	1.98			
2	12.5	0.9	15% of the rest organic N	0.69			
3	6.25	0.45	7.5% of the rest organic N	0.27			
4	3.12	0.225	3.75% of the rest organic N	0.13			
	1.56	0.112	1.8752% of the rest organic N	0.05			
Total for 4years		3.375	-	3.07			

^a, The US Composting Council (www.compostingcouncil.org); ^b, The Ohio State University Extension and Purdue University (www.ohioline.osu.edu; www.agriculture.purdue.edu).

Table 4.	Crude	protein	content	of luceri	ie after	[•] mineral	and	manure	fertilizati	on on
			avera	age for th	e perio	d of stud	ly.			

Treatments						
	First year	Second year	Third year kg	Fourth year	Average	Increase
	kg ha⁻¹	kg ha⁻¹	ha ⁻¹	kg ha⁻¹	yield/year	%
$N_0P_{300}K_{150}$ - C	225.1	2195.2	1144.6	1698.5	1315.9±366.03	0
MN70 P300K150	265.3	2250.5	1259.2	1719.2	1373.6 ± 292.32	4.4
MN140 P300K150	296.2	2371.3	1176.9	1769.0	1403.3±383.53	6.6
MN210 P300K150	260.2	2264.8	1143.7	1616.3	1321.2±365.62	0.4
ON70 P300K150	309.9	2538.0	1315.7	1817.1	1495.2 ± 405.93	13.6
ON140 P300K150	300.3	2612.7	1354.4	1854.6	1530.5±420.24	16.3
ON210 P300K150	315.1	2670.8	1310.5	1805.6	1525.5±423.16	15.9
Mean± SE(P<0.05)	281.7±13.03	2414.8 ± 69.43	1243.6 ± 33.57	1754.3 ± 32.72	1423.6 ± 35.56	

It is recorded that dry matter yields were low in the first year of experiment when lucerne had not yet fully developed the root biomass and meteorological conditions were extremely unfavorable ((Vasileva, 2004)). The temperature during study period was warmer (Fig.1). This was also a period of two years of strong drought (critical in the first year), and relatively good moisture stocking in the second half of the experiment. The effect of mineral fertilization was significantly lower in the second year only for the dose of 140 kg N ha⁻¹. Increasing the doses of manure the crude protein content increased from 15.6% to 21.7%.

In the third year mineral fertilization only at the dose of 70 kg N ha⁻¹ affected the crude protein content which was increased by 17.0%. Manure increased crude protein content in the three doses tested from 6.3 to 18.3%. Mineral fertilization increased crude protein in the fourth year slightly by 4.2 and 4.8% in the treatments applying doses of 140 and 210 kg N ha⁻¹. The effect of manure application was also decreased from 6.3 to 9.2%. Mineral fertilization had the strongest influence on crude protein content in the first year of development of

lucerne. Effect of both types of fertilizers decreased with increasing the age of sward which was more significant for mineral fertilizers.

When compared the data for crude protein content for mineral and manure fertilization it appears that application of manure led to increase the amounts of crude protein content depending on doses used for the whole experimental period. Mineral fertilization increased crude protein yield less, by 4.4 and 6.6% for the doses of 70 and 140 kg N ha⁻¹. Manure increased crude protein content from 13.6 to 15.9%. The tendency found for the crude protein content was confirmed by the data of dry matter yield. The curves of dry matter yield on average for the period and quantities of fertilizer applied are presented in Fig.2.

During the fourth year, the plants treated with manure showed significantly higher yields compared with the plants received mineral fertilizer. It was observed that under mineral fertilization dry matter content increased with the dose of 140 kg N ha⁻¹, and then strongly decreased at the highest dose (Fig.2). For the manure the curve was polynomial (r = 0.998) and when applied the highest dose the increase was relatively least. A positive correlation was found between dry matter yield and quantity of fertilizers (r = 0.982). These curves allowed determining the critical quantities of mineral fertilizers for dressing the lucerne, after which the yields decreased. For manure even high doses did not decreased the yields.



Fig.1. Agro-meteorological characteristics of period of study.



Fig.2. Curve, prognostic equation and relationship between the quantity of mineral (i) and organic fertilizer in Lucerne (ii).

The results obtained recommend that lucerne should not be dressed with doses exceeded 140 kg mineral N ha⁻¹ for the particular soil subtype – leached chernozem. Curve of tendency for manure demonstrated that quantity of nitrogen or nutrients coming from organic fertilizing did not reduced yields. There was a strong positive correlation between dry matter yield and crude protein yield (Fig 2).

Our data indirectly confirm the opinion of Hartwig and Soussana (2001) that regardless the nitrogen fixing ability, lucerne using fertilizer nitrogen from the soil in the initial development, because nitrogen assimilation required lower rates of CO_2 and energy, than for nitrogen fixation. This gives an impact on the crude protein yield from plants. In the next years the role of biological nitrogen increases gradually, enhancing the process of demineralization thereby the nitrogen fertilization has less influence on the synthesis of nitrogen compounds.

Conclusions

Mineral nitrogen fertilization and manure at the doses of 70, 140 and 210 kg N ha⁻¹ increased the crude protein content most strongly in the first year of development of lucerne - for mineral fertilization from 15.6 to 31.6% compared with the unfertilized control, and for manure – from 33.4 to 47.0%. With increasing the age of sward the effect of fertilization decreased, more significant for mineral one. Manure had positive effect on crude protein yield during the whole experimental period. Average for the period of study mineral nitrogen fertilization at the doses of 70 and 140 kg N ha⁻¹ increased crude protein yield by 4.4 and 6.6%, manure fertilization at the doses of 70, 140 and 210 kg N ha⁻¹ - from 13.6 to 16.3%.

References

- Barnes, R.F., Miller, D.A. and Nelson, C.J. (1995). *Forages*. Vol. I: *An Introduction to Grassland Agriculture*. Iowa State University Pres, Ames, Iowa, USA.
- Frame, J. (2005). Forage legumes for temperate grasslands. FAO, Rome.
- Frame, J. and Laidlaw, A.S. (2005). Prospects for temperate forage legumes. In: *Grasslands: Developments Opportunities Perspectives*. Reynolds S.G. and Frame J. (eds.). FAO, Rome and Science Publishers, Inc., Enfield, New Hampshire, USA, Chapter 1, pp. 1-28.
- Hartwig, U.A. and Soussana, J.F. (2001). Ecophisiology of symbiotic N₂ fixation in grassland legumes. In: Organic Grassland Farming, Isselstein J., Spatz G., Hofmann M. (eds.), Grassland Science in Europe 6:23-26.
- Jarvis, S.C. (1998). Nitrogen management and Sustainability Grass for Dairy Cattle. In: *Grass for Dairy Cattle*. Cherney J.H. and Cherney D.J.R. (eds.), CAB International, Wallingford 161-192.
- Michaud, R., Lehman, W. and Runbaugh, M. (1988). World distribution and historical development. In: *Alfalfa and Alfalfa Improvement*, Hansa A., Barnes D. and Hill R. (eds.). Madison USA.
- Nelson, D. W. and Sommers, L.E. (1980). Determination of total nitrogen in plant material. Agron. J. 65: 109-112.
- Nyfeler, D., Huguenin-Elie, O., Frossard, E., Luscher, A. (2006). Regulation of symbiotic nitrogen fixation in grass-clover mixtures. In: *Sustainable Grassland Productivity*. Lloveras J., Gonzales-Rodríguez A., Vazquez-Yañez O., Piñeiro J., Santamaría O., Olea L. and Poblaciones M. J. (eds.). Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, 3-6 April, 2006. *Grassland Science in Europe* 11: 246-248.
- Oliveira, W.S., Oliveira, P.P.A., Corsi, M., Duarte, F.R.S. and Tsai, S.M. (2004). Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti*. *Scientia Agricola* 61: 433-438.
- Porqueddu, C., Parente, G. and Elsaesser, M. (2003). Potential of grasslands. In: Kirilov A., Todorov N. and Katerov I. (eds.). Grassland Science in Europe 8: 11-20.
- Vasilev, E. (2003/2004). Comparative testing of Bulgarian alfalfa varieties in mixtures with grasses. *Pastagens e Forragens* 24: 35-43.
- Vasilev, E. (2004). Forage productivity of some Bulgarian lucerne cultivars in mixtures with grasses. In: Land Use Systems in Grassland Dominated Regions, Luscher A., Jeangros B., Kessler W., Huguenin O., Lobsiger M., Millar N. and Suter D. (eds.). Proceedings of the 20th General Meeting of the European Grassland Federation, Luzern, Switzerland, 21-24 June, 2004. Grassland Science in Europe 9: 401-403.
- Vasileva, V. (2004). Effect of nitrogen fertilization on growth, nodulation and productivity of lucerne (Medicago sativa L.) at optimal and water deficit conditions. Ph.D. thesis. Sofia, Bulgaria.
- Vasileva, V., Kostov, O. and Vasilev, E. (2006). Development of lucerne (*Medicago sativa* L.) treated with mineral fertilizer and manure at optimal and water deficit conditions. *Comm. Appl. Biol. Sci.* Ghent University 71: 5-17.
- Vessey, J. K., Pawlowski, K. and B. Bergman, B. (2004). Root-based N₂-fixing symbiosis: Legumes, actinorhizal plants, *Parasponia* sp. and cycads. *Plant and Soil* 266: 205-230.