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THE EFFICIENCY OF NITROGEN FROM FERTILIZER IN LUCERNE CULTIVATED AS A PURE SWARD OR AS A MIXTURE WITH GRASSES

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ABSTRACT

The aim of the study was to investigate the effect of N fertilization on dry matter and nitrogen yield and botanical composition of lucerne and its mixtures with grasses, as well as the effect of different levels of N fertilizer on nitrogen utilization efficiency. The study included pure lucerne and lucerne mixtures with grasses and legumes (cocksfoot, tall fescue and sainfoin) and four fertilization treatments with nitrogen (0, 70, 140 and 210 kg ha^{-1}). In a two-year study, it was concluded that lucerne uses nitrogen in fertilizers more efficiently, compared to its mixtures and that the utilization efficiency increases with the increasing share of legumes in the mixture. The four component mixture of lucerne, cocksfoot, tall fescue and sainfoin, of all studied mixtures, was characterized by the largest increase in yield per unit of added nitrogen and the percentage of nitrogen utilization from mineral fertilizers in the relative value of 54.52%. Although the addition of nitrogen lead to a progressive increase in yields, the value of the realized yield per unit of added N decreased. The highest utilization of N from mineral fertilizers was recorded in the treatment with 70 kg N ha^{-1} (51.2%).

Key words: nitrogen, use efficiency, fertilizer, grass-legumes mixtures,

INTRODUCTION

Lucerne is an important plant that is commonly used as a roughage, hay and silage for feeding ruminants and non-ruminants. It achieves high yields of green mass, very resistant to drought conditions, and due to its N_2 fixation ability, the need for the application of N fertilizers is reduced. Growing lucerne in a mixture with grasses expands the range of its utilization and enhances its importance as leguminous plants. Part of N taken by the lucerne through nitrogen fixation is transported to the grasses in the mixture, so that the grasses compensate part of their nitrogen requirements from N_2 fixation. Bowman et al. (2005) reported that lucerne meets 72-81% of its nitrogen requirements through nitrogen fixation. However, growing of lucerne in grass mixtures increases the utilization efficiency of N through nitrogen fixation, compared to monoculture, and it amounts to 92-95% of lucerne nitrogen requirements (Brophy et al., 1987) and increases with increasing share of grasses (Carlsson and Huss-Danell, 2003). Transfer of N from lucerne to grasses in the mixture ranges from 10% (Gebhart et al., 1993), to 68% of the total N in the grasses (Brophy et al., 1987). Part of the nitrogen that can not be satisfied from nitrogen fixation is compensated by applying N fertilizer. N fertilizers increases the yield and quality of the mixture, however, a large amount of N can have a depressive effect on the yield, disturb the balance of species in the mixture (Bijelić et al., 2013), reduce the intensity of the biological nitrogen fixation (Hǿgh-Jensen and Schjoering, 1997), leading to the water-pollution by nitrates, algal blooms, soil and water acidification (Rockström et al., 2009). To avoid this, each application of N has to be controlled in accordance with the needs of the plants. The best effect of the fertilization of mixtures, which is reflected in a stimulating effect on biological N_2 fixation, adoption of non-symbiotic nitrogen and efficient transformation of the adopted nitrogen in biomass is achieved with 50 and 150 kg N ha-1 and with the proportion of legumes in the

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mixture of 40-60% (Nyfeler et al., 2011). Nitrogen-use efficiency (NUE) is one of the basic guidelines of the environmental and economic sustainability of farm production systems (Ryan et al., 2012). It is the plant's ability to adopt nitrogen from the environment and convert it into biomass (Mohd-Radzman et al., 2013). There are many indices that can define the efficiency of nitrogen utilization: partial factor productivity of applied N (NUE), agronomic efficiency of applied N (AE), physiological efficiency of applied N (PE), percent of fertilizer recovery (PFR). They are based on differences in crop yield and total N in aboveground plant mass between fertilized plots and an unfertilised control. On a plot of uniform structural and chemical composition in the same production system, NUE is generally reduced by the addition of large amounts of N fertilizer (Dobermann, 2005). For perennial plants, Barth et al. (2014), quoting other authors states that NUE may be increased in three ways, by increasing the amount of biomass produced per unit of nitrogen, by increasing the amount of soil nutrients that are taken up by the plant and increasing the fraction of nitrogen translocated out of the leaf and stems into the root and rhizomes.

The aim of the study was to investigate the effect of nitrogen fertilization on dry matter and nitrogen yield as well as botanical composition of lucerne and its mixtures with grasses. Also focus of our research was to investigate the effect of different levels of nitrogen fertilization on nitrogen utilization efficiency, estimated through various efficiency indexes.

MATERIAL AND METHODS

Experimental design

The experiments were carried out at the experimental field of the Institute for Animal husbandry, Zemun, Serbia, during 2008 and 2009. Soil analyses have shown that the soil is silty clay loam and that contained 6.87% of organic matter, 0.4% of $CaCO_3$, 1956 mg kg^{-1} of total N, 91.35 mg 100 g^{-1} of P_2O_5, 16.35 mg 100 g^{-1} K_2O and the pH (KCl) was 7.08. The experiment was established in spring 2007 in four replications in a split - plot design, with a plot area of 10 m^2. Certified lucerne seed of commercial name K-28, cocksfoot cultivar K-40, tall fescue K-20 and sainfoin Krajina were used. The study included following factors: lucerne mixtures with grasses in different ratios and lucerne in pure crop as a control variant (Table 1), as well as different levels of nitrogen fertilizer. N was applied in split doses, in early spring and after first cut, in the quantity of: 0, 70, 140 and 210 kg ha^{-1}.
Fertilizer KAN (27% N) was used for fertilization.

Table 1. Studied fodder mixture sructure and sowing rate

Species	Ratio	Sowing rate, kg ha^{-1}			
		Lucerne	Cocksfoot	Tall fescue	Sainfoin
Lucerne	100	20	-	-	-
Mixture 1	50:50	10	20	-	-
Mixture 2	33.3:33.3:33.3	6.7	13.3	13.3	-
Mixture 3	25:25:25:25	5	10	10	35

Measurements

The year of sowing – 2007, was not considered. Yield measurements and chemical analyses were observed in first and second production years. In both years, all plots were cut four times. All cut mass of the plots was measured for the calculation of the yield of green mass per unit area, and then 1 kg samples were taken for drying and recalculating the yield of dry matter per unit area. Samples of the green mass were dried at a temperature of 60°C in laboratory dryer for three days. Dry weight was measured by digital balance and converted into above ground biomass yield.

The botanical composition was determined in samples taken from 1 m^2, by separating each plant species, measuring and calculating the percentage share of the yield in each harvest.

Samples for chemical analyses were dried at a temperature of 105°C, then milled and analysed for nitrogen concentration (g kg^{-1} DM) by Kjeldahl analyses. Nitrogen use was defined as nitrogen use efficiency (NUE) agronomic efficiency (AE), physiological efficiency of applied nitrogen (PE), percent of fertilizer recovery (PFR), calculated by using the following formulas according to Dobermann, (2005) and Varvel and Peterson (1990):

NUE = Y_N / F_N, (kg yield per kg N applied);

AE = (Y_N – Y_0) / F_N, (kg yield increase per kg N applied);

PE = (Y_N – Y_0) / (U_N – U_0), (kg yield increase per kg increase in N uptake from fertilizer);

PFR= (U_N – U_0) / F_N) x 100 (% of N uptake from kg of N applied).

F_N – amount of applied N (kg ha^{-1});

Y_N – crop yield in a treatment with applied N (kg ha^{-1});

Y_0 – crop yield in a treatment with no N (kg ha^{-1});

U_N – total plant N uptake in a plot that received N (kg ha^{-1});

U_0 – the total N uptake in a plot that received no N (kg ha^{-1}).

Statistical analyses

STATISTICA 8 software (StatSoft. Inc. 2007) was used for all analyses. The results of the study regarding the dry matter yield, nitrogen yield, botanical composition and parameters of efficiency of nitrogen utilization were analysed by two-factorial analysis of variance (ANOVA), while the difference between means were tested using Fisher's (LSD) test ($p<0.05$). The correlation and regression analysis ($p<0.05$) was conducted between the studied factors and NUE parameters, and the botanical composition and NUE parameters. All the results are presented in tables and figures.

Agro-meteorological conditions

The climatic conditions of the area, in which the research was conducted, correspond to a temperate continental climate, characterized by warm summers, very cold winters, with snow, sharp shifts from winter into the summer with an average mean annual temperature of 11.5°C, temperature during the growing seasons of 16.0°C and an annual average of precipitation of 650.0 mm.

The average annual precipitation in the studied years was 587.0 and 804.4 mm, and mean annual temperature 14.0 and 13.6°C. During vegetation period, from mid-March until the end of October, 64.6% of total precipitation occurs, with the average temperature of 18.2°C. The hottest months are July and August. The average monthly temperature in 2008. and 2009., as well as the monthly sum of rainfall and drought periods, are shown graphically below (Figure 1).

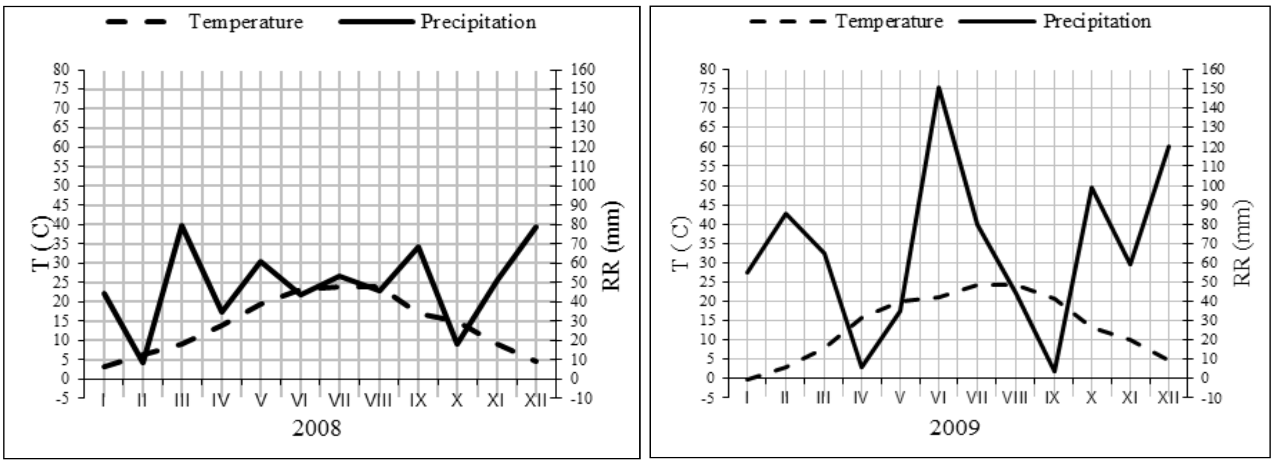

Figure 1. Climate diagram for 2008 and 2009 years

In both years of the investigation the mean annual and temperature during the growing season, were higher than the long-term average. In regard to the total annual rainfall, 2009 year, they exceeded the multiannual average; however uneven distribution of rainfall caused the emergence of droughts, which acted adversely on the growth and development of plants.

RESULTS AND DISCUSSION

The measure of the efficiency of nitrogen from fertilizers is expressed as harvested biomass per unit of N. From shown results (Table 2), we see that the dry matter yield of lucerne and its mixtures was highly dependent on the type of mixture, the level of applied N fertilization, and their interactions. The yield of lucerne was significantly higher than the yield of its mixtures with grasses. All mixtures achieved yields that do not show statistically significant differences. Dry matter yield proportionately increased with the addition of N through fertilization. So the control had significantly lower yield than the fertilized variants. The level of added nitrogen of 210 kg ha^{-1} reached a significantly higher yield compared to treatments with lower levels of fertilization.

The yield of nitrogen and dry matter yield had the same statistical variations in relation to the type of mixture and in relation to the level of N fertilization. The interaction of the two studied factors had no significant impact. The yield of nitrogen ranged from 399.11 in mixtures to 475 kg ha^{-1} in lucerne, or from 362.55 in the control treatment to 485.45 kg ha in the treatment with the 210 kg N ha^{-1}. If it is assumed that the yield of N from the control treatment comes from nitrogen fixation and N soil mineralization, then generated additional yield of N in fertilization treatments from low-to-high N rates is less than the amount of applied nitrogen, which leads to the eventual loss of nitrogen in agro-ecosystems. Minimum loss was in the treatment with 70 kg N ha^{-1}.

Table 2. Yield of dry matter (DM) and N, nitrogen use efficiency (NUE), agronomic efficiency (AE) and percent fertilizer recovery (PFR) of lucerne and its grass mixtures in dependence of level of N fertilization

Factor	DM yield (t ha^{-1})	N yield (kg ha^{-1})	NUE (kg kg^{-1})	AE (kg kg^{-1})	PFR %
Mixture (M)					
Lucerne	15.90^{a}	475.00^{a}	143.29^{a}	19.95^{a}	68.72
Mixture 1	14.65^{b}	416.35^{b}	129.85^{b}	10.27^{b}	34.87
Mixture 2	14.38^{b}	399.11^{b}	126.76^{b}	14.28^{ab}	38.93
Mixture 3	14.35^{b}	412.52^{b}	130.15^{b}	20.25^{a}	54.52
Level of significance M	**	**	**	**	ns
Fertilization (N)					
0	13.32^{c}	362.55^{c}	-	-	-
70	15.02^{b}	421.97^{b}	214.54^{a}	24.19^{a}	73.16^{a}
140	14.96^{b}	433.01^{b}	106.83^{b}	11.65^{b}	38.30^{b}
210	16.00^{a}	485.45^{a}	76.17^{c}	12.72^{b}	36.31^{b}
Level of significance N	**	**	**	**	*
Level of significance M x N	**	ns	**	ns	ns

Means in a column with different letters are statistically different at $p<0.05$ (*) and $p< 0.01$ (**); ns – non significant.

Realized yield of dry matter per kilogram of added nitrogen (NUE) was significantly higher in lucerne in relation to its mixtures. There were no significant variations between mixtures. The level of N fertilization had a highly significant impact, as well as the interaction of two tested parameters. Addition of N reduced the efficiency of N utilization. The highest yield per 1 kg of added nitrogen was recorded in the treatment with 70 kg ha^{-1}.

Addition of the next 70 kg N ha^{-1} decreased the yield achieved per unit of N fertilization by 50%. Similar to our research, Comakli et al. (2005) obtained in their research the highest efficiency of N used in the treatment with the least amount of nitrogen added 75 kg ha^{-1} compared to the doses of 150 and 225 kg ha^{-1}.

The type of mixture and the level of nitrogen fertilization had a significant effect on agronomic efficiency (AE). The highest increase of yield per 1 kg of added nitrogen was recorded in case of lucerne mixture with cocksfoot, tall fescue and sainfoin, while the lowest was recorded in the mixture of lucerne and cocksfoot. Similar to other studies (Aynehband et al., 2012), our results indicated that AE decreased with increasing N rate.

Cost effectiveness threshold was recognized (Jankowski et al., 2014) as 7 kg of hay per 1 kg of nitrogen as fertilizer. From our own calculations, average price of KAN (27%) was 25.600,00 din. t^{-1} and average price of hay was 6.666,00 din. t^{-1}, agricultural efficiency stands at 7 kg too. All mixtures and all treatments of fertilization yielded over the threshold of profitability.

The most efficient increase of the yield was in the treatment with 70 kg N ha^{-1} in pure lucerne, as well as, in its mixture with grasses and sainfoin.

The capability of a plant to get nitrogen from the soil depends of soil type, environment and species (Masclaux-Daubresse et al., 2010). PFR is defined as the percentage of utilization of nitrogen from fertilizers. The level of N fertilization, of all parameters examined, had no significant effect. Lucerne and its mixture with grasses and sainfoin had the highest percentage of use of N fertilizers (68.72 and 54.52%). In the nitrogen treatments, the highest percentage of N used from the fertilizer was in the treatment with 70 kg N ha^{-1}. This means that in the treatment with 70 kg N ha^{-1}, 73.16% was used or 51.2 kg N ha^{-1} and the rest was nitrogen which originated from fixation and N soil mineralization. Any subsequent addition of nitrogen significantly reduced its utilization from fertilizers. In the treatment with 210 kg N ha^{-1} only 36.31 and 76.2 kg N ha^{-1} are used, so that the remaining added nitrogen represents a potential threat to the environment (Roberts, 2008).

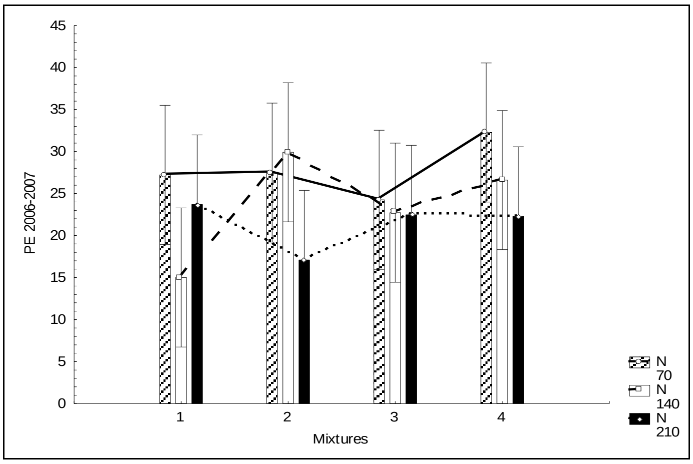

Figure 2. Impact of mixture structure and level of nitrogen fertilization on physiological effectiveness

The physiological effectiveness (PE), as an increase of hay yield per 1 kg of nitrogen taken by the plant, was not influenced by the investigated factors (Figure 2). The highest PE was observed in cultivation of lucerne with grasses and sainfoin and at level of nitrogen fertilization of 70 kg ha^{-1}. In the research of Jankowski et al. (2014), the highest increase in yield per unit of adopted N was recorded in the fertilization treatment with 90 kg N ha^{-1}.

Correlation analysis of examined factors and the efficiency indicators of utilization of nitrogen from N fertilizer led to the results which confirm that the fertilization factor was significantly negatively correlated with test indicators, especially with NUE (Table 3).

Table 3. Correlation analysis of dependence of the tested factors and efficiency index of N exploitation

Factor	PFR	NUE	AE	PE
Mixture	-0.10	-0.08	0.05	0.21
Fertilization	-0.36*	-0.94*	-0.45*	-0.36*

*significant at $p<0.05$.

Regression analysis showed that the addition of 1 kg N reduced the efficiency of nitrogen utilization by an average of 0.98, AE by 0.08, PE by 0.04 kg kg^{-1} and PFR by 0.26% (Table 4).

Table 4. Regression analysis of the levels of N fertilization and N utilization efficiency index

Parameters	Level of N fertilization
PFR	y = 86.11 - 0.26x
NUE	y = 270.88 - 0.98x
AE	y = 27.65 - 0.08x
PE	y = 30.67 - 0.04x

Botanical composition and NUE parameters

The structure of grass-legume mixtures is variable and depends on many parameters such as: tolerance of species to climatic and soil conditions, the number of species in the mixture, their competitive abilities, application of agricultural measures etc. In the present study, of all parameters examined, only the level of fertilization showed a significant effect on the share of individual species in the mixture (Table 5). The share of legumes decreased, and the share of grasses and weeds increased with the addition of N. Similar to our research, Yolcu et al. (2014) reported that the share of legumes decreased due to nitrogen fertilization. The reason for reduction of the legume component of the grassland by applying N fertilizer is explained by the reduction of the nitrogen fixation process and assimilation of soil nitrogen in legumes compared to the grass component (Frankow-Lindberg, 1987).

Table 5. The effect of the structure of the mixture and N fertilization on the proportion of individual species in the mixture

Factor	Share of legumes	Share of grasses	Share of weeds
Mixture (M)			
Mixture 1	59.80	38.35	1.85[a]
Mixture 2	59.82	39.10	1.09[b]
Mixture 3	62.03	36.73	1.37[b]
S.E.	±3.00	±3.00	±0.12
Level of significance M	ns	ns	**
Fertilization (N)			
0	71.23[a]	27.56[c]	1.10[b]
70	63.33[ab]	35.62[b]	1.39[ab]
140	56.30[bc]	42.17[ab]	1.54[a]
210	51.34[c]	46.88[a]	1.73[a]
S.E.	±3.47	±3.46	±0.14
Level of significance N	**	**	*
Level of significance M x N	ns	ns	ns

Means in a column with different letters are statistically different at $p<0.05$ (*) and $p<0.01$ (**); ns – non significant.

The newly created legumes and grasses ratio in the mixture had an impact on efficiency of the nitrogen utilization. The share of legumes had a significant positive effect on NUE and AE, while the share of grasses and weeds had a negative impact. Increase in the share of legumes by 1% generally increased dry matter yield by an average of 2.32 kg per kg of N and the increment of yield per unit of added N fertilizer, by 0.41 kg kg^{-1}N. With increasing share of grasses the NUE decreased by 2.16, and AE by 0.39 kg kg^{-1}N (Tables 6 and 7).

Table 6. Correlation analysis of dependance of the share of species and N utilization efficiency indexes

Parameters	PFR	NUE	AE	PE
Share of legumes	0.23	0.41*	0.46*	0.24
Share of grasses	-0.21	-0.38*	-0.44*	-0.24
Share of weeds	-0.24	-0.47*	-0.45*	-0.23

Table 7. Regression analysis of the share of individual species and NUE and AE

Parameters	NUE	AE
Share of legumes	y = 3.33 + 2.32x	y = -7.26 + 0.41x
Share of grasses	y = 224.64 - 2.16x	y = 32.28 - 0.39x
Share of weeds	y = 233.71 - 62.01x	y = 30.75 - 9.36x

This can be explained by the fact that N fertilization has a depressive effect on NUE as well as the share of legumes in the mixture, and hence there is a positive correlation between the efficiency of nitrogen utilization and the share of legumes in the mixture, or negative between NUE and share of grasses and weeds. Also, the share of legumes was the highest in the mixture 3, which was the most efficient in regard to the use of nitrogen fertilizers, with the value of 54.52%.

CONCLUSION

Based on the analysis of the presented results, the following was concluded:

Lucerne achieved significantly higher dry matter yield, nitrogen yield and dry matter yield per 1 kg of added nitrogen, compared to its mixtures with grasses. In percentage, it used significantly more N from fertilizer.

The mixture of lucerne, cocksfoot, tall fescue and sainfoin, as compared to other mixtures, was characterized by the highest increase in yield per unit of added nitrogen, as well as the percentage of nitrogen utilization from mineral fertilizers (54.52%).

The increase of share of legumes in mixtures leads to the increase of NUE and AE, while the increase in the share of grasses caused the decrease of these two parameters.

Nitrogen fertilization lead to a significant increase in dry matter yield, however, it reduced the value of the achieved yield and increase in the yield per unit of added nitrogen. The highest use of nitrogen fertilizers was recorded in the treatment with 70 kg N ha^{-1}, being 51.2%.

The improvement in the utilization of N from nitrogen fertilizers should be the main purpose of future agriculture. Achievement of this goal should not burden farmers and the environment and must be in accordance with the three basic rules of fertilization: adequate timing, adequate amounts and in appropriate place. This would benefit the farmers, the economy, as well as the environment.

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