QUALITY OF ALFALFA(*Medicago sativa* L.), IN THE FIRST YEAR OF VEGETATION

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Abstract

In a bifactorial experience, during March-October 2010, at the Research Station Ezăreni from Iasi, we studied alfalfa in the first year of vegetation, analizing the effect of seed inoculation with symbiotic bacteria *Rhizobium meliloti* Dangeard and the effect of fertilization on the dry matter (DM) yield, the amount of crude protein per hectare, the leaves/stems ratio and the forage quality indicators (CP - crude protein content, NDF - neutral detergent fiber content, ADF - acid detergent fiber content and RFV - relative feed value). The results showed that under experimental conditions, the interaction of two factors led to DM yields ranging from 5.04 to 7.75 t·ha^{-1} and a quantity of crude protein of 1121-1539 kg·ha^{-1}. CP content in leaves ranged from 24.04 to 30.29% and 10.51 to 19.80% between the strains, the leaves/stems ratio having the biggest influence on CP content of the whole plant. Analyzing the data, we observed that seed inoculation had an insignificant influence on the forage quality indicators. Fertilization positively influenced DM yield and amount of CP produced and had a negative impact on the leaves/stems ratio, plant content in CP, NDF, ADF and RFV.

Key words: alfalfa, inoculation, fertilizer, productivity, quality

Alfalfa (*Medicago sativa* L.) is one of the most valuable forage due to high production of green mass or hay and high quality forage.

Lucerne has a high quality potential and ability to control factors can affect the quality and will improve production quality. Factors affecting quality are represented by soil fertility, cultivar, the presence of other species in culture, the use of pesticides, climatic conditions, harvesting (season, time of day and stage of development at harvest) and the method of preservation (Lloveras J. et al., 2004; Stancheva I. et al., 2008; Dragomir Carmen and Moisuc A., 2007).

Evaluating the amount of crude protein produced per unit area, alfalfa can not be surpassed by any other forage crop in specific agro-ecological conditions of our country.

Inoculation of seeds and providing the necessary nutrients through fertilization (mineral or organic) are two important technological links to improve productivity and quality of alfalfa (Oliveira W. S. et al., 2004; Berg W. K. et al., 2009; Dragomir N. and colab., 2010**)**.

MATERIAL AND METHODS

The research was conducted during March-June 2010, on the Ezăreni farm (47°05'-47°10' North latitude and 27°28'-27°33' East longitude), farm belonging to the University of Agricultural Sciences and Veterinary Medicine Iaşi. The soil from the region is a cambic chernozem characterized by the indicators presented in *table 1*.

Table 1

Physico-chemical characteristics of soil that was placed experience

Horizon	Clay (<0.002)	pH	Humus (%)	N total (%)	P-Al (ppm)	K mobile (ppm)	Ca exch. (me)
Ap 0-20 cm	41.8	6.68	2.24	0.178	26.00	242	15.21
Atp 20-28 cm	38.8	6.78	2.40	0.149	10.43	178	15.38

Research has sought influence of inoculation and fertilization on the dry matter yeld (DM) per hectare, the amount of crude protein per hectare, the leaves/stems ratio and the quality indicators (CP - crude protein content, NDF - neutral detergent fiber content, ADF - acid detergent fiber content and RFV - relative feed value) of alfalfa (*Medicago sativa* L.) in the first year of vegetation. The experiment was

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bifactorial, with a design type 2x4, arranged in subdivided plots in three replications, which have a 10 m^2 harvesting area (2m x 5m). The factors were as follows: A-seed inoculation with two graduations (a_1-uninoculated and a_2-inoculated) and B-fertilization with four graduations (b_1-unfertilized, b_2-$N_{50}P_{50}$, b_3-$N_{75}P_{50}$ and b_4-30 $t\cdot ha^{-1}$ manure).

Yield production was determined by weighing the yield harvested from an area of 10 m^{-2} then reported per hectare.

Dry matter was determined by treating samples at 105°C for 3 hours.

The leaves/stems ratio was determined by separating the stem, leaflets, buds and flowers by the stem, weighing them separately and report their amount to the amount of strain (leaves/stems ratio).

Nitrogen content was determined by Kjeldahl method, and NDF and ADF content were determined by Van Soest method.

The amount of CP per hectare was calculated based on DM production per hectare and its content in the CP.

RFV was calculated using the formula (Sheaffer C.C. et al., 1995; Boman R. L., 2010):

$$RFV = \frac{88.9 - (0.779 \cdot ADF) \cdot \frac{120}{NDF}}{1.29}$$

Depending on the content CP, NDF, ADF and RFV, alfalfa hay can be classified into six quality classes (*table 2*), used by many authors (Mirzaei-Aghsaghali A. et al., 2007; Kiraz A.B., 2011; Redfearn D. and Zhang H., 2011).

The biological material used was represented by Sandra alfalfa variety (F 660-94) registered in 2003 to I.N.C.D.A. Fundulea. (Schitea Maria and Martura T., 2004).

Table 2

Quality standards of grasses, legumes and grasses-legumes mixtures

Quality standard	CP	NDF	ADF	RFV
	(% from DM)			
0-Prime	>19	<40	<31	>151
1	17-19	40-46	31-40	125-151
2	14-16	47-53	36-40	103-124
3	11-13	54-60	41-42	87-102
4	8-10	61-65	43-45	75-86
5	<8	>65	>55	<75

The inoculation of seeds happened one hour before sowing, with selected strains of *Rhizobium meliloti* Dangeard. Manure used had the following chemical composition: N-0.445%, P_2O_5-0.212% and K_2O-0.695%.

Fertilizers were applied, and incorporated into the soil before seeding. Harvesting was performed with Bertolini 411 harvester, at a height of 7 cm from the ground. The timing of the first cut was at 10% flowering, second cut was harvested at full flowering and third cut at 50% flowering.

In the area where research was conducted, the 2009-2010 crop year, monthly average temperatures had no signifficant deviations from the multiannual mean values.

During the growing season shortages of water was recorded in the following periods: third decade of April and May, the second and third decade of July, and the first decade of August.

In the third decade of June there were a quantity of 132 mm rainfall, well above normal.

The results were interpreted statistically by analysis of variance and calculation of least square difference (LSD).

RESULTS AND DISCUSSION

If investigations were highlighted distinct and very significant increases in all fertilized variants, each of the 3 cuts (*table 3*), (*figure 1*).

The interaction between two factors caused maximum DM production increases at a_2b_3 variant, each of the three cuts in the first year of vegetation. Production differences were due to fertilization, especially phosphorus. Phosphorus is a essential nutrient for alfalfa plants development in the first year of growing. Also, nitrogen applied in small quantities (20-50 $kg\cdot ha^{-1}$) when sowing, provide the necessary plants nitrogen to the formation of nitrogen fixing nodules. The lack of these elements in the first development phenophases is not compensated for future fertilization, negative effects are manifested throughout the entire life of the alfalfa crop. This was confirmed by research conducted by other authors (Mullen R.W. et al., 2000; Delgado I. et al., 2001; Stancheva I. et al., 2008).

Inoculation, in the Ezăreni experimental conditions, did not cause significant production increases. Inoculation efficiency is influenced by various factors such as presence of *Rhizobium meliloti* Dangeard soil bacteria and other mycorrhizal microorganisms (fungi), cultivated variety, bacterial strain used, soil N, Ca, B, Co, Mo content, soil aeration, previous crop,etc.. (Kuykendall L.D. et at., 1999; Maćešić D. et al., 2007).

Other authors (Varga P, et al., 1973; Moga I. et al., 1996**)** show that there is a close relationship between the efficiency of alfalfa seed inoculation and soil content of exchangeable calcium. This relationship indicates alfalfa seed inoculation with *Rhizobium meliloti* Dangeard selected strains in all cases where the soil content of exchangeable calcium is less than 2 ‰. Then, the exchangeable calcium content of soil is more than 2 ‰, inoculation is ineffective.

Table 3

Production of dry matter (DM), alfalfa culture in the first year of vegetation

Experimental plot			***Dry matter production (kg·ha^{-1})***					***% as reffered to control***
			First cut	**Second cut**	**Third cut**	**Total**	***Dife-rences***	
a_1- without inoculation (control)	b_1- unfertilized (control)		1.87	2.39	0.77	**5.04**	**control**	**100**
	b_2- $N_{50}P_{50}$		2.25*	2.79***	0.88	**5.92****	**0.88**	**118**
	b_3- $N_{75}P_{50}$		2.73***	3.53***	0.97*	**7.23*****	**2.19**	**144**
	b_4- 30 Mg·ha^{-1} manure		2.24*	3.04***	0.88	**6.15*****	**1.12**	**122**
a_2- inoculated	b_1- unfertilized		2.00	2.63**	0.79	**5.42**	**0.39**	**108**
	b_2- $N_{50}P_{50}$		2.41**	3.03***	0.88	**6.31*****	**1.28**	**125**
	b_3- $N_{75}P_{50}$		3.02***	3.73***	0.99*	**7.75*****	**2.71**	**154**
	b_4- 30 Mg·ha^{-1} manure		2.41**	3.27***	0.89	**6.57*****	**1.54**	**131**
LSD		*5%*	*0.33*	*0.16*	*0.17*	*0.46*		
		1%	*0.47*	*0.22*	*0.24*	*0.64*		
		0.1%	*0.66*	*0.31*	*0.34*	*0.91*		

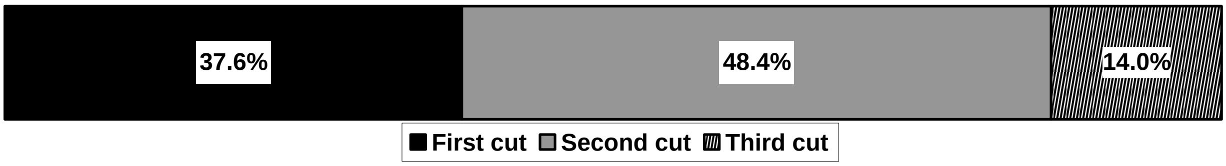

Figure 1 **The proportion of each cut from total DM production**

Research has shown that the leaves/stems ratio was different, depending on the experimental plot analyzed and the number of cut.

At each of the three cuts, the leaves/stems ratio was better (meaning a higher percentage of leaves), for the unfertilized and organically fertilized plots (*table 4, figure 2*). Percentage of leaves was lower in mineral fertilized plots, due to more vigorous strains and larger strains diameter.

Table 4

Influence of interaction between inoculation and fertilization on the alfalfa leaves/stems ratio

Experimental plot			***Leaves/stems ratio***		
			First cut	**Second cut**	**Third cut**
a_1- without inoculation (control)	b_1- unfertilized (control)		1.30	0.90	1.86
	b_2- $N_{50}P_{50}$		0.97^{oo}	0.87	1.81^{o}
	b_3- $N_{75}P_{50}$		0.85^{oo}	0.71^{oo}	1.72^{oo}
	b_4- 30 Mg·ha^{-1} manure		1.07^{o}	0.88	1.76^{oo}
a_2- inoculated	b_1- unfertilized		1.25	0.83	1.76^{oo}
	b_2- $N_{50}P_{50}$		0.88^{oo}	0.74^{oo}	1.81^{o}
	b_3- $N_{75}P_{50}$		0.81^{oo}	0.67^{ooo}	1.63^{ooo}
	b_4- 30 Mg·ha^{-1} manure		1.08^{o}	0.79^{o}	1.68^{ooo}
LSD		*5%*	*0.11*	*0.07*	*0.03*
		1%	*0.26*	*0.16*	*0.09*
		0.1%	*0.56*	*0.23*	*0.16*

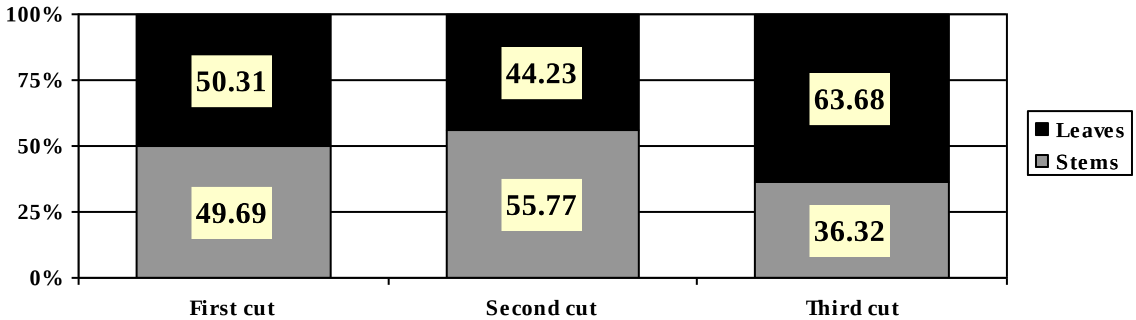

Figure 2 **Cycle of vegetation influence on the leaves/stems ratio**

The leaves/stems ratio varied depending on the number of cut. Thus, at the first cut the average percentage of leaves was approximately equal to that of strains, to the second cut leaves were 10% less than the stems, and at the third cut the leaf percentage was 27% higher than that of strains. The results confirm the research of other authors in similar experimental conditions (Bender A. and Tamm S., 2010; Stanćev Vidica et al., 2010).

The leaves/stems ratio is an important

quality indicator, because of this depends quality of hay obtained. Percentage of leaves is desirable to be as high as possible, because in the leaves are found a crude protein content at least twice then in the strains, as shown by many authors (Popovic S. et al., 2001; Petkova D. and Panayotova G., 2007).

Following results were found, although fertilization positively influenced the content of the leaves and stems in CP. CP content in plants was lower in fertilized plots due to leaves/stems ratio, negatively affected by fertilization (*table 5*).

Although plants CP content in the fertilized variants was lower, the amount of CP was higher, statistically assured, due to higher yields obtained from these plots (*table 6, figure 3*).

Table 5

Influence of interaction between inoculation and fertilization on the alfalfa CP content

Experimental plot		***CP content (% from DM)***								
		Leaves			**Stems**			**Whole plant**		
		First cut	**Second cut**	**Third cut**	**First cut**	**Second cut**	**Third cut**	**First cut**	**Second cut**	**Third cut**
a_1 -c	**b_1-c**	24.04	26.26	28.98	10.51	14.09	19.57	19.28	22.13	29.95
	b_2	24.30*	26.69*	29.63***	10.76	14.28	19.71***	19.19	20.85^{oo}	28.65
	b_3	24.51**	27.80***	30.29***	11.09**	14.45*	19.73***	17.92^{oo}	20.17^{ooo}	27.45^{oo}
	b_4	24.19	26.25	29.84***	10.77	14.22	19.73***	18.55	21.60	28.22^{o}
a_2	**b_1**	24.20	26.88**	29.59***	10.59	13.90	19.71***	19.55	22.24	28.66
	b_2	24.44**	26.95**	29.67***	10.87	14.30	19.75***	17.98^{oo}	20.85^{oo}	27.17^{ooo}
	b_3	24.48**	27.17***	30.14***	11.06**	14.48**	19.80***	17.67^{oo}	19.89^{ooo}	26.51^{ooo}
	b_4	24.36*	26.46	30.03***	10.77	14.17	19.72***	18.84	20.97^{o}	27.66^{oo}
LSD	*5%*	*0.25*	*0.41*	*0.30*	*0.38*	*0.27*	*0.07*	*0.84*	*0.89*	*1.33*
	1%	*0.36*	*0.57*	*0.43*	*0.54*	*0.38*	*0.09*	*1.17*	*1.24*	*1.87*
	0.1%	*0.50*	*0.81*	*0.60*	*0.76*	*0.54*	*0.13*	*1.66*	*1.75*	*2.64*

Table 6

Anount of crude protein (CP), alfalfa culture in the first year of vegetation

Experimental plot		***Amount of CP (kg·ha^{-1})***					***% as reffered to control***
		First cut	**Second cut**	**Third cut**	**Total**	***Diferences***	
a_1- without inoculation (control)	b_1- unfertilized (control)	361	528	231	**1121**	**control**	**100**
	b_2- $N_{50}P_{50}$	430	581*	253	**1265***	**144**	**113**
	b_3- $N_{75}P_{50}$	488**	711***	267	**1466*****	**346**	**131**
	b_4- 30 Mg·ha^{-1} manure	416	656***	247	**1318****	**198**	**118**
a_2- inoculated	b_1- unfertilized	390	584*	226	**1200**	**79**	**107**
	b_2- $N_{50}P_{50}$	432*	632***	239	**1302****	**182**	**116**
	b_3- $N_{75}P_{50}$	533***	742***	263	**1539*****	**418**	**137**
	b_4- 30 Mg·ha^{-1} manure	455*	685***	247	**1387*****	**266**	**124**
LSD	*5%*	*70*	*46*	*54*	*117*		
	1%	*98*	*64*	*76*	*164*		
	0.1%	*139*	*91*	*107*	*231*		

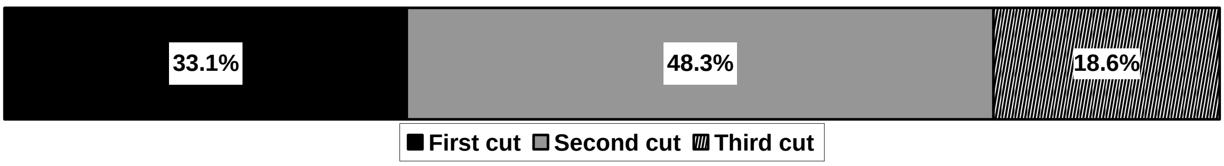

Figure 3 **Amount of crude protein (CP) distribution**

By analyzing the influence of inoculation and fertilization on alfalfa fiber content and relative feed value, it appears that inoculation had little influence on these indicators of quality feed. The results confirm the research of other authors who have come to similar conclusions, in conditions close to those present study (Hristozkova M. et al., 2009; Stevoviš Vladeta et al., 2010).

Organic fertilization showed a statistically positive effect on the content of plant leaves and stems in NDF and ADF. The same trend was manifested in the case of mineral fertilization strains except the ADF content, where the influence was reduced at the first and third cut (*table 7, 8*).

Relative feed value of alfalfa plants and leaves was negatively influenced and very significant to all of fertilization treatments (*table 9*). These trends, in terms of quality alfalfa under the influence of organic and mineral fertilization, confirming the results of research by other authors (Showalter J., 2000; Ketterings Q.M. et al., 2008**)**. The forage obtained from the first and third can be classified as prime class (0), while the forage from the second cut can be classified as class 2.

Table 7

Influence of interaction between inoculation and fertilization on the alfalfa NDF content

Experimental plot		NDF content (% from DM)								
		Leaves			Stems			Whole plant		
		First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
a_1 -c	b_1-c	18.0	29.9	24.3	56.3	56.9	42.1	35.2	43.1	30.7
	b_2	19.1***	30.6*	25.2**	57.8*	59.7***	43.6***	38.7***	45.6***	31.6**
	b_3	20.2***	32.2***	25.8***	58.8**	61.2***	44.2***	40.9***	48.7***	33.3***
	b_4	19.5***	30.6*	24.7	57.8*	59.2**	42.3**	38.2***	45.1***	31.5*
a_2	b_1	18.2	29.7	24.5	56.8	58.1	41.4	36.1	44.0	31.8
	b_2	19.4***	30.6*	25.3**	57.8*	60.7***	41.7***	39.4***	47.2***	31.7**
	b_3	20.4***	31.6***	26.5***	59.0***	60.9***	44.2***	41.7***	48.7***	33.7***
	b_4	19.4***	30.1	24.9*	57.8*	59.5**	41.3**	38.1***	45.4***	32.6***
LSD	*5%*	*0.4*	*0.7*	*0.6*	*1.3*	*1.4*	*1.0*	*0.9*	*1.1*	*0.6*
	1%	*0.6*	*1.0*	*0.8*	*1.8*	*1.9*	*1.4*	*1.2*	*1.5*	*0.9*
	0.1%	*0.9*	*1.3*	*1.1*	*2.5*	*2.6*	*1.9*	*1.7*	*2.0*	*1.2*

Table 8

Influence of interaction between inoculation and fertilization on the alfalfa ADF content

Experimental plot		ADF content (% from DM)								
		Leaves			Stems			Whole plant		
		First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
a_1 -c	b_1-c	13.9	19.5	19.6	46.1	47.7	36.0	28.5	33.1	27.5
	b_2	15.1***	20.6***	20.8***	46.8	48.9*	35.8	30.6***	35.4***	28.6***
	b_3	16.3***	21.6***	20.9***	47.4*	51.4***	36.1	31.5***	36.2***	29.4***
	b_4	15.0***	19.9	19.7	46.1	50.4***	36.1	30.1***	34.4**	28.5***
a_2	b_1	13.8	19.1	19.4	44.7º	48.8	35.5	29.1	34.0*	28.6***
	b_2	15.3***	20.7***	20.1*	46.4	50.5***	35.8	30.6***	35.3***	29.7***
	b_3	16.4***	21.4***	21.0***	46.0	53.1***	36.4	32.0***	37.5***	30.9***
	b_4	15.3***	20.1**	20.0	44.5ºº	50.4***	35.7	29.9***	34.5**	30.5***
LSD	*5%*	*0.4*	*0.5*	*0.5*	*1.0*	*1.2*	*0.8*	*0.7*	*0.8*	*0.5*
	1%	*0.5*	*0.7*	*0.6*	*1.4*	*1.6*	*1.1*	*1.0*	*1.1*	*0.7*
	0.1%	*0.7*	*0.9*	*0.9*	*2.0*	*2.2*	*1.6*	*1.3*	*1.5*	*1.0*

Table 9

Influence of interaction between inoculation and fertilization on the alfalfa relative feed value (RFV)

Experimental plot		RFV								
		Leaves			Stems			Whole plant		
		First cut	Second cut	Third cut	First cut	Second cut	Third cut	First cut	Second cut	Third cut
a_1 -c	b_1-c	404	230	282	88	85	134	176	136	205
	b_2	377ººº	221º	268ººº	85	79ºº	130	157ººº	125ººº	196ºº
	b_3	352ººº	208ººº	262ººº	82ººº	74ººº	128ºº	146ººº	116ººº	185ººº
	b_4	369ººº	223º	277	85	78ººº	133	160ººº	128ººº	197ºº
a_2	b_1	400	232	280	88	81º	138	171º	132º	195ººº
	b_2	368ººº	221º	269ºº	85	76ººº	136	154ººº	121ººº	193ººº
	b_3	348ººº	213ººº	255ººº	84º	73ººº	128ºº	143ººº	114ººº	179ººº
	b_4	369ººº	226	274º	87	78ººº	138	160ººº	127ººº	186ººº
LSD	*5%*	*9*	*6*	*7*	*3*	*3*	*4*	*5*	*4*	*5*
	1%	*13*	*8*	*10*	*4*	*4*	*6*	*7*	*6*	*7*
	0.1%	*18*	*11*	*14*	*6*	*6*	*8*	*9*	*8*	*9*

CONCLUSIONS

The first year alfalfa, inoculation resulted in no significant differences in productivity and quality of alfalfa, because the farm Ezăreni conditions (soil containing calcium by 3 ‰) *Rhizobium meliloti* Dangeard symbiotic bacteria has a sufficient presence in the soil.

Research has shown that the leaves/stems ratio was different, depending on the experimental plot analyzed and the number of cut. At each of the three cuts, the leaves/stems ratio was better (meaning a higher percentage of leaves), for the unfertilized and organically fertilized plots

Following results were found, although fertilization positively influenced the content of the leaves and stems in CP, CP content in plants was lower in fertilized variants, do to leaves/stems ratio, negatively affected by fertilization. Although plants CP content in the fertilized variants was lower, the amount of CP was higher due to higher yields obtained from these plots.

Fertilization showed a statistically positive effect on the content of plant leaves and stems in NDF and ADF (lower values of these indicators).

Relative feed value of alfalfa plants and leaves was influenced distinctive significantly and very significantly in all of fertilization plots.

The forage obtained from the first and third can be classified as prime class (0), while the forage from the second cut can be classified as class 2.

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