Assessment of surface and injection fertilization on various grass hybrids in grass-clover mixture

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ABSTRACT

In a small-plot trial, grass hybrids Perseus (loloid) and Felina (festucoid) were compared in a mixture with red clover (*Trifolium pratense* L.) and their reactions to surface application of nitrogen fertilizers or injection into soil according to CULTAN method were investigated. Both fertilizer application methods were used with three levels of nitrogen. Mixtures containing the Felina hybrid had higher yields (12.1 t/ha) compared to the Perseus hybrid (11.0 t/ha), and they also showed higher clover abundance. Increasing dosage of nitrogen resulted in slightly higher yield, whereas the trend in clover abundance was opposite. Nitrogen injection resulted in slightly higher dry matter yields and slightly higher clover abundance as compared to surface fertilization. The N content in the grass-clover mixture was balanced for both types of fertilizer application and did not change significantly with increasing dosage of fertilizer. A slightly higher N content was observed at the Felina hybrid treatments; the dependence of this element on clover abundance in the mixture was set up to 50%.

Keywords: dry matter yield; nitrogen uptake; fibre content; clover abundance; N application technique

Grass-clover mixtures combine the advantages of both their components; their yield usually exceeds those of the components grown in monocultures (Annicchiarico and Tomasoni 2010). Moreover, the production of dry matter throughout the year is balanced; grasses yield more in spring whereas clover crops produce more dry matter in summer (Mooso and Wedin 1990). Clover crops have a higher content of protein than grass (Evans et al. 1996, Gökkus et al. 1999); yet, their duration in the mixture is lower as compared to grass (Hejduk and Knot 2010). More erect leaves of grasses and horizontal leaves of clover crops minimize any interspecific competition (Lantinga et al. 1999). Another advantage of grass-clover mixture is a possibility of lower input of nitrogen fertilizer due to atmospheric nitrogen fixation by root-nodule bacteria at the roots of clover crops (Erkovan et al. 2008); yet, to obtain the maximum yield of dry matter, it is necessary to fertilize the grass-clover stands with nitrogen. At the conventional surface application of nitrogen fertilizers, the plants, both grass and clover, take up nitrogen mainly in the nitrate form. At the same time, clover, similar to other legumes fertilized with mineral nitrogen, produces nodules as a prerequisite of N_2 fixation. Nodule formation and therefore N_2 fixation may be reduced when N is supplied as mineral ferti-

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QH71077, and by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6046070901.

lizer. This behaviour is based on the characteristics of nitrate form. After reduction and assimilation in the leaves of clover, it supports growth of the above-ground parts at the expense of root development. Saccharides are preferentially used for growth before being transformed at rhizobia in roots. As a result of insufficient supply of saccharides, rhizobia discontinue N_2 fixation. An alternative method of fertilization is the CULTAN (Controlled Uptake Long Term Ammonium Nutrition) method; it uses a special injection technique to apply nitrogen solutions rich in ammonium ions into soil, into a depth of 7–20 cm, where it is retained in the spots with high concentration of ammonia, so-called depots (Sommer 2005, Kücke and Scherer 2006, Kozlovský et al. 2009). This process does not influence the function of rhizobia, as they cannot uptake ammonium ions. Saccharides produced in the above-ground parts are faster transported to the nodules, before roots get in contact with ammonium ions at the place of injection. The herbage of grass-clover mixtures at conventional fertilization may also suffer from necroses of clover leaves margins after harvest and follow-up nitrogen fertilization; it strongly inhibits the clover growth. Yet, similar necrotic spots were not observed at the CULTAN system. A possible explanation is the fact that ammonia is not uptaken by the clover plants. Therefore, it is supposed that the decline of the clover ratio in the herbage is not a result of dominant and aggressive growth of grasses at fertilization, but of toxic effect of mineral N fertilization on the clover growth. This toxicity results from high amounts of N; after harvest, clover must store much nitrogen to a rather limited leaf apparatus, as the nodule bacteria do not reduce their activity and the fixation of atmospheric nitrogen still continues. Such disproportion may be further depened by subsequent nitrogen fertilization. Yet, at this stage the number of bacteria at roots and their development is optimal because the mineral nitrogen from fertilization was used by grasses before harvest and all the nitrogen supply to clover is derived from symbiotic fixation. This is the reason why the dosage of nitrogen (at conventional fertilization) after the first harvest significantly exceeds clover demand. Nitrate taken up by clover is abundant, causes 'latent toxicity' and weakens the clover development. These are the main reasons why clover disappears from the grass-clover mixtures at conventional fertilizer application. The CULTAN method should reduce the negative effects of fertilizer N; this technique enables to provide a sufficient supply of mineral N to grasses and herbs, without being toxic to clover. Moreover, it should eliminate the marks of toxicity on the clover leaves. The distance of application points should be 15–20 cm, which more or less responds to medium length of lateral roots of grasses.

The aim of the presented investigation is to compare the influence of surface and injection fertilizer application on dry matter yield, clover abundance, N uptake, N and fibre content between two grass-clover mixtures.

MATERIALS AND METHODS

In 2007 at three different sites (Červený Újezd, Jevíčko, Troubsko; Czech Republic) an experiment was established in order to compare two systems of nutrition of grass-clover mixture. Characteristics of the experimental sites are given in Tables 1 and 2.

The experiment consisted of two sowing cycles: 1. cycle: sowing in 2007 and the harvesting in 2008 and 2009; 2. cycle: sowing in 2008 and harvesting in 2009. Sowing was done without a cover crop (plot size of 30 m^2 (20 × 1.5 m)) in a randomised block design with four replications. Prior to sowing 30 kg P/ha as finely ground apatite (11% P) and 90 kg K/ha as KCl (42% K) were applied. Plots were sown with a mixture of red clover (*Trifolium pratense* L., Vltavín variety) and the loloid hybrid Perseus (*Lolium multiflorum* Lam. × *Festuca pratensis* Huds.) or the festucoid hybrid Felina (*Lolium multiflorum* Lam. × *Festuca arundinacea* Schreb.). The ratio was 70% grass component and 30% clover. The experiment consisted of a control without N fertilizer and two N levels (90 kg N/ha; 180 kg N/ha). N was applied as calcium ammonium nitrate (27.5% N) on the soil surface and

Table 1. Characteristics of experimental sites

Site	Altitude (m)	Annual average		Soil type	Soil class	$pH/CaCl_2$
		precipitation (mm)	temperature (°C)			
Červený Újezd	405	549	7.6	luvisol	loam	7.4
Jevíčko	366	545	7.4	chernozem	loam	6.6
Troubsko	280	547	8.4	chernozem	loam	6.9

Table 2. Content of nutrients in soils at experimental sites (mg/kg), Mehlich III

Site	Ca	Mg	K	P
Červený Újezd	4139	131	166	111
Jevíčko	4116	241	143	55
Troubsko	4236	290	162	44

as urea ammonium nitrate (30% N), respectively, according to the CULTAN method using the GFI 3A machine (Maschinen- und Antriebstechnik, Güstrow, Germany). While 90 kg N were applied in spring, 180 kg N were applied in 3 rates: 90 kg N in spring, 60 kg N after the first harvest and 30 kg after the second harvest. Characteristics of the plots at field trial are in Table 3. At each experimental plot an assessment of ability of the sown species to cover the ground using the method of reduced projective dominance (sown species, other species, empty spaces = 100%) was done prior to each harvest. In the experiment, the 3-cut system was used. Experimental plots were harvested with an MPZ-115 small-plot forage harvester, with a finger cutter of 1.50 m width. The height of stubble was set to 50 mm as standard. The harvester was equipped with a tensometric weight VZT-3 (Cressto, Rožnov pod Radhoštěm, Czech Republic). An average mixed sample was taken from each plot. After drying at 60°C, dry matter yield was determined and the samples were homogenized in a laboratory knife mill Pulverisette 15 (Fritsch, Idar-Oberstein, Germany) equipped with normalised mesh with circle holes to sieve the particles < 1 mm. The analysis of samples with the NIRS method determined the following parameters of forage: N content and fibre content using the FOSS NIR Systems 6500 instrument (FOSS NIRSystems, Laurel, USA). Forage quality was predicted with the WinISI II software (Infrasoft International, State College, USA). Nitrogen uptake was determined as dry matter yield multiplied by N content. Quantitative and qualitative characteristics were statistically evaluated using a three-factor analysis of variance. The differences among average values were tested with the Tukey's test. Values within the column marked with the same letter are not statistically different at the level of significance $P < 0.05$.

RESULTS AND DISCUSSION

The total annual yield of dry matter from three harvests of grass-clover mixture is given in Table 4. At the Troubsko site, a dependence of dry matter production on the fertilizer dosage was observed in all harvests. It confirmed the results of Lynch et al. (2004) who reported that the increasing dosage of fertilizer resulted in increased dry matter production in both systems. Yet, dry matter yield in the injection system was slightly higher as compared to conventional fertilization. At the other sites, similar results were obtained in the second harvest year of the first sowing cycle. This contradicts the results of Neuberg et al. (2010) in pot experiments, who always obtained lower yields in treatments with injection fertilization of grass-clover mixture (*Festulolium*: *Trifolium pratense* L.); these plants were more stressed after injection fertilization, because the higher root ratio is connected with the depot in the pot than field experiment. Comparing individual hybrids in our experiment, grass-clover mixtures containing the Felina hybrid seem to be more productive. Higher productivity was caused by higher clover abundance in the mixture with Felina hybrid and by higher dry matter yield in the second and the third harvest. Higher dry matter yield of the Felina hybrid, compared to mixtures with the Perseus hybrid, was observed in both fertilization systems. In the second harvest year of the first sowing cycle we observed an interannual decrease of dry matter yield in all treatments and mixtures. In mixtures with the Perseus hybrid, this decrease was more significant. In an experiment with grass-clover mixture Estavillo et al. (1996) obtained higher dry matter yield in treatments with fertilization compared to unfertilized control, even though the ratio of clover in the mixture decreased with

Table 3. Characteristics of the plots at the field trial

Treatment	Type of application	Dosage (kg N/ha)	Mixture composition
1	conventional	0	Perseus: Vltavín
2		90	
3		180	
4		0	Felina: Vltavín
5		90	
6		180	
7	injection	0	Perseus: Vltavín
8		90	
9		180	
10		0	Felina: Vltavín
11		90	
12		180	

Table 4. Dry matter yield of grass-clover mixtures in three harvests (t/ha)

No.	Treatment	Červený Újezd			Jevíčko			Troubsko		
		A1	A2	B2	A1	A2	B2	A1	A2	B2
1	K, P, 0	11.0^{a}	5.6^{a}	11.5^{a}	22.2^{cde}	6.0^{a}	12.0^{a}	9.1^{a}	2.8^{a}	8.0^{a}
2	K, P, 90	10.7^{a}	7.9^{abc}	11.1^{a}	20.5^{bcd}	7.3^{abc}	12.2^{a}	12.4^{cdef}	5.9^{b}	9.5^{ab}
3	K, P, 180	11.6^{a}	8.0^{abcd}	12.2^{a}	24.1^{e}	8.8^{bcd}	13.5^{a}	13.5^{f}	6.4^{b}	10.8^{bc}
4	K, F, 0	11.0^{a}	9.2^{bcdef}	12.5^{a}	19.6^{abc}	8.00^{abc}	12.5^{a}	11.2^{bc}	5.5^{b}	10.4^{bc}
5	K, F, 90	12.2^{a}	10.8^{cdef}	11.5^{a}	21.0^{bcd}	8.9^{bcd}	13.7^{a}	12.6^{cdef}	7.7^{c}	11.8^{cd}
6	K, F, 180	11.9^{a}	11.7^{ef}	11.8^{a}	19.6^{abc}	10.3^{d}	15.1^{a}	13.4^{ef}	9.9^{d}	14.0^{e}
7	C, P, 0	11.1^{a}	6.7^{ab}	10.1^{a}	21.0^{bcd}	6.6^{ab}	12.7^{a}	10.6^{ab}	3.4^{a}	8.8^{ab}
8	C, P, 90	10.7^{a}	8.7^{abcde}	10.6^{a}	22.3^{cde}	8.0^{abcd}	14.0^{a}	12.2^{bcdef}	6.0^{b}	11.7^{cd}
9	C, P, 180	11.1^{a}	8.7^{abcde}	10.4^{a}	23.0^{de}	8.5^{bcd}	13.9^{a}	13.1^{def}	6.4^{b}	11.7^{cd}
10	C, F, 0	10.8^{a}	10.8^{cdef}	11.4^{a}	18.9^{ab}	7.8^{abc}	13.6^{a}	11.7^{bcd}	5.6^{b}	11.8^{cd}
11	C, F, 90	13.3^{a}	12.3^{f}	10.8^{a}	19.7^{abc}	8.6^{bcd}	14.3^{a}	11.9^{bcde}	8.1^{c}	13.7^{de}
12	C, F, 180	11.6^{a}	11.4^{def}	10.7^{a}	17.3^{a}	9.1^{cd}	14.2^{a}	12.1^{bcdef}	9.8^{d}	14.7^{e}

K – conventional; C – CULTAN; P – Perseus; F – Felina; 0, 90, 180 – dosages of N/ha; A – sowing 2007; B – sowing 2008; 1 – harvest 2008, 2 – harvest 2009

the increasing amount of ammonium nitrate. The decrease of clover ratio in the mixture is assumed to be a result of a great competition of grass components for uptake of applied nitrogen. Thomet et al. (2007) found that the total annual yield of grass-clover mixture dry matter under good moisture conditions and the dosage of nitrogen of 150 kg/ha is not related to the distribution of nitrogen dosages during the vegetation. However, the system of divided nitrogen application enabled to shift up to 10% of the total dry matter yield from the spring to the autumn period.

Table 5 shows a trend of decreasing clover abundance in the mixture with increasing dosage of fertilizer applied conventionally. At the CULTAN system, no difference was observed between the control treatment and the treatment with 90 kg N/ha. However at the rate of 180 kg N/ha applied according to the CULTAN, a decrease of the clover ratio in the mixture was also observed. This trend was observed at all experimental sites. Ledgard (2001) reported that the ratio of clover and the grass-clover mixture has a major influence on accessibility of soil nitrogen, as clover is more

Table 5. Average clover abundance in three harvests (%)

No.	Treatment	Červený Újezd			Jevíčko			Troubsko		
		A1	A2	B2	A1	A2	B2	A1	A2	B2
1	K, P, 0	7^{a}	42^{abc}	52^{ab}	39^{ab}	25^{a}	52^{abc}	18^{a}	32^{a}	34^{a}
2	K, P, 90	1^{a}	11^{a}	45^{ab}	22^{a}	15^{a}	45^{abc}	20^{a}	26^{a}	30^{a}
3	K, P, 180	2^{a}	20^{ab}	40^{a}	25^{ab}	15^{a}	40^{ab}	21^{a}	25^{a}	28^{a}
4	K, F, 0	27^{ab}	67^{c}	82^{b}	79^{c}	24^{a}	82^{c}	17^{a}	41^{a}	47^{a}
5	K, F, 90	37^{b}	57^{abc}	72^{ab}	73^{c}	17^{a}	72^{abc}	18^{a}	36^{a}	42^{a}
6	K, F, 180	24^{ab}	40^{abc}	81^{b}	71^{c}	11^{a}	81^{c}	18^{a}	37^{a}	45^{a}
7	C, P , 0	7^{a}	43^{abc}	50^{ab}	42^{b}	35^{a}	50^{abc}	21^{a}	35^{a}	35^{a}
8	C, P, 90	4^{a}	32^{abc}	50^{ab}	30^{ab}	31^{a}	50^{abc}	21^{a}	34^{a}	35^{a}
9	C, P, 180	4^{a}	17^{ab}	38^{a}	26^{ab}	31^{a}	38^{a}	25^{a}	31^{a}	31^{a}
10	C, F, 0	44^{b}	63^{bc}	83^{b}	80^{c}	21^{a}	83^{c}	19^{a}	41^{a}	48^{a}
11	C, F, 90	48^{b}	68^{c}	79^{b}	75^{c}	20^{a}	79^{bc}	20^{a}	40^{a}	46^{a}
12	C, F, 180	38^{b}	52^{abc}	75^{ab}	77^{c}	23^{a}	75^{abc}	12^{a}	37^{a}	41^{a}

A – sowing 2007; B – sowing 2008; 1 – harvest 2008; 2 – harvest 2009

Table 6. Average N content of grass-clover mixture in three harvests (%)

No.	Treatment	Červený Újezd			Jevíčko			Troubsko		
		A1	A2	B2	A1	A2	B2	A1	A2	B2
1	K, P, 0	1.62[ab]	1.99[a]	2.39[a]	2.28[a]	2.24[a]	2.67[a]	2.04[a]	1.88[a]	1.68[a]
2	K, P, 90	1.64[abc]	1.81[a]	2.57[a]	2.20[a]	2.24[a]	2.44[a]	2.10[a]	1.76[a]	1.82[a]
3	K, P, 180	1.81[abc]	1.93[a]	2.54[a]	2.34[a]	2.44[a]	2.54[a]	2.13[a]	1.91[a]	1.98[a]
4	K, F, 0	1.92[abc]	2.35[a]	2.97[a]	2.69[a]	2.26[a]	2.91[a]	2.11[a]	1.75[a]	1.70[a]
5	K, F, 90	2.27[c]	2.35[a]	2.95[a]	2.68[a]	2.30[a]	2.83[a]	2.31[a]	1.69[a]	1.82[a]
6	K, F, 180	2.07[abc]	2.09[a]	2.76[a]	2.57[a]	2.38[a]	2.60[a]	2.36[a]	1.90[a]	2.10[a]
7	C, P, 0	1.54[a]	2.12[a]	2.43[a]	2.38[a]	2.35[a]	2.55[a]	2.00[a]	1.92[a]	1.73[a]
8	C, P, 90	1.78[abc]	2.02[a]	2.36[a]	2.30[a]	2.22[a]	2.52[a]	2.05[a]	1.84[a]	1.79[a]
9	C, P, 180	1.56[ab]	2.00[a]	2.39[a]	2.27[a]	2.38[a]	2.49[a]	2.12[a]	1.91[a]	1.94[a]
10	C, F, 0	2.15[abc]	2.40[a]	2.67[a]	2.52[a]	2.25[a]	2.73[a]	2.20[a]	1.78[a]	2.00[a]
11	C, F, 90	2.20[bc]	2.38[a]	2.85[a]	2.64[a]	2.23[a]	2.94[a]	2.20[a]	1.77[a]	1.92[a]
12	C, F, 180	2.13[abc]	2.26[a]	2.69[a]	2.64[a]	2.30[a]	2.95[a]	2.24[a]	1.85[a]	2.00[a]

A – sowing 2007; B – sowing 2008; 1 – harvest 2008, 2 – harvest 2009

able to compete at stands with low soil nitrogen. In contrast, on stands rich in nitrogen, grass components are dominant. Davidson and Robson (1986) explain it partly as a higher effectiveness of nitrogen uptake from soil compared to combined uptake from soil and fixation. In our experiment, the clover abundance in mixtures was higher in the CULTAN treatments than at conventionally fertilized plots. In both fertilization systems, the mixtures containing the Felina hybrid had higher clover abundance, compared to combination with the Perseus hybrid. This trend was more significant in the first harvest year of both sowing cycles. It was due to faster Perseus seeds emergence compared to Felina seeds that led to higher competition of Perseus hybrid with the clover. The worst and delayed emergence of clover seeds was observed in the first sowing cycle at the Červený Újezd site. Combination of previous facts caused extremely low clover abundance in Perseus treatments in the first sowing cycle at this site. There was no interannual decrease in the clover ratio in mixture. Annicchiarico and Tomasoni (2010) succeeded to increase the clover ratio in mixture by increasing the number of inter-rows with grass components.

N content in grass-clover mixtures (Table 6) was balanced in both systems of fertilization, which is in compliance with the results of Neuberg et al. (2010); in our experiment, the average N content in plants was 2.22% for both methods. Nitrogen fertilization did not increase N content, which confirmed the results of Lynch et al. (2004); it applies to both sowing cycles and all sites. High N content at non-fertilized plots is a result of a higher ratio of clover in the mixture (Figure 1). In comparison of Perseus and Felina hybrids, the

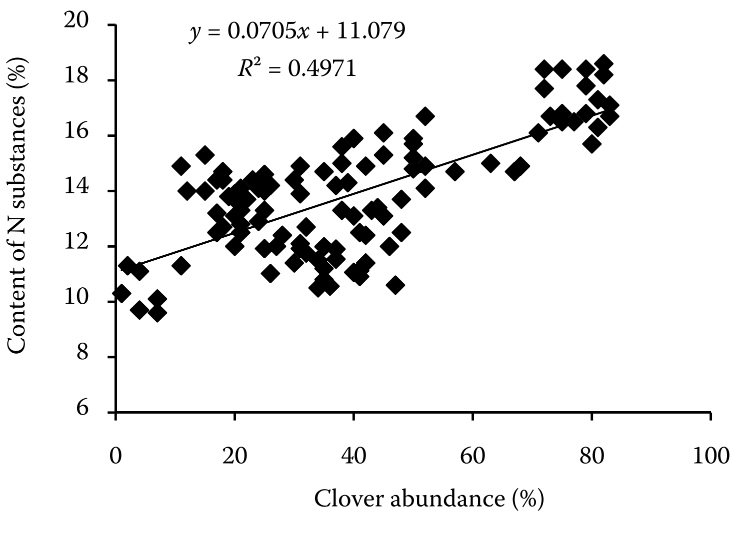

Figure 1. Relation between the N content of the mixture and clover abundance

Table 7. Total N uptake by grass-clover mixture in three harvests (kg N/ha)

No.	Treatment	Červený Újezd			Jevíčko			Troubsko		
		A1	A2	B2	A1	A2	B2	A1	A2	B2
1	K, P, 0	180[ab]	111[a]	274[ab]	498[cd]	133[a]	324[ab]	162[a]	52[a]	128[a]
2	K, P, 90	179[ab]	143[ab]	291[bc]	441[a]	171[b]	300[a]	242[d]	92[c]	172[b]
3	K, P, 180	209[bc]	158[b]	312[cd]	545[e]	219[d]	360[bcd]	262[ef]	123[e]	218[d]
4	K, F, 0	205[bc]	213[c]	378[e]	525[d]	183[bc]	368[cd]	221[c]	92[c]	177[b]
5	K, F, 90	265[ef]	253[d]	346[de]	556[e]	213[d]	393[de]	295[g]	129[f]	215[cd]
6	K, F, 180	241[de]	247[cd]	335[d]	503[cd]	251[e]	392[de]	308[h]	191[i]	295[g]
7	C, P, 0	167[a]	141[ab]	245[ae]	492[cd]	159[b]	324[ab]	183[b]	62[b]	144[a]
8	C, P, 90	191[ab]	174[b]	256[a]	489[bcd]	180[bc]	353[bcd]	224[c]	99[d]	201[c]
9	C, P, 180	171[a]	175[b]	255[a]	505[cd]	198[cd]	346[bc]	258[e]	117[e]	221[d]
10	C, F, 0	226[cd]	258[d]	317[cd]	477[abc]	179[bc]	374[cd]	246[d]	98[cd]	238[e]
11	C, F, 90	283[f]	293[e]	312[cd]	519[de]	198[cd]	425[e]	263[ef]	145[g]	261[f]
12	C, F, 180	244[de]	257[de]	295[bc]	452[ab]	214[d]	418[e]	270[f]	181[h]	292[g]

A – sowing 2007; B – sowing 2008; 1 – harvest 2008; 2 – harvest 2009

higher N content in plants was observed in grass-clover mixtures with the Felina hybrid; yet, at the Troubsko site, no such difference was observed. Average N content in mixtures with the Perseus hybrid was 2.1%, compared to 2.3% in mixtures with the Felina hybrid; average N content in our experiment was 2.22%, but this parameter was to a great extent dependent on site climatic conditions. Mela (2003) determined a linear regression of N content in relation to cumulative temperature sum. According to Knezevic et al. (2009), a possibility to increase the N content in grass-clover mixtures may be a harvest in the earlier growth stages of grasses.

Total N uptake by the upper biomass from three harvests is shown in Table 7. Felina treatments always achieved higher N uptake as compared to Perseus treatments. This was due to higher clover ratio in Felina treatments, because the higher clover ratio caused slightly higher dry matter yield and slightly higher N content of Felina treatments. The N uptake decreased in all treatments in the second harvest year of the first sowing cycle, due to the dry matter yield depression. Increasing dosage of fertilizer resulted in increased N uptake in all cases at the Troubsko site and in Perseus treatments after conventional surface fertilizer at the other sites. In other cases, increase of fertilizer dosage from 90 to 180 kg N/ha had no effect on the N uptake. N uptake was similar in both fertilization systems.

Neither the influence of the fertilizer dosage nor of the system of application on the fibre content of the plants was observed in this experiment. However, at the Červený Újezd and Jevíčko sites slightly higher fibre contents were observed in mixtures with the Perseus hybrid (25.0%) compared to Felina (24.3%). At the Troubsko site, an opposite trend was observed. The experiment revealed a negative relationship between the fibre content and N content, which is confirmed by the results of Skuodiene et al. (2005). A slightly higher fibre content in mixture with the Perseus hybrid may be also influenced by a lower ratio of clover in the stands sown with this mixture, as the legumes have a lower fibre content than grasses (Fulkerson et al. 2007).

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Received on July 13, 2010

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