

## EFFECT OF ORGANIC FERTILIZERS ON BOTANICAL COMPOSITION OF GRASSLAND, HERBAGE YIELD AND QUALITY

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The aim of this study was to assess the effects of organic fertilizers (cow manure + dung water; cattle slurry) applied in different annual doses of nitrogen (54, 84 and 120 kg/ha) by different intensities of grassland utilization (extensive – two cuts per year, medium intensive – three cuts per year, intensive – four cuts per year) on percentage of plant functional groups, dry matter yield, and forage quality. The study was performed on small-plot trial over 7 years on moderately moist grassland in the Czech Republic. The proportion of legumes was significantly higher in the treatments fertilized with cow manure + dung-water combined with medium intensive utilization and intensive utilization (10.2% and 10.3%, respectively). Fertilization significantly increased dry matter yields by 51.9% (cow manure + dung water) and 56% (cattle

slurry) compared with unfertilized controls (4.81 t/ha). Grasslands fertilized with cattle slurry showed significantly higher concentration of crude protein (142.9 g/kg) compared with unfertilized (126.4 g/kg). Extensive grassland utilization significantly affected the increase of crude fibre concentration (up to 282.1 g/kg), and decrease of the energy value (up to 4.68 MJ g/kg of NEL). Organic matter digestibility was also negatively influenced by extensive grassland utilization (61.0%, 65.42% and 67.44% for the extensive, medium intensive and intensive utilization, respectively). Our findings suggested that medium intensive and intensive grassland utilization by the organic fertilization, which corresponded to annual doses of nitrogen of 84 and 120 kg/ha were the most suitable from the viewpoint of animal nutrition.

Key words: dry matter production, herbage quality, permanent grassland, manure, cattle slurry, fertilization

Permanent grasslands are an important part of the landscape in all European countries. In the Czech Republic, permanent grasslands cover a relatively large area of 992 thousand ha, which represents 23.47% of total agricultural land (Czech Statistical Office 2013). They formed and evolved under the influence of abiotic and biotic ecological factors of environment, plus the overwhelming influence of anthropogenic factor. Their importance consists in the ability to conserve biodiversity, reduce environmental pollution, including nitrogen oxide and sulphur in the air and prevent soil erosion. They are also an important source of forage for a large group

of ruminants, but only if improvement measures are provided, along with a rational use (Czembor 2013).

As Müller *et al.* (2005) emphasized, grassland yield and quality is necessary to assess in relation to changes in botanical composition. The plant composition and species richness of permanent grasslands are determined by management practices and site characteristics such as topography, water and nutrient availability, and light conditions (Sebastiá 2004; Tziialla *et al.* 2006; Wellstein *et al.* 2007). As a result, high variability is seen in floristic composition of the vegetation and hence the productivity and quality of forage pro-

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duced from these categories of agricultural land (Criste *et al.* 2013).

The productive potential and quality of permanent grasslands could be increased by different fertilization regimes and types of mineral/organic fertilizers. A typical characteristic of fertilizers is that they affect (directly and indirectly) the growth and development of plants (Pozdíšek *et al.* 2008). Freely available chemical fertilizers over the past half-century gave rise to a period in which efficient nutrient recycling was not prioritized. However, the emphasis on organic fertilizers as a nutrient resource has been re-established following recent increases in fertilizer prices and the increased focus on manure management within European Union (EU) and national environmental policies (Lalor *et al.* 2012).

Organic agriculture relies on ecosystem management and ecological processes rather than on the external flow of agricultural inputs (Foissy *et al.* 2013). Synthetic inputs are replaced with site-specific management practices to balance input and output nutrients to ensure short-term productivity and long-term sustainability. Hence, organic fertilizers are the irreplaceable foundation for rational agriculture. If applied rationally to grasslands, they can entirely replace mineral fertilizers. In addition, organic fertilizers support soil fertility and have other positive effects (Samuil *et al.* 2009). Cattle slurry, in particular, is a commonly used fertilizer in many countries, and its effect on grassland has been studied (e.g. Liu *et al.* 2010; Lalor *et al.* 2012; Duffková & Libichová 2013).

In the Czech Republic, the systematic utilization of organic fertilizers in permanent grasslands is not common, because of their preferred application in intensive arable crops; however, their importance progressively develops mainly in connection with the development of the organic sector, which induces a spatial decoupling of livestock and crop production. Existing methodological recommendations for the application of organic fertilizers in the Czech Republic do not take into account many important criteria such as type of fertilizer and its recommended dose in relation to type of the grassland, altitude or time of application. Long-term experience from other countries (particularly Austria) is used in this issue. Experimental research is therefore necessary to elucidate this knowledge for Czech local conditions.

The objective of this study was to evaluate how are the botanical composition and the forage yield and quality of permanent grassland influenced by different applications of organic fertilizers (cow manure + dung water; cattle slurry) on the basis of the experimental research conducted in the Czech Republic.

## MATERIAL AND METHODS

### *Study site*

A long-term small plot experiment (one plot size: 12.5 m<sup>2</sup>) in completely randomized blocks with four replicates was investigated during 2005–2011 on permanent grassland in the locality of Rapotín (50°00'32"N and 17°00'83"E). The experimental site is situated at 390 m above sea level on the east decline (with 5.1–6.2° declination) in a moderately warm region without temperature extremes (Quitt 1971). Average annual temperature is 7.7°C and annual precipitation 693 mm. Further meteorological data are given in Table 1. The soil is sandy-loam, *Haplic Cambisol* with horizons Am–Bv–Bv/Cc–Cc (classification system according to IUSS Working Group WRB, 2006). Table 2 shows agro-chemical parameters of soil horizons determined in spring 2005. The vegetation of the experimental pasture was classified as *Cynosurion* with some elements of *Arrhenatherion* (Moravec *et al.* 1995). Before the experiment setup, the grassland had been used for cattle grazing for over 30 years.

### *Treatments*

Two types of organic fertilizers were applied during 2005–2011: (M) combination of cow manure + dung water and (S) cattle slurry. Organic fertilizers were used in annual doses of nitrogen: 54, 84 and 120 kg/ha, which approximately corresponded to 0.9 LU/ha (LU = livestock unit), 1.4 and 2.0 LU/ha. The first 50% dose of the cattle slurry (diluted with water in a ratio 1:3) was applied early in spring and the second 50% after the first cut. Cow manure was applied in autumn, dung water after the first cut. These organic fertilizers originated from the stables of VÚCHS Rapotín Ltd. (cow manure and dung water) and from the Dairy farm in České Petrovice (cattle slurry). The fertilizers were analysed for the

content of nutrients before their application, which was conducted annually during 2005–2011.

The plots were cut 2–4 times per year depending on the given dose of fertilizer. Unfertilized plots (F-0) with three types of utilization were also observed as the control treatments: two- (extensive), three- (medium intensive) and four- (intensive) cuts

per year. Treatments of the fertilization and cutting regime are given in Table 3.

*Experimental measurements*

The proportion of three functional groups (grasses, legumes and forbs) was visually estimated directly in percentages in each plot in May (before the

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Meteorological data of the experimental site

Year	Months												Average		Deviation <sup>1</sup>
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual	Vegetation period	
	Daily average temperature [°C]														
2005	-1.3	-4.5	-0.7	8.9	12.7	15.6	18.3	15.7	13.4	4.9	3.1	-1.7	7.03	14.10	-0.17
2006	-8.4	-2.6	-1.8	9.3	11.2	15.8	19.3	14.2	12.6	7.8	4.9	1.6	6.99	13.73	-0.21
2007	2.4	1.8	3.3	6.9	12.9	16.8	17.0	15.8	9.5	6.3	1.0	-2.0	7.64	13.15	0.44
2008	0.3	0.5	1.4	6.5	11.7	16.1	16.7	15.0	10.5	7.0	4.7	0.5	7.58	12.75	0.38
2009	-5.3	-1.5	2.3	8.5	11.4	14.2	16.8	16.0	12.6	6.1	4.0	-1.4	6.98	13.25	-0.23
2010	-6.2	-2.6	0.9	6.2	11.4	16.3	18.9	16.0	10.1	4.4	5.4	-5.8	6.25	13.15	-0.95
2011	-2.9	-3.8	3.0	10.5	12.8	17.5	17.0	18.1	14.2	7.8	2.3	1.1	8.13	15.02	0.93

  

Year	Months												Total		Deviation <sup>1</sup>
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual	Vegetation period	
	Precipitation [mm]														
2005	90.0	45.0	27.5	23.5	76.0	50.0	78.0	69.0	19.0	56.0	120.0	74.6	728.6	315.5	35.6
2006	36.1	63.7	62.7	62.2	84.7	89.2	41.3	125.3	22.9	31.3	73.7	32.5	725.6	425.6	32.6
2007	85.6	47.2	40.1	4.0	66.4	61.5	86.6	68.3	67.9	35.9	67.0	39.9	670.4	354.7	-22.6
2008	69.4	14.3	90.9	34.2	71.1	92.9	96.4	78.3	18.6	31.0	44.3	19.8	661.2	391.5	-31.8
2009	37.3	56.3	70.3	16.2	66.9	88.7	120.6	35.1	10.6	78.1	41.2	68.1	689.4	338.1	-3.6
2010	62.6	45.3	28.2	25.8	176.6	68.5	135.1	128.9	93.1	5.5	73.9	54.7	898.2	628.0	205.2
2011	39.8	7.7	25.0	29.5	52.0	94.0	154.0	98.1	36.1	37.2	2.0	84.0	659.4	463.7	-33.6

<sup>1</sup>Deviation from long-term annual average [1961–1990]

T a b l e 2

Soil agro-chemical parameters of soil horizons determined in spring 2005

Horizon <sup>1</sup>	pH <sub>KCl</sub>	C <sub>ox</sub> [%]	Ratio (C : N)	N <sub>tot</sub> [g/kg]	P [mg/kg]	K [mg/kg]	Ca [mg/kg]	Mg [mg/kg]
Am	4.63	1.34	10.0	1.34	53	109	1799	124
Bv	4.60	0.73	9.5	0.77	78	62	1442	97
Bv/Cc	4.41	0.33	8.4	0.39	27	53	1753	131
Cc	4.44	0.19	10.5	0.18	29	45	1875	166

C<sub>ox</sub> – oxidizable organic carbon; N<sub>tot</sub> – total nitrogen in soil

<sup>1</sup>Classification system of soil horizons according to IUSS Working Group WRB (2006)

T a b l e 3

Description of treatments with different grassland managements

Treatment	Fertilization	Annual dose of nitrogen [kg/ha]	Application	First cut	Second cut	Third cut	Fourth cut
F-0-ext	Nil-fertilization	0	–	June 15	Sept. 30	–	–
F-0-med. int.	Nil-fertilization	0	–	May 30	July 30	Sept. 30	–
F-0-int.	Nil-fertilization	0	–	May 15	June 30	Aug. 15	Sept. 15
M-0.9-ext.	Cow manure + dung water	54	Cow manure – in autumn; dung water – after the 1 <sup>st</sup> cut	June 15	Sept. 30	–	–
M-1.4-med. int.	Cow manure + dung water	84	Cow manure – in autumn; dung water – after the 1 <sup>st</sup> cut	May 30	July 30	Sept. 30	–
M-2.0-int.	Cow manure + dung water	120	Cow manure – in autumn; dung water – after the 1 <sup>st</sup> cut	May 15	June 30	Aug. 15	Sept. 15
S-0.9-ext.	Cattle slurry (diluted with water 1:3)	54	50% of dose – in spring, 50% of dose – after the 1 <sup>st</sup> cut	June 15	Sept. 30	–	–
S-1.4-med. int.	Cattle slurry (diluted with water 1:3)	84	50% of dose – in spring, 50% of dose – after the 1 <sup>st</sup> cut	May 30	July 30	Sept. 30	–
S-2.0-int.	Cattle slurry (diluted with water 1:3)	120	50% of dose – in spring, 50% of dose – after the 1 <sup>st</sup> cut	May 15	June 30	Aug. 15	Sept. 15

first harvest) each year of the study, whereas cover estimates ranged from 0.5% to 100%.

Dry matter (DM) annual yield and quality of grasslands were also measured. In each plot, the sward was mown by machine leaving a stubble height of approximately 5 cm. The harvested biomass was immediately weighed and the percentage of DM determined in the laboratory after 48 h of drying at 65°C. It was then expressed as DM yield in t/ha. Nutrients in samples collected during the vegetation seasons 2005–2009 depending on the term of the cut were analysed according to Czech State Standard 46 7092 (Testing methods of feeding-stuffs). Crude protein (CP) was determined by the Kjeldahl procedure using the device Kjeltac Auto Distillation 2200 and ether extract (EE) by the Soxhlet method. The Fibertec System 2023 FiberCap (FOSS Comp.) was used

to analyse crude fibre (CF). Ash (A) content was measured gravimetrically by igniting samples in a muffle furnace at 450°C for 4 h. The concentration of nitrogen-free extract (NFE) was calculated according to the formula:

$$\text{NFE [g/kg DM]} = 1000 - (\text{CP} + \text{EE} + \text{CF} + \text{A}).$$
 The *in-vitro* organic matter digestibility (OMD) was determined by the Tilley & Terry method (1963) modified according to Resch (1991). The energy value (ME – metabolizable energy; NEL – net energy of lactation) was predicted by means of the equations officially used in the Czech Republic and the Slovak Republic, which corresponds with the system INRA (Jarrige *et al.* 1989).

Analysis of variance (ANOVA) and LSD test ( $P < 0.05$ ) was used for the statistical data analysis by means of the software Statistica v. 10.

RESULTS AND DISCUSSION

*Functional groups*

Data about the proportion of functional groups (grasses, legumes, forbs) as percentage are given in Table 4. It was found that grassland management had an influence on the botanical composition with

some differences being significant ( $P < 0.05$ ). The mean proportion of grasses was positively influenced by cattle slurry application (53.4%), compared with fertilization with cow manure + dung water (48.8%), and to control (42.9%), whereas this percentage significantly increased with decreasing cutting frequency and decreasing doses of fertiliz-

T a b l e 4

Botanical composition (%) over the treatments and years

Functional group	Grasses	Legumes	Forbs
Treatments <sup>1</sup>			
F-0-ext.	49.0 <sup>ac</sup>	1.9	49.1
F-0-med. int.	40.5 <sup>b</sup>	4.5	54.9
F-0-int.	39.2 <sup>b</sup>	4.2	55.9
M-0.9-ext.	59.6 <sup>c</sup>	4.1	36.2
M-1.4-med. int.	45.3 <sup>ad</sup>	10.2	44.5
M-2.0-int.	41.8 <sup>bd</sup>	10.3	47.5
S-0.9-ext.	63.6 <sup>c</sup>	1.7	34.5
S-1.4-med. int.	53.2 <sup>c</sup>	3.9	42.6
S-2.0-int.	43.5 <sup>bd</sup>	4.7	50.1
Means of fertilization types			
(F) nil-fertilization	42.9 <sup>a</sup>	3.5 <sup>a</sup>	53.3 <sup>a</sup>
(M) cow manure + dung water	48.8 <sup>b</sup>	8.2 <sup>b</sup>	42.7 <sup>b</sup>
(S) cattle slurry	53.4 <sup>c</sup>	3.4 <sup>a</sup>	42.7 <sup>b</sup>
Means of intensities of utilization			
(ext.) extensive	57.4 <sup>a</sup>	2.6 <sup>a</sup>	39.9 <sup>a</sup>
(med. int.) medium intensive	46.3 <sup>b</sup>	6.2 <sup>b</sup>	47.3 <sup>b</sup>
(int.) intensive	41.5 <sup>c</sup>	6.4 <sup>b</sup>	51.5 <sup>c</sup>
Means of years			
2005	51.1 <sup>a</sup>	14.7 <sup>a</sup>	34.1 <sup>ab</sup>
2006	52.1 <sup>ab</sup>	11.7 <sup>b</sup>	35.7 <sup>a</sup>
2007	68.8 <sup>c</sup>	0.8 <sup>c</sup>	29.8 <sup>b</sup>
2008	56.2 <sup>b</sup>	0.5 <sup>c</sup>	43.3 <sup>c</sup>
2009	32.8 <sup>d</sup>	0.6 <sup>c</sup>	66.4 <sup>d</sup>
2010	37.0 <sup>e</sup>	1.5 <sup>c</sup>	60.8 <sup>e</sup>
2011	40.6 <sup>e</sup>	5.6 <sup>d</sup>	53.6 <sup>f</sup>
Factor	<i>P</i> value		
Year	<0.001	<0.001	<0.001
Fertilization	<0.001	<0.001	<0.001
Intensity of utilization	<0.001	<0.001	<0.001
Fertilization × intensity of utilization	0.011	0.073	0.076

The values in the same column with different superscript letters are significantly different at  $P < 0.05$  level for each variable

<sup>1</sup>See Table 3



ers. In contrast, the unfertilized treatments showed significantly higher proportion of forbs (53.3%) in the mean of years, and this proportion significantly increased with increasing intensity of utilization (from 39.9% in the extensive utilization to 51.5% in the intensive utilization). The mean proportion of legumes was significantly higher in the treatments with application of cow manure + dung–water combined with medium intensive utilization and intensive grassland utilization (10.2% and 10.3%, respectively).

As for the results of other studies, Čunderlík *et al.* (2012) showed the effects of organic fertilizers on the botanical composition at permanent grassland and they found that sward proportion of grasses increased with increasing rates of fertilizers. At manure application, the proportion of grasses was dominant in the first year, but decreased later and the forbs and legumes proportions were increasing, which corresponds to our results, as well.

In contrast to our results, Seihyung *et al.* (2006) found that the percentage of legumes in the grassland increased with increased application rate of swine slurry. The reason of this finding could be that pig slurry supplied more phosphorus than did cow slurry (Christie 1987) used in our study. On average, the pig slurry is twice as rich in the content of phosphorous as the cattle slurry (Regulation No. 274/1998 of the Ministry of Agriculture), whereas phosphorus has a positive influence on legumes dominance in grasslands as it was proved by studies such as Skládanka and Hrabě (2008), Veselá *et al.* (2009) or Raus *et al.* (2012).

The long-term application of organic fertilizers resulted in the significant changes in the sward botanical composition also in the studies of Liu *et al.* (2010). Elsaesser *et al.* (2008) investigated the effects of different fertilization systems (organic and mineral fertilizers) on permanent grasslands and found that fertilization with slurry increased the proportions of grasses, whereas farmyard manure increased forbs; the proportion of legumes was increased by PK and by fertilization with slurry with lime. Søegaard *et al.* (2008) referred that the cattle slurry application decreased the proportion of legume species, increased the grass proportion, but hardly affected the non-leguminous herbs in grasslands.

Some differences in the scientific findings could be caused by different soil-climatic conditions and types of grasslands of the experimental study sites and also by some differences in treatments (e.g. doses of manures, ways of their application, etc.).

#### *Dry matter yield*

Data about DM yields of grasslands by different management, as well as the significance of differences, are given in Table 5. Significantly higher DM yields were found for the fertilized treatments, whereas cow manure + dung water increased DM yield by 51.9% and cattle slurry by 56.0%, compared with the unfertilized control (4.81 t/ha). Differences between the used types of organic fertilizers (cattle slurry vs. cow manure) on DM yield were not significant within our study; however, we found a slightly higher DM yield in the slurry treatment (7.51 t/ha) than in the treatment fertilized with the cow manure + dung water (7.31 t/ha).

An effect of intensity of utilization was also found within our study. In the grasslands utilized extensively (two cuts per year) fertilized with the lowest doses of organic manures, there was found the significantly lower mean DM yield (6.14 t/ha) in contrast to grasslands with medium intensive and intensive utilization (6.82 and 6.68 t/ha, for three and four cuts per year, respectively). This finding is not in agreement with Gruber *et al.* (2011) or Parsons *et al.* (2011), who documented that the treatment of four cuts is unfavourable in terms of production, which was explained in relation to phenological development of the swards.

Based on our results, we can state that DM yields were significantly influenced by the level of organic fertilization, which is in accordance with Jong-Won & Jacob (1997), Seihyung *et al.* (2006), Samuil *et al.* (2009) or Müller *et al.* (2011). Komárek *et al.* (2005) who investigated the same experiment at two different localities in the Czech Republic (Jevíčko, Vysoké nad Jizerou) also arrived at similar results. In contrast, Szewczyk *et al.* (2004) found that the addition of organic manure produced no increment in DM yields, which was probably caused by significant changes in the botanical composition.

In our study, DM yields were further influenced by the year. As apparent from Table 1, April 2007 was characterized by an exceptionally low level of

precipitation (4.0 mm), which negatively affected spring growth of the plants in our study site. It subsequently resulted not only in the significant decrease of the mean total seasonal DM yield (4.15 t/ha) but also in the significant decrease of the forage quality (113.8 g/kg DM of CP; 4.68 MJ/kg DM of NEL) in this year 2007 (Tables 5 and 6). From Table 4 it is evident that the exceptionally dry year 2007 had an influence also on the botanical composition

(significantly higher proposition of grasses to the detriment of forbs), which is in line with Valkó *et al.* (2012). As published by Kramberger *et al.* (2014), the amount of annual herbage DM yield correlated highly with the amount of precipitation during the March–August period. This finding is mentioned and described in relation to climatic conditions also in the paper of Criste *et al.* (2013).

T a b l e 5

Dry matter yields of grasslands at different levels of intensity of utilization and fertilization with organic fertilizers

Treatments <sup>1</sup>	Dry matter yield [t/ha]
F-0-ext.	4.90 <sup>a</sup>
F-0-med. int.	4.93 <sup>a</sup>
F-0-int.	4.62 <sup>a</sup>
M-0.9-ext.	6.75 <sup>b</sup>
M-1.4-med. int.	7.65 <sup>c</sup>
M-2.0-int.	7.54 <sup>c</sup>
S-0.9-ext.	6.78 <sup>b</sup>
S-1.4-med. int.	7.88 <sup>c</sup>
S-2.0-int.	7.88 <sup>c</sup>
Means of fertilization types	
(F) Nil-fertilization	4.81 <sup>a</sup>
(M) Cow manure + dung water	7.31 <sup>b</sup>
(S) Cattle slurry	7.51 <sup>b</sup>
Means of intensities of utilization	
(ext.) Extensive	6.14 <sup>a</sup>
(med. int.) Medium intensive	6.82 <sup>b</sup>
(int.) Intensive	6.68 <sup>b</sup>
Means of years	
2005	6.14 <sup>a</sup>
2006	6.06 <sup>a</sup>
2007	4.15 <sup>b</sup>
2008	7.42 <sup>c</sup>
2009	6.75 <sup>d</sup>
2010	7.37 <sup>c</sup>
2011	7.95 <sup>e</sup>
Factor	<i>P</i> value
Year	<0.001
Fertilization	<0.001
Intensity of utilization	<0.001
Fertilization × intensity of utilization	<0.001

The values with different superscript letters are significantly different at  $P < 0.05$  level

<sup>1</sup>See Table 3

*Forage quality*

Regarding the effect of different grassland management on the forage quality (Table 6), we have found in our study the significant increase of the concentration of CP with increasing intensity of grassland utilization up to 152.1 g/kg DM in the

mean of years, whereas grasslands fertilized with cattle slurry showed the significantly higher concentration of CP (142.9 g/kg DM) compared with unfertilized (126.4 g/kg DM). Further, it was found that the extensive grassland utilization significantly affected the increase of the concentration of CF (up

T a b l e 6

Forage quality (content of nutrients in dry matter) of grasslands at different levels of intensity of utilization and fertilization with organic fertilizers

Traits	CP [g/kg]	CF [g/kg]	EE [g/kg]	A [g/kg]	NFE [g/kg]	OMD [%]	ME [MJ/kg]	NEL [MJ/kg]
Treatments <sup>1</sup>								
F-0-ext.	108.9	274.9	27.8	99.8	488.5	61.37	8.34	4.78
F-0-med. int.	120.8	258.1	29.8	111.5	479.8	65.07	8.84	5.13
F-0-int.	139.4	237.9	32.2	110.6	480.0	66.83	9.17	5.36
M-0.9-ext.	112.6	284.1	25.7	96.8	480.9	60.65	8.11	4.62
M-1.4-med. int.	130.4	249.6	31.4	106.3	482.3	65.47	8.81	5.11
M-2.0-int.	154.9	225.9	34.8	112.0	472.3	67.73	9.12	5.32
S-0.9-ext.	116.2	287.1	25.8	99.3	471.6	61.05	8.16	4.66
S-1.4-med. int.	135.2	253.8	31.5	108.2	471.3	65.71	8.83	5.12
S-2.0-int.	162.0	236.2	33.7	120.1	448.0	67.76	9.02	5.26
Means of fertilization types								
(F) Nil-fertilization	126.4 <sup>a</sup>	257.0	30.4	107.3	482.8	64.42	8.78	5.09
(M) Cow manure + dung water	137.4 <sup>b</sup>	253.2	31.7	105.0	478.5	64.62	8.68	5.01
(S) Cattle slurry	142.9 <sup>b</sup>	259.0	31.2	109.2	463.6	64.84	8.67	5.01
Means of intensities of utilization								
(ext.) Extensive	112.6 <sup>a</sup>	282.1 <sup>a</sup>	26.4 <sup>a</sup>	98.6	480.3	61.02 <sup>a</sup>	8.21 <sup>a</sup>	4.68 <sup>a</sup>
(med. int.) Medium intensive	128.8 <sup>b</sup>	253.8 <sup>b</sup>	30.9 <sup>b</sup>	108.7	477.8	65.42 <sup>b</sup>	8.83 <sup>b</sup>	5.11 <sup>b</sup>
(int.) Intensive	152.1 <sup>c</sup>	233.3 <sup>c</sup>	33.6 <sup>c</sup>	114.2	466.7	67.44 <sup>b</sup>	9.11 <sup>b</sup>	5.31 <sup>b</sup>
Means of years								
2005	138.4 <sup>ab</sup>	256.8	28.4	100.5	480.5	66.4 <sup>a</sup>	8.99 <sup>a</sup>	5.24 <sup>a</sup>
2006	143.8 <sup>ab</sup>	251.6	33.9	102.4	474.7	66.4 <sup>a</sup>	9.01 <sup>a</sup>	5.25 <sup>a</sup>
2007	113.8 <sup>c</sup>	269.3	31.2	112.4	478.8	62.5 <sup>b</sup>	8.20 <sup>b</sup>	4.68 <sup>b</sup>
2008	132.3 <sup>a</sup>	262.4	32.2	118.8	459.1	65.5 <sup>ab</sup>	8.96 <sup>a</sup>	5.21 <sup>a</sup>
2009	149.4 <sup>b</sup>	242.0	29.8	101.7	481.8	65.9 <sup>a</sup>	8.89 <sup>a</sup>	5.16 <sup>a</sup>
Factor	<i>P</i> value							
Year	<0.001	0.283	0.052	0.170	0.359	0.038	0.009	0.009
Fertilization	0.024	0.840	0.874	0.833	0.122	0.952	0.860	0.859
Intensity of utilization	<0.001	<0.001	<0.001	0.089	0.283	<0.001	0.001	0.001
Fertilization × intensity of utilization	0.822	0.931	0.702	0.938	0.844	0.992	0.995	0.995

The values in the same column with different superscript letters are significantly different at  $P < 0.05$  level for each variable

<sup>1</sup>See Table 3

Abbreviations: CP – crude protein; CF – crude fibre; EE – ether extract; A – ash; NFE – nitrogen free extract; OMD – organic matter digestibility; ME – metabolizable energy; NEL – net energy of lactation



to 282.1 g/kg DM), the decrease of the concentration of EE (up to 26.4 g/kg DM) and the decrease of the energy value (up to 8.21 MJ g/kg DM of ME; or up to 4.68 MJ g/kg DM of NEL). The OMD was negatively influenced by the extensive grassland utilization (61.0%, 65.42% and 67.44% for the extensive, medium intensive and intensive grassland utilization, respectively).

These results about the forage quality are in agreement with Szewczyk *et al.* (2004), who found that the fertilized (including organic fertilizers) swards produced 55–60% more protein within their investigations. In the experiment of Vintu *et al.* (2008), the influence of organic fertilization was investigated on the content of CP, crude fibre, phosphorus and raw ash. The applied fertilization systems resulted in an increased fodder yield and CP content, as compared with the unfertilized control, by 14–29% on *Nardus stricta* grassland and by 9–22% on *Agrostis capillaris* + *Festuca rubra* grassland.

Usually, the intensive grassland utilization is connected with the decrease of DM yield by the simultaneous increase of the forage quality (Gruber *et al.* 2011). Within our study, it was found that higher doses of organic fertilizers (especially of cattle slurry) by the increasing grassland utilization had more significant effect than the intensity of utilization. Our finding could be explained by the fact that cattle slurry provided a source of fast-release nitrogen, which could be immediately utilized by the plants for their growth. As Cabrera and Gordillo (1995) referred, slurries typically contain more inorganic than organic N, whereas most of the inorganic N in slurries is present as  $\text{N-NH}_4^+$ .

## CONCLUSIONS

Based on our results, we can conclude that the extensive grassland utilization and the organic fertilizers' application (cattle slurry in particular) significantly affected the increase of the proportion of grasses. The application of cow manure + dung–water together with the intensive and medium intensive grassland utilization positively influenced the proportion of legumes. DM yields were significantly influenced by the year and by the organic fertilization compared with unfertilized controls, whereas the

increasing doses of both types of organic fertilizers significantly increased DM yields, even though the treatments with higher doses of fertilizers were utilized more intensively.

Appropriate grassland management through the number of cuts and fertilization makes it possible to improve botanical composition, and amount and quality of the forage. In our study, cattle slurry application significantly increased the concentration of CP in DM compared with unfertilized control. Extensive grassland utilization significantly affected the increase of the concentration of CF, and the decrease the energy value as well as the OMD.

Our findings suggested that medium intensive (three cuts per year) and intensive grassland utilization (four cuts per year) by the fertilization with doses of organic fertilizers, which corresponded to 84 kg N/ha/year and 120 kg N/ha/year, respectively, were the most suitable management practices from the viewpoint of animal nutrition. There are other relevant environmental factors that could influence these parameters, including climatic conditions, which are different for each locality and vegetation season. For the specific sites, it is necessary to take into account also the possible environmental risks, which could arise from the application of high doses of organic fertilizers (e.g. decrease of species diversity, leaching of nutrients into ground water).

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## REFERENCES

- CABRERA, M.L. – GORDILLO, R.M. 1995. Nitrogen release from land-applied animal manures. In HATCHER, K.J. (Ed.) *Proceedings of the 1995 Georgia Water Resources Conference*, University of Georgia, Athens, pp. 175–179. ISBN 0-935835-04-0.
- CHRISTIE, P. 1987. Long term effects of slurry on grassland. In VAN DER MEER, H.G. (Ed.) – UNWIN, R.J. – VAN DIJK, T.A. – ENNIK, G.C. – MEER, H.G. *Animal manure on grassland and fodder crops. Fertilizer or waste?* Dodrecht : Martinus Nijhoff Publishers, pp. 301–304. ISBN 90-247-3568-8.
- CRISTE, D. – MIHAI, G. – SIMA, N. – MEDREA, I. – BOTIS, A. – SIMA, R. 2013. Studies regarding the

- influence of organic and mineral fertilization on the permanent grassland from Maramures depression – Petrova. In *Bulletin UASVM Animal Science and Biotechnologies*, vol. 70, no. 2, pp. 240–243.
- Czech Statistical Office, 2013. *Land use in regions* [electronic resource]. Data released on 05.08.2013. Available at: <http://www.czso.cz/eng/redakce.nsf/i/home>.
- CZEMBOR, E. 2013. Effects of organic fertilization on agronomic traits of perennial grass species recommended for conventional and low input farming. In *Biuletyn Instytutu Hodowli i Aklimatyzacji Roślin*, no. 270, pp. 85–107.
- ČUNDERLÍK, J. – KIZEKOVÁ, M. 2012. The application of mineral and organic fertilizers and its impact on the quality and production of herbage at semi-natural grassland. In KOVÁČIKOVÁ, Z. (Ed.) – VARGOVÁ, V. – JENDRIŠÁKOVÁ, S. *Ecosystems and their functions*. Banská Bystrica, Plant Production Research Centre Piešťany, pp. 108–109. ISBN 978-80-89417-40-7.
- DUFFKOVÁ, R. – LIBICHOVÁ, H. 2013. Effects of cattle slurry application on plant species composition of moderately moist *Arrhenatherion* grassland. In *Plant, Soil and Environment*, vol. 59, no. 11, pp. 485–491.
- ELSAESSER, M. – KUNZ, H.G. – BRIEMLE, G. 2008. Strategy of organic fertilizer use on permanent grassland – results of a 22-year-old experiment on meadow and mowing-pasture. In *Grassland Science in Europe*, vol. 13, pp. 580–582.
- FOISSY, D. – VIAN, J.F. – DAVID, C. 2013. Managing nutrient in organic farming system: reliance on livestock production for nutrient management of arable farmland. In *Organic Agriculture*, vol. 3, no. 3–4, pp. 183–199. DOI: 10.1007/s13165-014-0060-8.
- GRUBER, L. – SCHAUER, A. – HÄUSLER, J. – URDL, M. – ADELWÖHRER, A. – SÜDEKUM, K.H. 2011. Influence of growth stage of permanent grassland on dry matter yield, nutritive value, feed intake and milk yield of dairy cows during the whole period of vegetation. In *Grassland Science in Europe*, vol. 16, pp. 136–138.
- IUSS Working Group WRB (2006): *World reference base for soil resources 2006*. World Soil Resources Reports, No. 103, FAO : Rome. 145 pp. ISBN 92-5-105511-4.
- JARRIGE, R. 1989. *Ruminant nutrition*. Paris-London-Rome : John Libbey Eurotext. 389 pp. ISBN 0-86196-247-8.
- JONG-WON, R. – JACOB, H. 1997. The effect of cattle slurry on the forage yield and grassland ecosystem. In *Journal of the Korean Society of Grassland Science*, vol. 17, no. 1, pp. 35–42.
- KOMÁREK, P. – KOHOUTEK, A. – FIALA, J. – ODSTRČILOVÁ, V. – NERUŠIL, P. 2005. Produkce a kvalita píče travních porostů v závislosti na zatížení skotem a frekvenci sečení [Production and quality of grassland forage in dependence on cattle load and cutting frequency]. In KOHOUTEK, A. (Ed.) – POZDÍŠEK, J. *Kvalita píče z travních porostů*, VÚRV Praha-Ruzyně, pp. 175–182. ISBN 80-86555-75-5.
- KRAMBERGER, B. – GSELMAN, A. – PODVRŠNIK, M. – LEŠNIK, M. – ŠKORJANC, D. 2014. Effects of low precipitation periods on the herbage yield of mesic semi-natural grasslands under different cutting regimes. In *Zemdirbyste-Agriculture*, vol. 101, no. 1, pp. 11–18. DOI: 10.13080/z-a.2014.101.002.
- LALOR, S.T.J. – HOEKSTRA, N.J. – MURPHY, P.N.C. – RICHARDS, K.G. – LANIGAN, G.J. 2012. *Practical advice for slurry application strategies for grassland systems*. International Fertilizer Society, 34 pp. ISBN 978-0853103493.
- LIU, W. – ZHU, Y.G. – CHRISTIE, P. – LAIDLAW, A.S. 2010. Botanical composition, production and nutrient status of an originally *Lolium perenne*-dominant cut grass sward receiving long-term manure applications. In *Plant and Soil*, vol. 326, no. 1–2, pp. 355–367. DOI: 10.1007/s11104-009-0016-z.
- MORAVEC, J. – BALÁTOVÁ-TULÁČKOVÁ, E. – BLAŽKOVÁ, D. – HADAČ, E. – HEJNÝ, S. – HUSÁK, Š. – JENÍK, J. – KOLBEK, J. – KRAHULEC, F. – KROPÁČ, Z. – NEUHÄUSL, R. – RYBNÍČEK, K. – ŘEHOŘEK, V. – VICHEREK, J. 1995. *Rostlinná společenstva České republiky a jejich ohrožení [Red list of plant communities of the Czech Republic and their endangerment]*. 2<sup>nd</sup>Ed, Litoměřice, 206 pp. ISBN 80-9000827-6-9.
- MÜLLER, M. – HRABĚ, F. – CHROUST, J. 2005. Změny v kvalitě píče pastevního porostu v průběhu pastevního období [Changes in forage quality of pasture sward in course of grazing period]. In KOHOUTEK, A. (Ed.) – POZDÍŠEK, J. *Kvalita píče z travních porostů*, VÚRV Praha-Ruzyně, pp. 106–111. ISBN 80-86555-75-5.
- MÜLLER, C. – LAUGHLIN, R. J. – CHRISTIE, P. – WATSON, C. J. 2011. Effects of repeated fertilizer and cattle slurry applications over 38 years on N dynamics in a temperate grassland soil. In *Soil Biology & Biochemistry*, vol. 43, no. 6, pp. 1362–1371. DOI: 10.1016/j.soilbio.2011.03.014.
- PARSONS, A. – ROWARTH, J. – THORNLEY, J. – NEWTON, P. – LEMAIRE, G. – HODGSON, J. – CHABBI, A. 2011. Primary production of grasslands, herbage accumulation and use, and impacts of climate change. In *Grassland productivity and ecosystem services*, CABI, Wallingford, pp. 3–18. ISBN 9781845938093. DOI: 10.1079/9781845938093.0003.
- POZDÍŠEK, J. – ŠTÝBNAROVÁ, M. – KOHOUTEK, A. – SVOZILOVÁ, M. – RŽONCA, J. 2008. Forage quality by animal fertilizer applications and by different grassland management. In *Grassland Science in Europe*, vol. 13, pp. 498–500.
- QUITT, E. 1971. *Klimatické oblasti Československa [Climatic regions of Czechoslovakia]*. Brno : Geografický ústav ČSAV, 73 pp.
- RAUS, J. – KNOT, P. – HRABĚ, F. 2012. Effect of fertilization and harvest frequency on floristic composition and yields of meadow stand. In *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 60, pp. 181–186. DOI: 10.11118/actaun201260050181.

- Regulation No. 274/1998 of the Ministry of Agriculture on the storage and manner of use of fertilizers.*
- RESCH, R. 1991. *In vitro* – Verdaulichkeitsuntersuchung nach Tilley und Terry, 1963 [*In vitro* – determination of organic matter digestibility by Tilley and Terry, 1963.] In *Bericht über die Tagung der ALVA Fachgruppe Versuchswesen*. Innsbruck 22–23. Mai 1991.
- SAMUIL, C. – VINTU, V. – IACOB, T. – SAGHIN, G.H. – TROFIN, A. 2009. Management of permanent grasslands in North-Eastern Romania. In *Grassland Science in Europe*, vol. 14, pp. 234–237.
- SEBASTIÁ, M.T. 2004. Role of topography and soils in grassland structuring at the landscape and community scales. In *Basic and Applied Ecology*, vol. 5, no. 4, pp. 331–346. DOI: 10.1016/j.baae.2003.10.001.
- SEIHYUNG, Y. – YOUNGCHUL, L. – JONGGEUN, K. – EUISOO, J. 2006. The study on the application level of swine slurry in grassland pasture. In *Journal of the Korean Society of Grassland Science*, vol. 26, no. 2, pp. 63–68.
- SKLÁDANKA, J. – HRABĚ, F. 2008: Effect of fertilization and cutting frequency on botanical composition, diversity and grassland quality. In *Agriculture (Poľnohospodárstvo)*, vol. 54, no. 1, pp. 1–8.
- SØEGAARD, K. – ERIKSEN, J. – ASKEGAARD, M. 2008. Herbs in grasslands – effect of slurry and grazing/cutting on species composition. In *Grassland Science in Europe*, vol. 13, pp. 200–202.
- SZEWCZYK, W. – KASPERCZYK, M. – KACORZYK, P. 2004. Role of farmyard manure on upland meadows. In *Grassland Science in Europe*, vol. 9, pp. 714–716.
- TILLEY, J.M.A – TERRY, R.A. 1963. A two stage technique for the *in vitro* digestion of forage crops. In *Grass and Forage Science*, vol. 18, no. 2, pp. 104–111. DOI: 10.1111/j.1365-2494.1963.tb00335.x.
- TZIALLA, C.E. – VERESOGLOU, D.S. – PAPAKOSTA, D. – MAMOLOS, A.P. 2006. Changes in soil characteristics and plant species composition along a moisture gradient in a Mediterranean pasture. In *Journal of Environmental Management*, vol. 80, no. 1, pp. 90–98. DOI: 10.1016/j.jenvman.2005.08.017.
- VALKÓ, O. – TÖRÖK, P. – MATUS, G. – TOTHMERESZ, B. 2012. Is regular mowing the most appropriate and cost-effective management maintaining diversity and biomass of target forbs in mountain hay meadows? In *Flora*, vol. 207, no. 4, pp. 303–309. DOI: 10.1016/j.flora.2012.02.003.
- VESELÁ, M. – MRKVIČKA, J. – HREVIŠOVÁ, Z. 2009. Species development of meadow stand related to yield. In *Grassland Science in Europe*, vol. 14, pp. 265–268.
- VINTU, V. – SAMUIL, C. – TROFIN, A. – POPOVICI, I.C. 2008. The influence of organic and mineral fertilizers on fodder quality in NE Romania. In *Grassland Science in Europe*, vol. 13, pp. 637–639.
- WELLSTEIN, C. – OTTE, A. – WALDHARDT, R. 2007. Impact of site and management on the diversity of central European mesic grassland. In *Agriculture, Ecosystems and Environment*, vol. 122, no. 2, pp. 203–210. DOI: 10.1016/j.agee.2006.12.033.

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