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NUTRITIVE VALUE AND MYCOTOXIN CONTAMINATION OF HERBAGE IN MOUNTAIN LOCALITY EXPOSED TO RENEWED CATTLE GRAZING

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Abstract

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The aim of the study was to estimate the forage quality and mycotoxin contamination in the locality of Švýcárna (1304 m a.s.l.), where the cattle grazing after the long-term management cessation was introduced on the pasture area of 3.6 ha in 2012. The pasture was divided into two grazing plots: P1 (Nar) (dominance of *Nardus stricta*) and P2 (Des) (dominance of *Deschampsia cespitosa*). Samples of forage were taken in June, July, August and September 2014 and 2015 from five permanent plots situated in different places of locality. Samples were analysed on the content of basic nutrients and macro- and micro- elements. Further, the content of deoxynivalenol (DON), zearalenone (ZEA) and T-2/HT-2 toxin was determined using ELISA method. From June to September the mean content of crude fibre significantly ($P < 0.05$) increased (from 243.9 g.kg^{-1} DM to 331.7 g.kg^{-1} DM, respectively) and the content of crude protein significantly ($P < 0.05$) decreased (from 166.9 g.kg^{-1} DM to 89.2 g.kg^{-1} DM, respectively). Mean concentration of DON in P1 (Nar) was numerically higher than in P2 (Des), being 715 and 620.63 µg.kg^{-1}, respectively ($P < 0.05$). Mean concentration of T-2/HT-2 toxin was 44.88 and 55.04 µg.kg^{-1} in P1 (Nar) and P2 (Des), respectively. The content of ZEA in P1 (Nar) of 54.65 µg.kg^{-1} was lower than in P2 (Des) being 78.72 µg.kg^{-1} ($P < 0.05$).

Keywords: suckler cows, pasture, forage, *Fusarium* mycotoxins, nature conservation

INTRODUCTION

Biodiversity of Central European semi-natural upland grasslands is steadily declining, due to either abandonment or management intensification (Merunková and Chytrý, 2012). The continuation of traditional ways of grassland management that would best preserve biodiversity is often not compatible with the requirements of intensive livestock production. Therefore, grassland is at risk of being abandoned from agricultural use (Isselstein *et al.*, 2005). The impacts of land abandonment and subsequent woody colonization on vegetation composition and plant traits were studied e.g. by Prévosto *et al.* (2011).

Grazing is, therefore, considered as a suitable tool to maintain biodiversity of grassland as documented by Metera *et al.* (2010). Czech authors, which referred on this topic, were e.g. Mládek *et al.* (2006) or Veselý *et al.* (2012). Due to above mentioned facts, grazing is renewed in the Czech Republic in protected areas where it was provably pursued in the past (e.g. Bílé Karpaty, Krkonoše, Beskydy). In 2012, it was restored also in Hrubý Jeseník Mts. (Praděd National Natural Reserve) in the Švýcárna lodge surroundings. The main aim of the cattle grazing reintroduction was the reinforcement of non-production functions of alpine grasslands (floristic diversity, anti-erosion function, landscape forming, attractiveness for the tourism etc.). However, it can not be dismissed also the production function of alpine grassland, which is closely connected with the proper cattle nutrition, and thus, with the animals' health and their condition.

Good health and production of farm animals depends on the feed quality. Green forage on pasture is exposed to many risk factors, which can have negative influences. One of the most important risks is the feed contamination by mycotoxins (Stryk and Křížová, 2013; Horký *et al.*, 2014). Mycotoxins are the toxic secondary metabolites produced by organisms of the fungi kingdom, commonly known as moulds (Křížová, 2009). Among these the moulds of the genus *Fusarium* are the most important under the central European agricultural conditions (Seeling and Dänicke, 2005). The most important *Fusarium* toxins from the point of view of animal health and productivity are deoxynivalenol (DON) and zearalenone (ZEA) (Fink-Gremmels, 2008). As Cheeke (1995) referred knowledge of various aspects of these toxins and toxicoses are necessary for optimal management and utilization of forage grasses.

The aim of the study was to estimate the forage quality (including the content of *Fusarium* mycotoxins DON, T-2/HT-2 toxin and ZEA) in the locality of Švýcárna (1304 m a.s.l., the Praděd National Natural Reserve), where the cattle grazing after the long-term management cessation was introduced in 2012.

MATERIAL AND METHODS

Soil-climatic Conditions and Site Characteristics

Our research was conducted in the mountain locality in surroundings of the Švýcárna lodge situated in the Hrubý Jeseník Mts. (Praděd National Natural Reserve; 1 304 m a.s.l.). In Praděd (1 491 m a.s.l., cca 2.5 km distant from the Švýcárna lodge) there is average annual temperature 0.9 °C and annual precipitation 1 231 mm. Experimental area is in the ownership of the Czech Republic with the right to manage for the Forests of the Czech Republic, state-owned enterprise. The grazed area is categorised as the open country according to the valid Forest Management Plan. Based on the declaration of The Regional Authority of the Olomouc Region (about the temporary revocation of the land devoted to fulfil the forest services) there was renewed the suckler cows (the Highland breed) grazing in 2012.

The rotational grazing system (three grazing cycles per year) was conducted in the locality, which was divided by the road into two grazing plots differing in dominant grass species. The plot **P1 (Nar)** with dominance of *Nardus stricta* was situated above the lodge while the plot **P2 (Des)** with dominance of *Deschampsia cespitosa* was situated below the lodge. The whole plot area was 3.6 ha. The stocking rate was up to 1 livestock unit per ha and year.

The amount of soil nutrients available to plants during the growing season was estimated in Mehlich III solution. On average, there was found the extremely acid soil reaction (pH = 3.7) and simultaneously the low content of calcium (232.8 $mg.kg^{-1}$), phosphorus (10.0 $mg.kg^{-1}$) and magnesium (69.8 $mg.kg^{-1}$) in the soil.

The vegetation in the lodge surroundings was characterized as mosaic of the close alpine grasslands, the subalpine *Vaccinium* vegetation, the subalpine tall grasslands and the subalpine tall-forb vegetation (Chytrý *et al.,* 2010), with the dominant species *Nardus stricta, Deschampsia cespitosa*, *Avenella flexuosa, Bistorta officinalis, Calamagrostis villosa, Festuca supine* and *Luzula sylvatica*.

Parameters Evaluated

Five permanent plots (one plot area: 5 m×5 m) were established in 2013 in different places of the grazed area (two in the sublocality P1 and three in the sublocality P2) to monitor changes in floristic composition. From the plots there were conducted samplings of the forage in four dates (June, July, August, and September) during 2014–2015. The samples were dried in 65 °C and milled through 1 mm screen. By the method of Weende analysis there was determined the content of crude protein (CP), ether extract (EE), crude fibre (CF) and ash (A) in $g.kg^{-1}$ DM (DM = dry matter). The content of nitrogen free extract (NFE) was calculated according to the equation:

$$NFE = 1000 - (CP + EE + CF + A).$$

Using the instrumental equipment of the accredited laboratory (atomic absorption spectrometry – AAS) there was further estimated the content of macroelements (Ca, Mg, P, K) in $g.kg^{-1}$ DM and some microelements (Zn, Pb, Cd) in $mg.kg^{-1}$ DM. A competitive enzyme-linked immunosorbent assay (ELISA) was used to determine the presence of fusarium mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and T-2/HT-2 toxin in samples using the NEO 9303 Stat – Fax Reader 4700 apparatus and commercially available quantitative ELISA assay kits Veratox (both Neogen Corp., Lansing, MI, USA) according to the manufacturer's instructions.

The three-factorial analysis of variance (with sublocality as factor A, date of harvest as factor B and year as factor C) and the LSD test ($P < 0.05$) were used for statistical computations.

RESULTS

Forage Nutritive Value

Tab. I shows results of estimated qualitative parameters, including the statistics. The swards situated above the lodge (P1 Nar) differed significantly ($P < 0.05$) from the swards situated below the lodge (P2 Des) only in the content of ash, I: *Effect of sublocality (S) and harvest date (HD) on nutrient contents (in dry matter) in the forage in 2014–2015*

Factor	Ash [g.kg^{-1}DM]	CF [g.kg^{-1}DM]	CP [g.kg^{-1}DM]	EE [g.kg^{-1}DM]	NFE [g.kg^{-1}DM]	P [g.kg^{-1}DM]	Mg [g.kg^{-1}DM]	Ca [g.kg^{-1}DM]	K [g.kg^{-1}DM]	Zn [mg.kg^{-1}DM]
Sublocality (S)										
P1 (Nar)	44.72^{a}	290.3^{a}	113.1^{a}	29.67^{a}	522.2^{a}	1.39^{a}	1.00^{a}	1.71^{a}	9.22^{a}	40.01^{a}
P2 (Des)	65.10^{b}	285.2^{a}	120.7^{b}	31.36^{a}	497.7^{b}	1.34^{a}	1.19^{b}	3.77^{b}	13.76^{b}	43.74^{a}
Significance	<0.001	0.465	0.038	0.731	0.004	0.530	0.043	< 0.001	< 0.001	0.459
Harvest date (HD)										
June	56.83^{a}	243.9^{a}	166.9^{a}	56.28^{a}	476.1^{a}	1.73^{a}	1.09^{a}	2.18^{a}	15.07^{a}	47.12^{a}
July	48.43^{a}	263.8^{a}	116.8^{b}	31.31^{b}	539.7^{b}	1.39^{b}	1.13^{a}	3.24^{a}	12.79^{b}	38.79^{a}
August	52.59^{a}	309.5^{b}	97.6^{c}	17.76^{c}	522.5^{b}	1.16^{b}	1.15^{a}	3.23^{a}	11.06^{b}	39.81^{a}
September	69.96^{b}	331.7^{c}	89.2^{c}	17.39^{c}	491.7^{a}	1.16^{b}	1.08^{a}	3.14^{a}	8.87^{c}	43.26^{a}
Significance	0.018	<0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.882	0.380	< 0.001	0.626
Year (Y)										
2014	58.24^{a}	295.1^{a}	119.4^{a}	39.31^{a}	487.9^{a}	1.54^{a}	1.08^{a}	2.87^{a}	12.29^{a}	44.05^{a}
2015	55.66^{a}	279.4^{b}	115.8^{a}	22.06^{b}	527.1^{b}	1.19^{b}	1.14^{a}	3.03^{a}	11.59^{a}	40.44^{a}
Significance	0.704	0.030	0.246	0.001	< 0.001	< 0.001	0.419	0.697	0.312	0.533
S x HD	0.691	0.498	0.267	0.302	0.476	0.987	0.875	0.655	0.734	0.949
S x Y	0.300	0.768	0.462	0.855	0.900	0.756	0.670	0.902	0.764	0.629
HD x Y	0.704	0.013	0.001	<0.001	0.068	0.147	0.814	0.698	0.356	0.197
S x HD x Y	0.946	0.654	0.189	0.282	0.276	0.640	0.710	0.918	0.412	0.685

P1 (Nar) grazing plot with dominance of *Nardus stricta* situated above the lodge
P2 (Des) grazing plot with dominance of *Deschampsia cespitosa* situated below the lodge
CF = crude fibre; CP = crude protein; EE = ether extract; NFE = nitrogen free extract
[a, b, c] Mean values in the same column with different superscripts differ significantly ($P < 0.05$)

CP, NFE, Mg, Ca and K. As for the content of crude fibre (CF), it was on the level of 290.3 g.kg^{-1} DM and 285.2 g.kg^{-1} DM for the swards above (P1 Nar) and below (P2 Des) the lodge, respectively. Concerning the concentration of crude protein, it was on the level of 113.1 g.kg^{-1} DM and 120.7 g.kg^{-1} DM for the swards above (P1 Nar) and below (P2 Des) the lodge, respectively.

The significant factor influencing the mean nutritive value of the swards in the locality was the date of harvest. In the course of the growing season the concentration of crude fibre significantly increased from 243.9 g.kg^{-1} DM in June to 331.7 g.kg^{-1} DM in September ($P < 0.05$). The concentration of crude protein simultaneously decreased from 166.9 g.kg^{-1} DM in June to 89.2 g.kg^{-1} in September ($P < 0.05$).

The estimation of the potential risk elements in our study brought the findings that the mean concentration of zinc was 40.01 mg.kg^{-1} DM (P1 Nar) and 43.74 mg.kg^{-1} DM (P2 Des), and the concentration of lead and cadmium was under the detection limit of instrumental technique AAS-ETA.

Mycotoxin Contamination of the Pasture Forage

All samples analyzed in our study were positive for DON. Although non-significant ($P > 0.05$) mean concentration of DON in P1 (Nar) was higher than in P2 (Des) as documented in Tab. II. Based on the month of sampling concentration of DON in P1 (Nar) increased from 622.5 μg.kg^{-1} in June to its maximum value of 990 μg.kg^{-1} in July and then gradually decreased to 577.5 μg.kg^{-1} in September (Fig. 1). On the other hand, in P2 (Des) concentration of DON increased gradually from 522.5 μg.kg^{-1} in June to 697.5 μg.kg^{-1} in September (Fig. 1).

All analysed samples were positive for T-2/HT-2 toxin. Mean concentration of T-2/HT-2 toxin was 44.88 and 55.04 μg.kg^{-1} in P1 (Nar) and P2 (Des), respectively (Tab. II). The effect of the term of sampling on T-2/HT-2 toxin content that is presented in Fig. 2 is not clear. While concentration of T-2/HT-2 toxin in P1 (Nar) was similar during June, July and September, being 46.71, 48.64 and 46.25 μg.kg^{-1}, respectively with decrease in August; content of T-2/HT-2 in P2 (Des) decreased in July (46.59 μg.kg^{-1}) and increased up to 65.65 μg.kg^{-1} in September.

In our study, the concentration of ZEA (Fig. 3) in both groups was almost the same at the beginning of the study in June, being 63.83 and 65.18 μg.kg^{-1} in P1 (Nar) and P2 (Des), respectively. The highest values were found in July, and then from July till September, content of ZEA gradually decreased in both groups.

Based on the evaluation of phytosociological relevés the vegetation in the lodge was characterized as a mosaic of the close alpine grasslands, the subalpine *Vaccinium* vegetation, the subalpine tall grasslands and the subalpine tall-forb vegetation composed of 110 vascular plant species. In connection with these facts it is necessary to note that animals (Highland cattle) used for the purpose of renewing grazing in that area consumed almost whole available vegetation in spite of its low forage quality. From this point of view additional information concerning the mycotoxin contamination of individual selected plants could be useful. As presented in Tab. II, all selected plants were positive for DON, T-2/HT-2 toxin and ZEA. The highest concentration of DON exceeding 2000 μg.kg^{-1} was found in *Bistorta officinalis* and in *Luzula sylvatica* in both terms of sampling. On the other hand the lowest concentrations of DON were measured in *Festuca supina* and *Calamagrostis villosa*. Concentration of T-2/HT-2 toxin ranged from 44.77 μg.kg^{-1} found in *Festuca supina* to 169.91 μg.kg^{-1} analysed in August sample of *Luzula sylvatica*. The same sample also contained the highest concentration of ZEA. The

II: *Concentration (in dry matter) of deoxynivalenol (DON), T-2/HT-2 toxin and zearalenone (ZEA) in pasture forage from two grazing plots and from selected individual plants*

	DON [μg.kg^{-1}]	T-2/HT-2 toxin [μg.kg^{-1}]	ZEA [μg.kg^{-1}]
Sublocality			
P1 (Nar) – mean	715.00	44.88	54.65
P2 (Des) – mean	620.63	55.04	78.72
Significance	0.397	0.824	0.229
Individual plants			
Avenella flexuosa	1 092.50	92.88	102.00
Bistorta officinalis	2 617.50	56.86	167.56
Calamagrostis villosa	585.00	51.19	139.18
Festuca supina	400.00	44.77	109.88
Luzula sylvatica – sampled in August	2 357.50	169.91	298.50
Luzula sylvatica – sampled in September	2 692.50	67.49	179.23

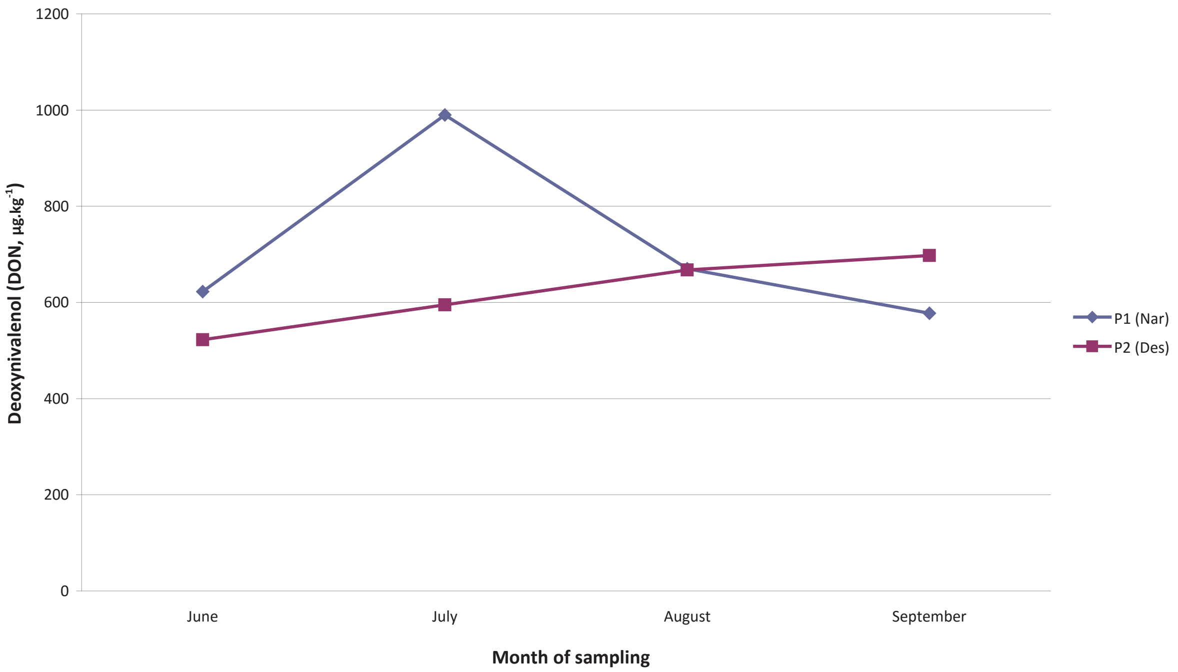
P1 (Nar) grazing plot with dominance of *Nardus stricta* situated above the lodge
P2 (Des) grazing plot with dominance of *Deschampsia cespitosa* situated below the lodge
DON = deoxynivalenol
ZEA = zearalenone

lowest content of ZEA was found in *Avenella flexuosa* and *Festuca supina*. To our knowledge no information is available on the occurrence of mycotoxins in above mentioned plants.

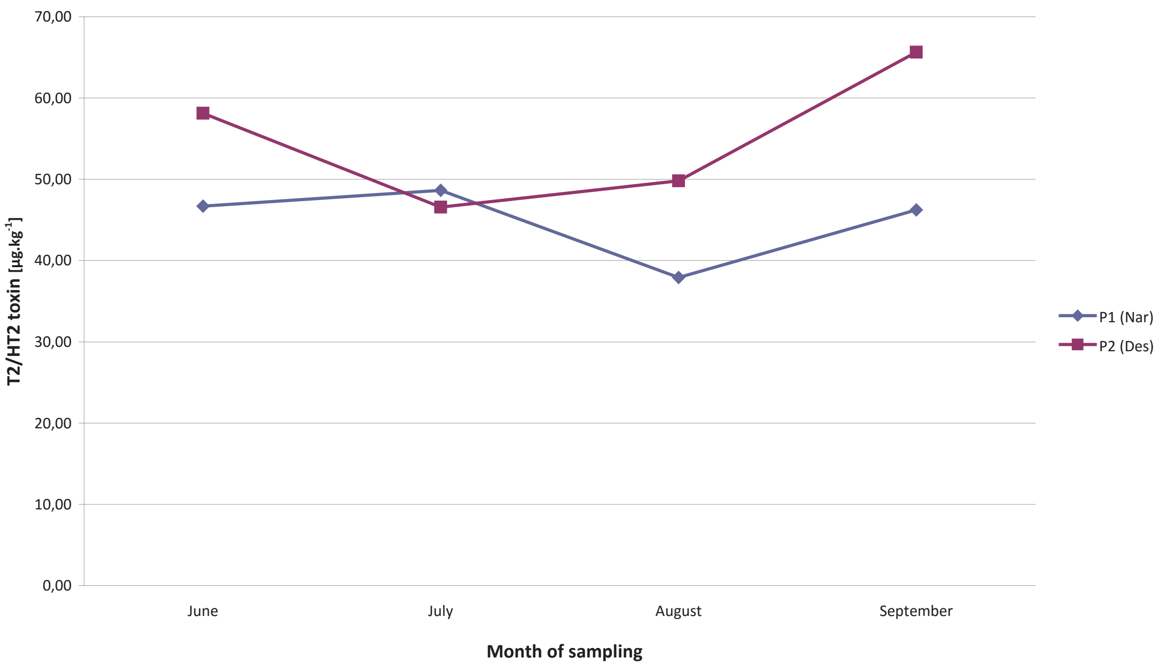
DISCUSSION

Forage Nutritive Value

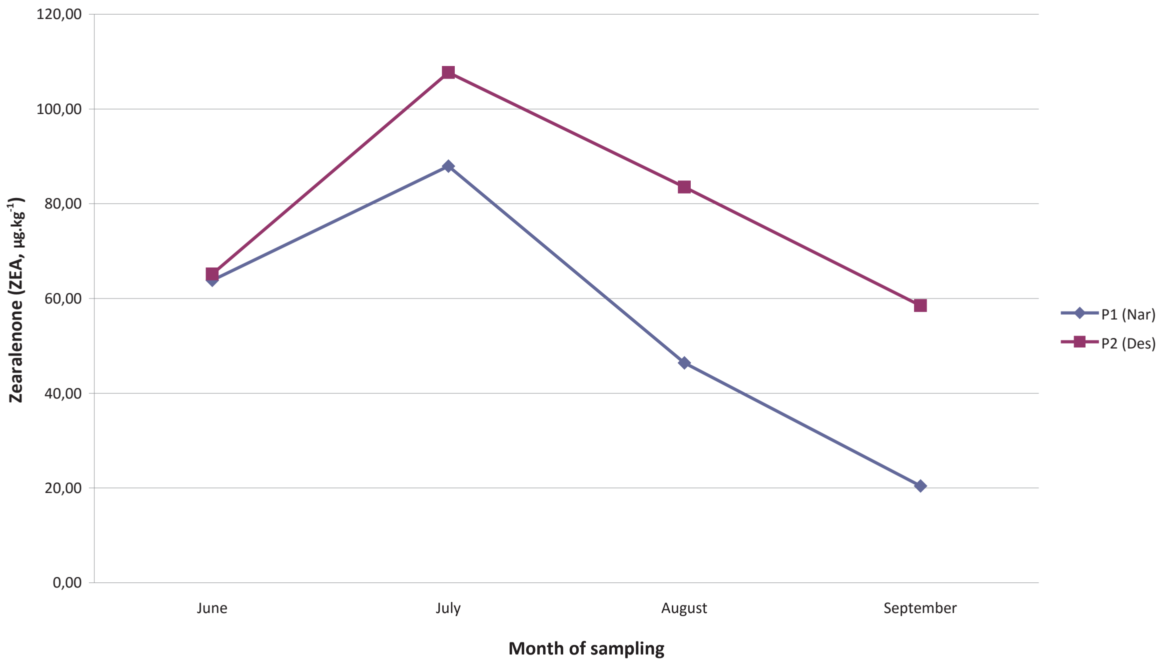
Grasslands that are distinguished by the higher forage quality contain: 150–200 g.kg^{-1} DM of CF and 180–200 g.kg^{-1} DM of CP, at least 3.0 g.kg^{-1} DM of phosphorous, at least 7 g.kg^{-1} DM of calcium, and at least 2 g.kg^{-1} DM of magnesium (Pavlů, 1994; Kováč, 2005; Pavlů *et al.*, 2006). The content of potassium is recommended being not higher than 25 g.kg^{-1} DM due to the possible dietetic problems in animals (Ryant and Skládanka, 2004). Taking into account our results, we can state that the pasture sward in the lodge Švýcárna surroundings was, on average, characterised by the lower nutritive value.



1: *Deoxynivalenol (DON) concentration [µg.kg^{-1}] in pasture forage differing in floristic composition in dependence on the month of sampling*



2: *T2/HT2-toxin concentration [µg.kg^{-1}] in pasture forage differing in floristic composition in dependence on the month of sampling*



3: *Zearalenone concentration [µg.kg^{-1}] in pasture forage differing in floristic composition in dependence on the month of sampling*

As Kobes (2010) mentioned, leaves and stems of *Nardus stricta* contain very high amounts of lignin and silicon. Hence, this species is characterised by the low organic matter digestibility (40–45%) and the low energy value. Owing to the progressive incrustation and lignification of plant tissues during the growing season the voluntary intake of the densely tufted grasses is more difficult in animals. Hejcman *et al.* (2005) documented that *Deschampsia cespitosa* is often passed by animals in later growing phases; however it is consumed without problems in the spring phase. Moreover, this species is characterised by the higher dry matter yield than in case of *Nardus stricta* (cca 1.80 t.ha^{-1}) (Hejcman *et al.*, 2004, 2006). Sipilä (1939) referred that the content of the crude protein in *Deschampsia cespitosa* decreased with the maturation and he recommended using these swards before the phase of flowering.

Cadmium and lead are the elements toxic for animals already in the lower concentrations in diets. Zinc, however, is the element which is tolerable to some extent. According Vrzgula *et al.* (1990) there is desirable concentration of zinc between 40–50 mg.kg^{-1} DM. Zinc can have toxic effects in the concentration that exceed 250 mg.kg^{-1} DM. In our case, from this viewpoint the concentrations of the potential risk elements (Zn, Pb, Cd) did not mean any dietetic risk for animals.

Mycotoxin Contamination of the Pasture Forage

Fungal contaminations of feedstuff cause severe problems for the health (Korosteleva *et al.*, 2009; Döll and Dänicke, 2011) and performance of farm animals (Yiannikouris and Jouany, 2002; Fink-Gremmels, 2008). Besides the grain and compound feedstuffs the occurrence of mycotoxins was also extensively studied in forage crops such as maize and grass silage. Data on mycotoxin occurrence in pastures are less documented. However, recent studies proved that pasture sward can be contaminated with significant amounts of mycotoxins (e.g. Laser *et al.*, 2001; Arslan and Essiz, 2009; Skládanka *et al.*, 2009, 2011; Ramirez *et al.*, 2014; Nichea *et al.*, 2015) suggesting that pasture may have a significant impact on the total intake of mycotoxins (Signorini *et al.*, 2012).

Similar trend in changes in DON concentrations in dependence on sampling terms was mentioned also by Skládanka *et al.* (2011), however, values measured for DON in our study were more than ten times higher than reported by those authors for *Lolium*, *Festulolium* and mixed grass swards.

Our values for T-2/HT-2 toxin are close to lower values reported by Engels and Krämer (1996) who determined wide range of T-2 toxin concentrations (from 40 to 2 780 µg.kg^{-1}) in several varieties of *Lolium* grasses. On the other hand, Ramirez *et al.* (2014) mentioned about 10 times higher concentration of T-2/HT-2 toxins in Argentinean pastures than those measured in our study.

Natural occurrence of ZEA in pasture forage has often been reported in the literature, mainly from New Zealand where it has been identified as an agent causing fertility problems in sheep (Di Menna *et al.*, 1987, 1991; Garthwaite *et al.*, 1994; Towers, 1993; Smith and Morris, 2006) and recently from Australia (Reed and Moore, 2009) or Argentina (Salvat *et al.*, 2013; Ramirez *et al.*, 2014; Nichea *et al.*, 2015). Although ZEA occurrence in pasture forage in Europe can also be expected (Summerell *et al.*, 2010; Szécsi *et al.*, 2013) only few studies are available (Engels and Krämer, 1996; Golinski *et al*., 2003; Skládanka *et al*., 2011). Furthermore, above mentioned studies has been performed on pastures intended for intensive pasture-based systems or on experimental swards composed of high-producing grasses belonging to genus *Lolium, Festuca* or *Poa* and/or their hybrids or mixtures. Studies on extensive mountain pastures are lacking.

Mean concentrations of ZEA determined in our study in P1 (Nar) and P2 (Des) were considerably higher than those reported by Arslan and Essiz (2009) or Golinski *et al*. (2003) but close to that determined in *Festulolium braunii* sward planted in the Czech Republic environment (Skládanka *et al*., 2011). On the other hand, higher values were found by Ramirez *et al*. (2012) in Argentinean pasture forages, by Reed and Moore (2009) in Australian pastures, by Skládanka *et al*. (2011) in mixtures of *Lolium/Festulolium* with *Festuca rubra* or *Poa pratensis,* or by Engels and Krämer (1996) in several *Lolium* varieties. In the latter study authors concluded that differences in infestation with *Fusarium* among varieties of *Lolium perenne* were dependent on location and did not correlate with yield. According to Smith and Morris (2006) there appears to be no close relation of pasture ZEA concentrations with a range of climatic parameters. Furthermore, Skládanka *et al*. (2009) observed significant effect of the species (*Festulolium*, *Dactylis glomerata* and *Arrhenatherum elatius)* on the zearalenone content but this finding was not confirmed in their subsequent study performed on *Lolium perenne*, *Festulolium pabulare*, *Festulolium braunii* and mixtures of these species with *Festuca rubra* and/or P*oa pratensis* (Skládanka *et al*., 2011). Thus, predicting the likely presence or severity of ZEA contamination of pasture sward is difficult.

CONCLUSION

Feed quality is a very important factor determining both animal health and productivity. In our study, the mean content of crude fibre (in mixed samples) significantly increased (from 242.3 $g.kg^{-1}$ DM to 327.2 $g.kg^{-1}$ DM) from June to September, respectively, and the content of crude protein significantly decreased (from 172.3 $g.kg^{-1}$ DM to 98.4 $g.kg^{-1}$ DM) from June to September, respectively. From the viewpoint of the potential risk elements (Zn, Pb, Cd) the concentrations of these elements in the forage did not mean any dietetic risk for animals.

The issue of moulds and, thus, contamination with mycotoxins is very topical, particularly in connection with forages from grass stands used at the end of the growing season. *Fusarium* mycotoxins deoxynivalenol (DON), T-2/HT-2 toxin and zearalenone (ZEA) were present in all samples of pasture forage as well as in individual selected plants. While the effect of the term of sampling on DON and T-2/HT-2 toxin content was not clear, concentration of ZEA in both groups increased from June to July, and then gradually decreased from July till September. The highest mean concentrations were noted for DON, ranging from 620 to 715 $\mu g.kg^{-1}$ in dependence on the locality of sampling, while concentration of DON in some individual plants exceeded 2600 $\mu g.kg^{-1}$ (all on dry matter basis). Mean concentrations of T-2/HT-2 toxin and ZEA were relatively low. However, in extensive grazing systems pasture may represent nearly 100 % of the dry matter intake resulting in a long time exposure of animals to mycotoxins that could be considerable. Thus, not only dry matter yield and nutritional quality but also mycotoxin contamination of the forage should be taken into account when planning a grazing as a suitable tool to preserve the biodiversity of grassland in protected areas.

It is necessary to take into consideration that the average annual dry matter yield in the mountain conditions and by the given floristic composition is only about 2–3 $t.ha^{-1}$. Our findings should be kept in mind for the optimal stocking rate and for usage of suitable additional feeds (mineral salts). Date of the beginning of grazing and its finishing should be chosen with the consideration of climatic conditions in particular years. Data published in this paper are important for the proper pasture management, for adequate cattle nutrition and for the following evaluation of changes in connection with the renewed cattle grazing.

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