

Influence of grass species on the mycotoxins content

Jiri Skladanka¹, Daniela Knotova², Pavel Knot¹, Lucie Hodulikova¹, Jan Nedelnik², Iva Klusonova¹, Monika Novotna¹

¹Mendel University in Brno, Brno, Czech Republic

²Agricultural Research, Ltd., Troubsko, Czech Republic

Corresponding author e-mail: jiri.skladanka@mendelu.cz

Keywords: Deoxynivalenol, Green matter, Silages, Zearalenone

Introduction

Clean and healthy phytomass is a prerequisite for producing high-quality forage. Development of microscopic fungi may lead to the formation of mycotoxins (Opitz von Boberfeld *et al.*, 2006), which are secondary metabolites produced especially by the fungi *Aspergillus*, *Penicillium* and *Fusarium* (Rodrigues and Naehrer, 2012). Mycotoxins are produced due to interactions and reactions of fungi to environmental conditions (Opitz von Boberfeld *et al.*, 2002). Mycotoxins naturally have negative impacts upon livestock, causing alterations in hormonal functions, poor feed utilization, lower rates of body weight gain, and possibly death (Duarte *et al.*, 2013).

Preventing the occurrence of mycotoxins in forage should begin in the field. These include the use of varieties or hybrids that are well adapted to the given growing area and that are resistant to fungal disease. Production and control of mycotoxins in silage are not well understood. General recommendations for limiting their occurrence include minimizing plant disease (Barnes *et al.*, 2007). The aim of the study was to assess the incidence of deoxynivalenol and zearalenone in green matters and silages of perennial grass species, evaluate the difference among grass species and the impact of fertilization and sites on the occurrence of mycotoxins.

Materials and Methods

Experimental sites were at the Agricultural Research Ltd. in Troubsko (49°10'N, 16°29'E, 270 m a.s.l.) and Research Station of Fodder Crops in Vatin, Mendel University in Brno (49°31'N, 15°58'E, 560 m a.s.l.), Czech Republic. The climate at the first station can be characterized by the mean annual precipitation of 543 mm and mean annual temperature of 8.3 °C. The soil type was Luvisol modal. The contents of soil nutrients were 37.3 mg kg⁻¹ P, 152.9 mg kg⁻¹ K, and 6548 mg kg⁻¹ Ca; pH was 7.33. The climate at the second station can be characterized by the mean annual precipitation of 617 mm and mean annual temperature of 6.9 °C. The soil type was Cambisol. The contents of soil nutrients were 89.1 mg kg⁻¹ P, 231.6 mg kg⁻¹ K, and 855 mg kg⁻¹ Ca; pH was 4.76.

First small-plot experiment (A) was established in 2007. The observation years were 2008-2010. Species used were *Lolium perenne* (cv. Kentaur), *Festulolium pabulare* (cv. Felina), *Festulolium braunii* (cv. Perseus). The experimental plots were fertilized with 50 kg ha⁻¹ N in March. Second small-plot experiment (B) was established 2013. The observation year was 2014. Species used were *Dactylis glomerata* (cv. Niva), *Festulolium braunii* (cv. Perseus) and *Lolium perenne* 4n (cv. Kertak). The experimental plots were without fertilization and/or with fertilization (80 kg ha⁻¹ N). Times of cutting were the beginning of June, at the earing stage. The experiments were carried out in triplicate. A split-plot design were used with plots of 1.5 × 10 m. Stubble height was 0.07 m.

Grasses after cut were allowed to wilt and dry for 20 to 30 h after mowing (experiment A), (content of dry matter from 41 to 44 %) and/or immediately after harvest (experiment B), (content of dry matter from 21 to 24 %). The wilted biomass was ensiled in containers with diameter and height 0.15 m and 0.64 m, respectively. Silages were sampled 90 days after closing the containers.

Green forage samples and silages were dried at 60 °C, ground to a particle size of <1 mm, then analyzed for content of the deoxynivalenol (DON) and zearalenone (ZEA) using enzyme-linked immunosorbent assay (ELISA) according to Skladanka *et al.*, (2011). The toxin concentration is expressed in parts per billion (ppb).

The data were processed statistically using STATISTICA.CZ Version 10.0 (ANOVA and Scheffe's method).

Results and Discussion

The experiment A (Table 1) showed similar contents of DON at evaluated grass species (*Lolium perenne*, *Festulolium braunii* and *Festulolium pabulare*). DON content was influenced ($P < 0.05$) by year. ZEA content in green matter was below the detection limit. DON content increased in silages. Similarly in silages was observed incidence ZEA that in samples from field has not been detected.

Follow experiment B (Table 2) showed the difference between the evaluated species. *Festulolium braunii* and *Lolium perenne* had a balanced DON content, as well as in the previous experiment, but significantly higher ($P < 0.05$) DON content was at *Dactylis glomerata*. In comparison with other species is aging faster and may have a higher fiber content. Higher incidence of fungal pathogens may be associated with a higher fiber content. Fertilization did not affect the mycotoxin content, although there is a trend of lower at a dose of 80 kg ha N. Difference ($P < 0.05$) was evaluated between sites. In the silages have not increased the levels of mycotoxins, unlike the experiment A. Silages were prepared from grass biomass immediately after harvest, while in the case of the previous experiment were made from wilted matter. Withering could lead to a rise of mycotoxins.

Table 1: Influence of species and year on the contents (ppb) of deoxynivalenol (DON) and zearalenone (ZEA) in the green matters and silages with wilted

Factor	Green matters		Silages	
	DON	ZEA	DON	ZEA
Species				
<i>Lolium perenne</i>	19.8	<LOQ	132.4	84.2
<i>Festulolium pabulare</i>	19.3	<LOQ	128.7	60.6
<i>Festulolium braunii</i>	20,8	0	130.1	53.6
p	0.7585	0.4444	0.9948	0.4584
Year				
2008	29.4 ^a	0	145.2	68.4
2009	0.07 ^b	0	125.2	81.9
1010	30.5 ^a	<LOQ	120.6	48.1
p	0.0002	0.1111	0.7848	0.4241

Mean values in the same column with different superscripts (^{a,b,c}) are significant at the $p < 0.05$ level

Table 2: Influence of species, fertilization and site on the contents (ppb) of deoxynivalenol (DON) and zearalenone (ZEA) in the green matters and silages without wilted

Factor	Green matter		Silages	
	DON	ZEA	DON	ZEA
Species				
<i>Dactylis glomerata</i>	1432.5 ^a	45.7	460.0 ^a	35.2
<i>Festulolium braunii</i>	262.5 ^b	46.6	317.5 ^{ab}	274.7
<i>Lolium perenne</i>	302.5 ^b	47.9	257.5 ^b	44.7
p	0.0005	0.9300	0.0299	0.4026
Fertilization				
0	796.7	47.6	365.0	191,7
80 N	535.0	45.9	325.0	44.6
p	0.1223	0.7326	0.4399	0.3702
Site				
Troubsko	488.3	29.4 ^a	333.3	44.2
Vatin	843.3	64.1 ^b	356.7	192.1
p	0.0486	0.0001	0.6475	0.3680

Mean values in the same column with different superscripts (^{a,b,c}) are significant at the $p < 0.05$ level

Conclusion

Deoxynivalenol was regularly found in green matters and silages. Zearalenone was not detected in the green matters regularly. Its occurrence was a regular in silages. Silage made from wilted materials had higher mycotoxin contents than the green matter during harvest. Silage made from non-wilted biomass had a content of mycotoxins comparable with the original green matter. The highest DON content was in the biomass *Dactylis glomerata*. Fertilization had not influence on the content of mycotoxins. Site affected the content of mycotoxins in green matter.

References

- Barnes, R. F., J. C. Nelson, K. J. Moore and M. Collins. 2007. *Forages: The Science of Grassland Agriculture, Volume II*. Blackwell Publishing, Ames, IA, USA.
- Duarte, S. C., A. M. Almeida, A. S. Teixeira, A. L. Pereira, A. C. Falcao, A. Pena and C. M. Lino. 2013. Aflatoxin M-1 in marketed milk in Portugal: Assessment of human and animal exposure. *Food Control* 30: 411-417.
- Opitz von Boberfeld, W. and K. Banzhaf. 2006. Yield and forage quality of different x *Festulolium* cultivars in winter. *J. Agron. Crop Sci.* 192: 239-247.
- Rodrigues, I. and K. Naehrer. 2012. Prevalence of mycotoxins in feedstuffs and feed surveyed worldwide in 2009 and 2010. *Phytopathol. Mediterr.* 51: 175-192.
- Skladanka, J., J. Nedelnik, V. Adam, P. Dolezal, H. Moravcova and V. Dohnal. 2011. Forage as a primary source of mycotoxins in animal diets. *Int. J. Environ. Res. Public Health* 8: 37-50.

Acknowledgement

The paper was support from Grant QJ1310100 "Development and optimization methods for the determination of biogenic amines in response to increasing health security of silage" founded by the National Agency for Agricultural Research and under support from Grant IGA TP 3/2015 "Influence of the specie of grass and harvest time on mycotoxin contents and impact on the health of animals".