ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 64 169 Number 5, 2016
http://dx.doi.org/10.11118/actaun201664051529

EFFECT OF SOIL CONTAMINATION OF FODDER AND WILTING ON THE OCCURRENCE OF FUNGI AND MYCOTOXINS IN ALFALFA SILAGES

Lucia Hodulíková[1], Jiří Skládanka[1], Veronika Mlejnková[1], Pavel Knot[1], Iva Klusoňová[1], Pavel Horký[1], Klára Konečná[2], Daniela Knotová[2], Jan Nedělník[2], Petr Sláma[3]

[1] Department of Animal Nutrition and Forage Production, Mendel University in Brno, Zemědělská 1665/1, 613 00, Brno, Czech Republic
[2] Research Institute for Fodder Crops, Ltd. Troubsko, Zahradní 1, 664 41, Troubsko, Czech Republic
[3] Department of Morphology, Physiology and Animal Genetics, Mendel University in Brno, Zemědělská 1665/1, 613 00, Brno, Czech Republic

Abstract

HODULÍKOVÁ LUCIA, SKLÁDANKA JIŘÍ, MLEJNKOVÁ VERONIKA, KNOT PAVEL, KLUSOŇOVÁ IVA, HORKÝ PAVEL, KONEČNÁ KLÁRA, KNOTOVÁ DANIELA, NEDĚLNÍK JAN, SLÁMA PETR. 2016. Effect of Soil Contamination of Fodder and Wilting on the Occurrence of Fungi and Mycotoxins in Alfalfa Silages. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 64(5): 1529–1536.

The aim of the work is to assess the effect of soil contamination of fodder and wilting on the occurrence of fungi in the biomass of alfalfa and subsequently consider the mycotoxin content in alfalfa silage. The alfalfa biomass of Jarka variety, harvested from two experimental plots in different climatic conditions, was evaluated. The total number of microorganisms and fungi were determined in silage biomass and silage. The content of deoxynivalenol and zearalenone in silages was treated with bacterial enzyme inoculant and chemical preservative, which is an acid. The total number of microorganisms was not influenced by the soil contamination of fodder. The occurrence of fungi was increased from 126,807 to 321,992 CFU·g^{-1}. The total number of microorganisms and fungi was raised by wilting. The occurrence of fungi was increased from 113,909 to 334,890 CFU·g^{-1}. Deoxynivalenol (increased from 101.9 to 131.5 ppb) was especially determined in alfalfa silage. The content of zearalenone reached up to 0.5442 ppb. The alfalfa silages, produced from wilted silage, contained lower levels ($P < 0.05$) of deoxynivalenol and zearalenone than alfalfa silages produced from no-wilted biomass. The content of mycotoxins was affected neither biological nor chemical preservative.

Keywords: deoxynivalenol, zearalenone, biological inoculant, organic acids, fodder safety

INTRODUCTION

Mycotoxins are dangerous metabolites that are often carcinogenic, and they represent a serious threat to both animal and human health. In the science, aflatoxins were discovered in 1960. The scientific community has attempted to limit the mycotoxin contamination in food and feed (Reverberi *et al.*, 2010).

Mycotoxins, produced by filamentous fungi, can evoke an acute or chronic disease in small concentrations in vertebrate animals (Gravesen *et al.*, 1994). Some compounds, usually classified among the mycotoxins, are not exactly suitable for the above-mentioned definition, e.g. zearalenone, which is an estrogen analogue (Mirocha *et al.*, 1978). The global occurrence of mycotoxins is considered to be a major risk factor because 25 % of the world

commodities are annually affected by known mycotoxins according to the Food and Agricultural Organization (FAO). In the United States, the average economic annual costs of crop losses, caused by mycotoxins, are estimated to be up to 932 million of USD (CAST, 2003). The contamination of food and feed occurs in fields before the harvest or during the storage despite of the efforts of prevention (Lillehoj *et al.*, 1983).

Fusarium mycotoxins are the largest group of mycotoxins, which includes more than 140 known metabolites of fungi. They are synthesized by many species of fungi, mainly by *Fusarium* (*F. graminearum and F. culmorum*). Due to high toxicity of *Fusarium* mycotoxins and the occurrence of the fungi species, which are producing them, these mycotoxins belong to the most animal and human health endangering ones (Yazar and Omurtag, 2008).

Deoxynivalenol (DON), zearalenone (ZEA) are included in the most frequently encountered mycotoxins (Vasatkova *et al.*, 2009, Vasatkova *et al.*, 2010) *Trichothecenes*, such as deoxinivalenol or nivalenol are wide family of chemically related sesquiterpenic mycotoxins produced by various species of *Fusarium* (Dohnal *et al.*, 2008, Wu *at al.*, 2010). The mycotoxin deoxynivalenol (DON) or vomitoxin is a major metabolite, produced by *Fusarium graminearum* (*Teleomorph = Gibberella zeae*), which is one of the most common fungi associated worldwide with grains in the field (Creppy, 2002).

DON was found out to be the compound responsible for emesis and feed refusal for sow (Mirocha *et al.*, 1976). DON has been proved to be a strong emetic agent in sows, dogs, and ducklings (Morgavi and Riley, 2007; Ueno *et al.*, 2007). They are all non-volatile, low-molecular-weight sesquiterpene epoxides sharing a tricyclic nucleus called trichothecene and usually contain an epoxide at C-12 and C-13, which are essential for toxicity (Desjardis *et al.*, 1993).

As other *Fusarium* toxins such as zearalenone (ZON) and fumonisins, the climatic conditions prove to have the major influence on the plant diseases during the growth. The occurrence of FHB is most affected by the rainfall at the timing of flowering, however, DON content is not always correlated with the severity of this symptom (Seitz and Bechtel, 1985).

Zearalenone is a non-steroidal oestrogenic mycotoxin produced by several *Fusarium* species (*Fusarium graminearum, Fusarium culmorum, Fusarium equiseti and Fusarium crookwellense*). All these species are worldwide regular contaminants of cereal crops (Hagler *et al.*, 2001). Legumes can enrich soils with biological nitrogen fixation (Eisenhauer and Scheu, 2008). Grasses show important role in grassland productivity and stability, and protein provides energy ratios with fixed nitrogen consumption (Akhavan *et al.*, 2013).

Fusarium root and crown rot is recognized as a significant disease of alfalfa (*Medicago sativa* L.) throughout eastern Canada (Martens *at al.*, 1988) and elsewhere in the world (Stuteville and Erwin, 1990). The infected plants do not withstand freezing as well as non-infected plants (Richard and Martin, 1993). The infection also reduces the yield and longevity of the crop, so the stands can be unproductive after a few years. Several *Fusarium* species are involved in the disease (Stuteville and Erwin, 1990; Aubé and Deschênes, 1967), including *Fusarium oxysporum* and *Fusarium solani*.

The harvesting of alfalfa can reduce the organic reserves in the roots during the critical period in the autumn (Couture *et al.*, 2002). The aim of the work is to assess the effect of soil contamination of fodder and wilting on the occurrence of fungi in the biomass of alfalfa and subsequently consider the mycotoxin content in alfalfa silage.

MATERIALS AND METHODS

The biomass of alfalfa (*Medicago sativa* L.) of Jarka variety, obtained from two experimental plots (Vatín and Troubsko), was evaluated. Each experiment was established in three repetitions. The experimental plot of Vatín is located at an altitude of 550 meters above sea level, with an annual rainfall of 617 mm and an average annual temperature of 6.9 °C. The experimental plot of Troubsko is situated at an altitude of 270 meters above sea level, annual rainfall of 305 mm and an average annual temperature of 8.6 °C. The total annual rainfall reached up to 305 mm in the year of monitoring.

The biomass of alfalfa, coming from the first cut, was used for the evaluation. The stands were founded in 2014. The first useful year of 2015 was observed. Alfalfa was harvested in the phase of butonization. The evaluated factors were soil contamination (contaminated and uncontaminated soil and wilting (wilting or no-wilting). Use of preservatives was evaluated as third factor (without preservative, bacterial-enzyme inoculum and chemical preservative). Bacterial-enzyme inoculum content bacterial species *Lactococcus lactis* $1.5 \cdot 10^{10}$ CFU·g^{-1}, *Lactobacillus plantarum* $2 \cdot 10^{10}$ CFU·g^{-1}, *Enterococcus faecium* $1.5 \cdot 10^{10}$ CFU·g^{-1}). Chemical preservative content formic acid 50 %, propionic acid 10 % and lignosulphopic acid 16 %. The presence of lignosulphonic acid reduces the aggressive nature of the organic acids leading to the reduced corrosion. The time of wilting lasted for 24 hours. The resulted dry matter reached up to 35 % of wilted biomass. The soil, coming from experimental plots, was used for the contamination. The biomass, prepared for the conservation (8 kg), was contaminated with the amount of 40 g·kg^{-1} of soil dry matter.

Micro-silage containers were used for the ensiling (Vyskočil *et al.*, 2011). The row of decimal dilution was prepared for the microbiological analysis. 1 ml of the dilution was inoculated in Petri dishes and watered with the nutrient medium. The culture medium of PCA agar (Biokar Diagnostics, France) was used for the determination of the total number of microorganisms (TNM). The incubation lasted

for 72 hours at 30 °C. Chloramphenicol Glucose Agar (Biokar Diagnostics, France) was used for the determination of fungi. The incubation lasted for 120 hours at 25 °C. After the incubation in thermostat, the grown colonies were counted in Petri dishes. The results of analyses were expressed in CFU per gram of silage.

The samples of green fodder and silages were dried at 60 °C grounded to a particle size of < 1 mm, then analyzed for the content of the mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEA) using the enzyme-linked immunosorbent assay (ELISA) according to Skládanka *et al.* (2011). ELISA is a competitive, direct enzyme-linked assay for quantitative analysis. The toxin concentration is expressed in parts per billion (ppb).

The data were statistically processed using STATISTICA.CZ Version 10.0 (Praha, the Czech Republic). The results are expressed as means (×). The obtained results were further analyzed using ANOVA and Scheffe's method. Cluster analysis was performed to create graphical representations.

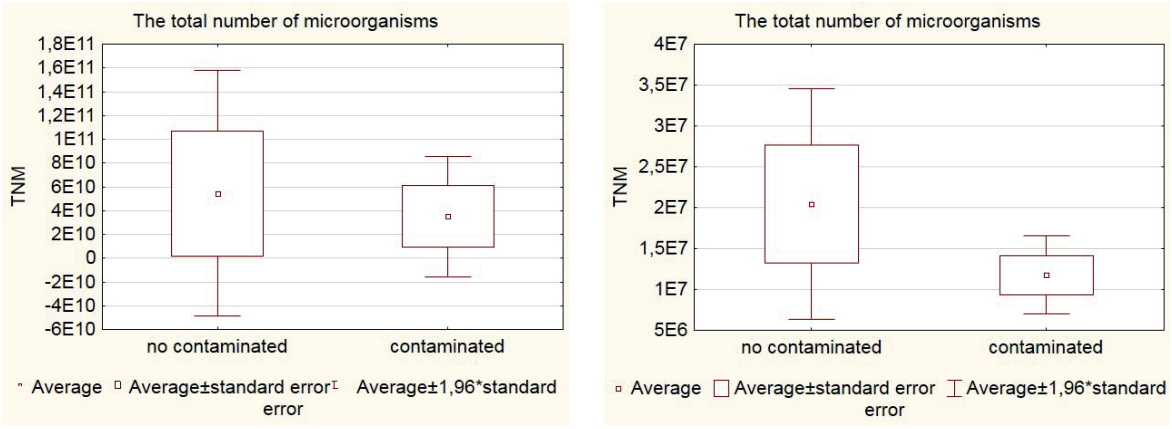
RESULTS

The total number of microorganisms was found out in the range of 10^{10} of CFU·g^{-1} in all variants before the ensiling. The total number of microorganisms (TNM) was decreased in 10^{7} of CFU·g^{-1} (Tab. I) by the effect of ensiling. This group of microorganism was determined to find out the total level of microbiological representatives in silage. Soil contamination did not influence the total number of microorganisms (Fig. 1). Higher occurrence of fungi was observed in contaminated biomass, which was evident from the relation with the content of fungi (Fig. 2). The total number of microorganisms was decreased by wilting (Fig. 3). However, the fungi were increased in their individual observation but only in biomass before the ensiling (Fig. 4). The higher occurrence of fungi was determined in the contaminated substance. The observed mycotoxins showed lower levels in contaminated than in uncontaminated substances. The wilting proved the significant effect on DON and ZEA content. In wilted substance, DON and ZEA were determined significantly lower ($P < 0.05$). The experimental plot did not significantly affect the amount of monitored mycotoxins. The content of deoxynivalenol (101.9–131.5 ppb) was detected higher than the content of zearalenone (0.3700–0.5442 ppb), (Tab. I) in produced silages. The soil contamination did not influence the content of mycotoxins. On the other side, the silages produced from no-wilted biomass proved higher ($P < 0.05$) content of mycotoxins than the silages produced from wilted biomass. The silage of wilted biomass showed the content of deoxynivalenol of 101.9 ppb. The silage of no-wilted biomass proved the content of deoxynivalenol of 131.5 ppb. Similarly, the content of zearalenone biomass reached up to 0.5442 ppb from no-wilted and up to 0.3700 ppb from wilted silage. Other monitored parameter was the use of preservatives (Tab. III). The use of preservatives partly influenced the number of microorganisms and the occurrence of fungi in the ensiling (Tab. III). Biological additive caused the increase of the total number of microorganisms. The content of fungi was fractionally increased before the use of preservation in silage biomass. On the other side, the use of preservation did not effect on the content of mycotoxin. The effect of biological silage additive and chemical preservative was not statistically significant on TNM and fungi (Tab. III).

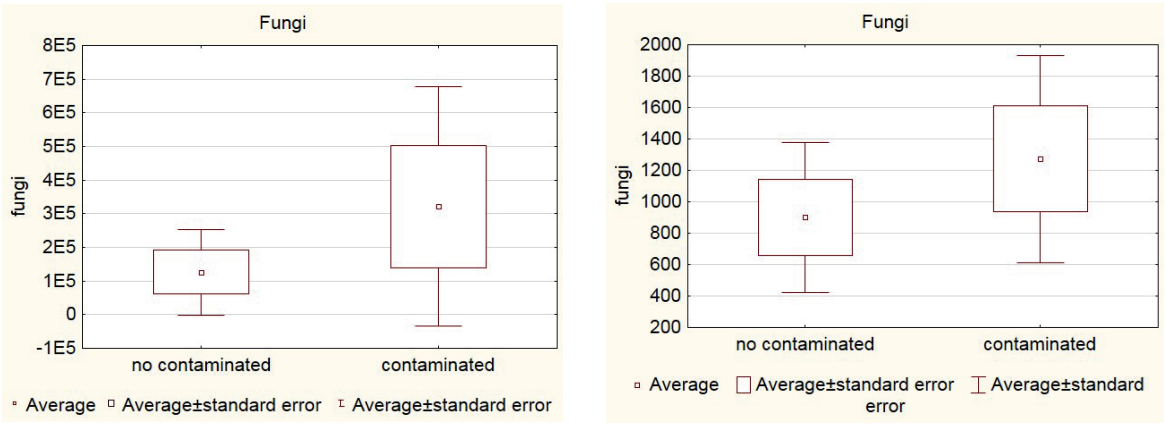
I: *Effect of contamination, wilting and experimental plot on the content of the total number of microorganisms and fungi in alfalfa biomass before the ensiling and in the silage in CFU·g^{-1}.*

Factor	TNM			Fungi		
	Before ensiling	Silage	Rel. %	Before ensiling	Silage	Rel. %
Contaminated						
No-contaminated	54548860000	20443182	0.0375	126807	901	0.7105
Contaminated	35054440000	11746212	0.0335	321992	1273	0.3953
P	0.7405	0.2173		0.2987	0.3979	
Wilted						
No-wilted	61046860000	21761364	0.0356	113909	1227	1.0772
Wilted	28556440000	10428030	0.0365	334890	947	0.2828
P	0.5820	0.1130		0.2411	0.5223	
Site						
Troubsko	87611210000	20583333	0.0235	383000	1068	0.2788
Vatin	19920910000	11606061	0.0583	65799	1107	1.6824
P	0.1559	0.2035		0.0983	0.9291	

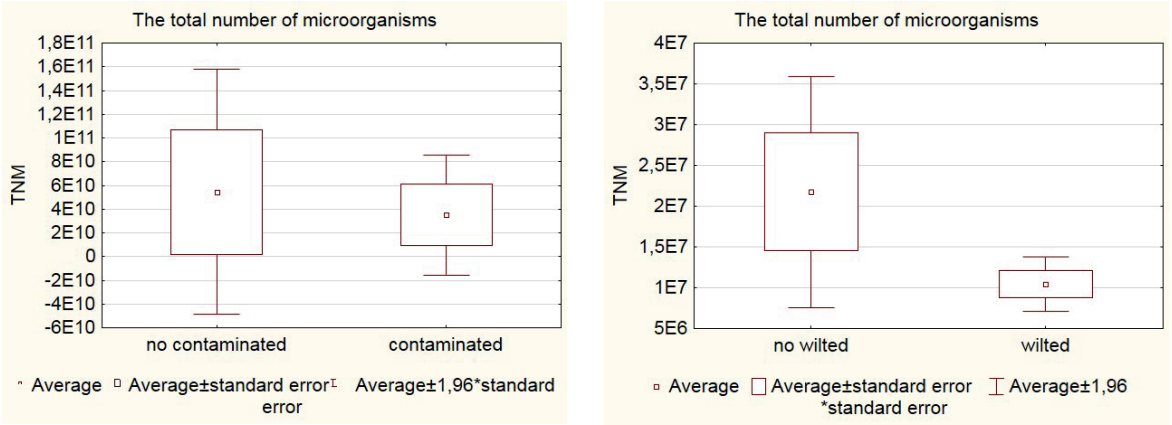
TNM – total number of microorganisms



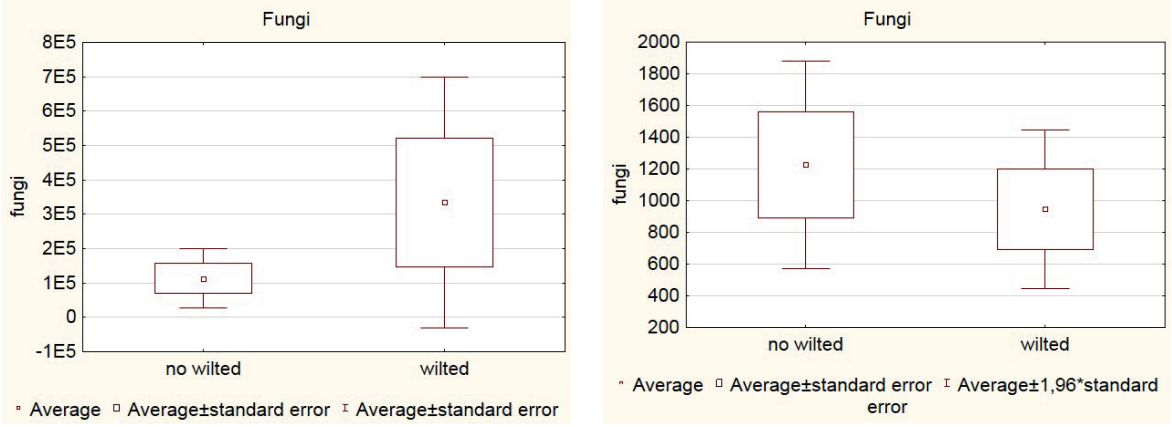
1: *Total number of microorganisms in uncontaminated and contaminated alfalfa biomass before the ensiling (a) and in the silage (b)*



2: *Fungi in uncontaminated and contaminated alfalfa biomass before the ensiling (a) and in the silage (b)*



3: *Total number of microorganisms in no-wilted and wilted alfalfa biomass before the ensiling (a) and in the silage (b)*



4: *Fungi in no-wilted and wilted alfalfa biomass before (a) the ensiling and in the silage (b)*

II: *Effect of contamination, wilting and experimental plot on the content (ppb) of deoxynivalenol and zearalenone in the alfalfa silage.*

Factor	DON	ZEA
Contaminated		
No-contaminated	122.6	0.4700
Contaminated	110.8	0.4442
P	0.1280	0.5560
Wilted		
No-wilted	131.5[a]	0.5442[a]
Wilted	101.9[b]	0.3700[b]
P	0.0008	0.0008
Site		
Troubsko	116.4	0.4183
Vatin	117.1	0.4958
P	0.9263	0.0887

Average values in the same column with different superscripts ([a,b]) are significant at the $P < 0.05$ level after Scheffe's method of analysis.

III: *Effect of the preservation on the content of the total number of microorganisms and fungi in CFU·g^{-1} and content (ppb) of deoxynivelenol (DON) and zearalenone (ZEA) in the alfalfa silage.*

Preservation	TNM	Fungi	DON	ZEA
Control	12443182	640	114.82	0.421250
Biology	26494318	1354	114.41	0.440000
Chemical	9346591	1268	120.96	0.510000
P	0.2173	0.3548	0.7246	0.2345

DISCUSSION

Currently, the tolerance limits of the fungi amount are not implemented in canned feeds in the Czech Republic. But the limits of the fungi number should be defined with the maximum concentration up to 10^5 CFU·g^{-1} according to Zeman *et al.* (2006) and Doležal *et al.* (2012). The highest number of fungi reached up to 10^5 CFU·g^{-1} in all experimental variants before the ensiling. The number of mold was decreased from 10^2 to 10^3 CFU·g^{-1}after the ensiling. So we can say that the number of fungi in the experimental silages did not exceed the recommended values. In the experimental contaminated silage, higher number of fungi was found out than in the uncontaminated silage. Simultaneously, higher number of fungi was detected in the experimental no-wilted silage than in the experimental wilted silage. No statistical significant difference was calculated between the variants. Particularly, higher number of fungi was determined in the wilted biomass before the ensiling than in no-wilted biomass.

The connection between wilting and the increasing number of fungi can be found out in dying of plant material. The population density of filamentous fungi is known to be positively associated with the senescence process of plants (Behrendt *et al.*, 2004). Significant reduction of fungi in silage, especially in the wilted biomass can be explained because of an anaerobic environment reduction of the fungi growth. From this perspective,

the ensiling is an effective strategy to prevent the production of mycotoxins (Cheli *et al.,* 2013).

The material for production of silage is already contaminated with the mycotoxin-producing fungi in fields. The contamination of soil increased the occurrence of fungi to a certain extent but the increase was not evident in the content of mycotoxins in silages. Similarly, the trend towards a higher incidence of fungi in the wilting but with quite different results achieved in the analysis of mycotoxins in silages. The wilting reduced the content of fungi but led to a higher content of mycotoxins. According to Laser *et al.* (2003), the high content of ergosterol need not necessarily indicate a high content of mycotoxins. In the comparison of the content of deoxynivalenol and zearalenone, the silage contained especially deoxynivalenol. Deoxynivalenol could be passed to milk if the content in feeds reached the level of 6 $mg \cdot kg^{-1}$ (Charmley *et al.,* 1993).

The observed mycotoxins of DON and ZEA were confirmed in all samples. The analysis of feeds, carried out with ELISA method for Central Europe, acknowledged the occurrence of ZEA in 17 % of samples from 759 tested ones and DON was detected in 59 % of the samples from 1,220 tested ones. The maximum amount of ZEA reached up to 522 ppb and DON was of 14,137 ppb. The highest amount was determined in barley (Grajewski *et al.,* 2012). Krnjaja *et al.* (2013) explored of 2,477.5 ppm of ZEA and up to 164 ppb of DON in alfalfa grass silage in her work. Higher values compared to those observed in our experimental samples were achieved (in untreated samples, the content was up to 0.421 ppb of ZEA and DON 114.82 ppb). The European Commission advisory guideline for deoxynivalenol reached up to 5 $mg \cdot kg^{-1}$ of dry matter. This limit was not exceeded in any sample. Due to the cumulative effect, the mycotoxins can be a security risk in silages. For zearalenone, the threshold of toxicity is even lower. The guidance value is up to 500 $\mu g \cdot kg^{-1}$ of dry matter for zearalenone in Europe. According to D'Mello (2003), zearalenone concentrations, ranging from 0.2 to 1.0 $mg \cdot kg^{-1}$, can be even toxic to rodents. *Fusarium* and *Alternaria* are major fungal strains occurred in the green mass (Rasmussen *et al.,* 2010) and surviving in the acidic environment and in the anaerobic fermentation (Samson *et al.,* 2002). It was found out that the content of DON or ZEA cannot be influenced by the preservation. This effect was not statistically significant. Interestingly, the wilting reduced the content of fungi but led to higher mycotoxin content in green mass.

And also the chemical preservation should suppress fungi but the increase even occurred. It was established that wilting can suppress both the development of fungi and the mycotoxins. In wilted mass, lower content of mycotoxins was found out compared to the samples of no-wilted forage. This can be justified by the fact that the wilted mass already partly developed in an anaerobic environment in which fungi are unable to live. Fungi, contained in the harvest of forage, probably persist during the storage even if further fungal growth is prevented by drying. The acidic anaerobic fermentation of suitable silage can also metabolise some of the mycotoxins already present at harvest (Scudamore *et al.,* 1998). Similarly, it was not obvious that the use of preservatives could affect the mycotoxin content. Moon (1983) states, that the organic acid and propionic acid particularly proved the antifungal effects.

CONCLUSION

The effect of soil contamination of fodder leaded to a higher occurrence of fungi in biomass of alfalfa determined for the ensiling. A similar trend toward higher occurrence of fungi was evident also in wilting. Silage, produced from wilted biomass, showed lower contain of deoxynivalenol and zearalenone than the silage made from no-wilted biomass. The predomination of deoxynivalenol was detected in alfalfa silage. The use of silage additives did not affect the content of mycotoxins. For the production of health and safety fodder for livestock feed, correct technological processes should be primarily complied with the harvesting biomass leading to the production of not only the quality but also the health and safety of fodder. The use of preservatives could not replace the mistakes in technological processes.

Acknowledgements

The paper was prepared under the support from Grant IGA IP 16/2015: Evaluation of health security perennial forage grasses and legumes.

REFERENCES

AKHAVAN, A. M., HASHEMI, M. AND AZARNIVAND, H. 2013. Physiological and morphological responses of three Bromus species to drought stress at seedling stage and grown under germinator and greenhouse conditions. *African Journal of Plant Science*, 7(5): 155–161.

AUBÉ, C. AND DESCHÊNES, J. 1967. Crown and root rots of alfalfa and red clover and the relative prevalence of associated fungi. *Plant Disease Report*, 51: 573–577.

BEHRENDT, U., STAUBER, T. AND MULLER, T. 2004. Microbial communities in the phyllosphere of grasses on fenland at different intensities of management. *Grass Forage Science*, 59(2): 169–179.

CAST. 2003. Mycotoxins, Risks in Plant, Animal and Human Systems. Task Force Report 139: 10. Iowa: Council of Agricultural Science and Technology.

CHARMLEY, E., TRENHOLM, H. L., THOMPSON, B. K., et. al. 1993. Influence of levels of deoxynivalenol in the diet of dairy cows on feed intake, milk production, and its composition. *Journal of Dairy Science*, 76(11): 3580–3587.

CHELI, F., CAMPAGNOLI, A. AND DELL'ORTO, V. 2013. Fungal populations and mycotoxins in silages: From occurrence to analysis. *Animal Feed Science and Technology*, 183(1–2): 1–16.

COUTURE, L., DHONT, C., CHALIFOUR, F. P. et. al. 2002. Fusarium root and crown rot in alfalfa subjected to autumn harvests. *Canadian Journal of Plant Science*, 82(3): 621–624.

CREPPY, E. E. 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters*, 127(1–3): 19–28.

DESJARDINS, A. E., HOHN, T. M. and MCCORMICK, S. P. 1993. Trichothecene biosynthesis in Fusarium species: Chemistry, genetics, and significance. *Microbiology Reviewes*, 57(3): 595–604.

D'MELLO, J. P. F. 2003. Food Safety Contaminants and Toxins. Wallingford, USA: CABI Publishing.

DOHNAL, V., JEZKOVA, A., JUN, D. et. al. 2008. Metabolic pathways of T-2 toxin. *Current Drug Metabolism*, 9(1): 77–82.

DOLEŽAL, P. (ed.). 2012. *Preservation of foods and their use in animal nutrition* [in Czech: *Konzervace krmiv a jejich využití ve výživě zvířat*]. 1st ed. Olomouc: Baštan.

EISENHAUER, N. and SCHEU, S. 2008. Earthworms as drivers of the competition between grasses and legumes. *Soil Biology and Biochemistry Journal*, 40(10): 2650–2659.

GRAJEWSKI, J., BLAJET-KOSICKA, A., TWARUZEK, M. et. al. 2012. Occurrence of mycotoxins in Polish animal feed in years 2006–2009. *Journal of Animal Physiology and Animal Nutrition* 96(5): 870–877.

GRAVESEN, S., FRISVAD, J. C. and SAMSON, R. A. 1994 *Microfungi*, 1st ed. Copenhagen: Munksgaard International Publishers Ltd.

HAGLER JR., W. M., TOWERS, N. R., MIROCHA, C. J. et. al. 2001. Zearalenone: mycotoxin or mycoestrogen? In: SUMMERELL, B. A., LESLIE, J. F., BACKHOUSE, D. et. al.(eds.), *Fusarium, Paul E. Nelson Memorial Symposium*. St. Paul: APS Press, 321–331.

KRNJAJA, V., NOVAKOVIĆ, Ž., STOJANOVIĆ, L. et. al. 2013. Kontaminacija hrane za goveda plesnima i mikotoksinima/Contamination of cattle feed with fungi and mycotoxins. *Veterinarski Glasnik*, 67(1–2): 129–138.

LASER, H., OPITZ VON BOBERFELD, W., WÖHLER, K. et. al. 2003. Effect of the botanical composition and weather conditions on mycotoxins in winter forage from grassland. *Mycotoxin Research*, 19(1): 87–90.

DIENER U. L., ASQUITH, R. L. and DICKENS, J. W. (eds.). 1983. *Aflatoxin and Aspergillus Flavus in Corn*. So. Coop. Series Bull. 279, Auburn University.

MARTENS, J. W., SEAMAN, W. L. and ATKINSON, T. G. 1988. *Diseases of field crops in Canada: an illustrated compendium*, 2nd ed. Harrow: Canadian Phytopathological Society.

MIROCHA, C. J., PATHRE, S. V., BEHRENS, J. et. al. 1978. Uterotropic activity of cis and trans isomers of zearalenone and zearalenol. *Applied and Environmental Microbiology*, 35(5): 986–987.

MIROCHA, C. J., PATHRE, S. V., SCHAUERHAMER, B. et. al. 1976. Natural occurrence of Fusarium toxins in feedstuff. *Applied and Environmental Microbiology*, 32(4): 553–556.

MOON, N. J. 1983. Inhibition of the growth of acid tolerant yeast by acetate, lactate and propionate and their synergistic mixtures. *Journal of Applied Bacteriology*, 55(3): 453–460.

MORGAVI, D. P. and RILEY, R. T. 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with Fusarium toxins. *Animal Feed Science Technolog*, 137, 201–212.

RASMUSSEN, R. R., STORM, I. M. L. D. and RASMUSSEN, P. H. 2010. Multimycotoxin analysis of maize silage by LC-MS/MS. *Analytical and Bioanalytical Chemistry*, 397(2): 765–776.

REVERBERI, M., RICELLI, A., ZJALIC, S. et. al. 2010. Natural functions of mycotoxins and control of their biosynthesis in fungi. *Applied Microbiology and Biotechnology*, 87(3): 899–911.

RICHARD, C. and MARTIN, J. G. 1993. The influence of Fusarium oxysporum f. sp. medicaginis on total soluble-sugar concentration of infected alfalfa roots. *Canadian Journal of Plant Science*, 73: 647–649.

SAMSON, R. A., HOEKSTRA, E. S., FRISVAD, J. C. et. al. 2002. *Introduction to food and airborne fungi.* 6th ed. Utrecht: Centraalbureau voor Schimmelcultures.

SCUDAMORE, K. A. and LIVESEY, C. T. 1998. Occurrence and Significance of Mycotoxins in Forage Crops and Silage: A Review. *Journal of the Science of Food and Agriculture*, 77(1): 1–17.

SEITZ, L. M. and BECHTEL, D. B. 1985. Chemical, physical and microscopical studies of scab-infected hard red winter wheat. *Journal of Agricultural and Food Chemistry*, 33(3): 373–179.

SKLÁDANKA, J., NEDĚLNÍK, J., ADAM, V. et. al. 2011. Forage as a primary source of mycotoxins in animal diets. *Interantional Journal of Environmental Resesearch and Public Health*, 8(1): 37–50.

STUTEVILLE, D. L. AND ERWIN, D. C. 1990. Compendium of alfalfa diseases, 2nd ed. St. Paul: APS Press.

UENO, Y., ISHII, K., SATO, N. et. al. 1974. Toxicological approaches to the metabolites of Fusaria. VI. Vomiting factor from moldy corn infected with Fusarium spp. *The Japanese journal of experimental medicine*, 44(1): 123–127.

VASATKOVA, A., KRIZOVA, S., ADAM, V. et. al. 2009. Changes in metallothionein level in rat hepatic tissue after administration of natural mouldy wheat. *International Journal of Molecular Sciences*, 10(3): 1138–1160.

VASATKOVA, A., KRIZOVA, S., KRYSTOFOVA, O. et. al. 2010. Effect of naturally mouldy wheat or fungi administration on metallothioneins-3 level in brain tissues of rats. *Neuroendocrinollogy Letters*, 30(1): 163–168.

VYSKOČIL, I., SKLÁDANKA, J., DOLEŽAL, P. et. al. 2011. *Methodology for the production of experimental microsilages* [in Czech: *Metodika výroby experimentálních mikrosiláží*]. Brno: Mendelova univerzita v Brně.

WU, Q. H., DOHNAL, V., HUANG, L. L. et. al. 2010. Metabolic pathways of trichothecenes. *Drug Metabolism Reviews*, 42(2): 250–267.

YAZAR, S. and OMURTAG, G. Z. 2008. Fumonisins, trichothecenes and zearalenone in cereals. *International Journal of Molecular Science*, 9(11): 2062–2090.

ZEMAN, L. (ed.). 2006. *Nutrition and feeding of farm animals* [in Czech: *Výživa a krmení hospodářských zvířat*]. 1st ed. Praha: ProfiPress.

Contact information

Lucia Hodulíková: lucia.hodulikova@mendelu.cz