ISAH 2005 - Warsaw, Poland
Vol 2

FEEDING GRAINS CONTAMINATION WITH FUNGI AND MYCOTOXINS AFTER HARVEST

Violeta Baliukoniene E-mail.: violeta.baliukoniene@lva.lt, Bronius Bakutis E-mail.: zoohig@lva.lt, Gediminas Gerulis E-mail.: gedulis@lva.lt

Lithuanian Veterinary Academy, Department of Food Safety and Animal Hygiene; Tilžės 18, 47181 Kaunas, Lithuania.

Introduction

Field fungi infect grain seeds before harvest and are involved in weather damage when harvests are delayed. Many researchers have divided fungal species into two groups: field fungi and storage fungi. Field fungi are those that invade the seeds while the crop is still in the field and require high moisture conditions (20-21%). These include species of *Fusarium*, *Alternaria*, *Cladosporium*, *Helminthosporium* and other [2].

Fungi imperfecti are known to produce variety of secondary metabolites that seem to improve their competitiveness in nature [11]. Secondary metabolites are formed in the final stages of the exponential growth phase [6]. Fungal metabolites exhibit an intrinsic toxicity even at low concentrations, resulting in their collective classification as mycotoxins [5]. Fungi of the genus *Fusarium* are common plant pathogens in a variety of crops, although they are mainly associated with cereals. *Fusarium* species can produce over one hundred secondary metabolites. The most important *Fusarium* mycotoxins that can frequently occur at biologically significant concentrations in cereals are trichothecenes (deoxynivalenol, nivalenol and T-2 toxin), zearalenone, fumonisins (mainly B_1 and B_2) [2].

The occurrence of mycotoxins contamination in feed is an important factor influencing feed safety and animal health. The effects in domestic animals include allergic reactions, reproductive failure, unthriftiness, loss of appetite, feed refusal, immunosuppression, decreased feed efficiency and mortality [4].

The aim of this study was determine the contamination with fungi and levels of deoxynivalenol, T-2 toxin, zearalenone in feeding grain in Lithuania.

Material and methods

The samples of wheat (*Triticum aestivum* L.) (n=10) and barley (*Hordeum distichon L.*) (n=10) were collected just after harvest in August-September 2003. The samples of wheat (n=13) and barley (n=11) were collected after harvest in August 2004. Each sample was collected automatically, taken directly from the truck.

The analysis of each sample was performed: direct plating and dilution plating were applied for isolation of fungi [9; 10]. Standart Czapek agar and SNA (Selective Nutrient Agar) were used to isolate and identify individual genera and *Fusarium* Link species. Fungi genera and *Fusarium* species were determined according to Nelson et al. [8], Lugauskas et al. [7], Samson et al. [9]. Detection frequency (%) of each identified genera and *Fusarium* species was calculated. The wheat and barley samples were analysed by the ELISA: VERATOX ® DON 5/5 (Neogen, USA), VERATOX ® T-2 toxin (Neogen, USA), VERATOX ® Zearalenone (Neogen, USA).

Meteorological data were obtained from Lithuanian Hydrometeorological service and Weather station in Dotnuva. The obtained results were processed using Prism 2.01 programme. Test results are statistically significant at $p<0.05$.

Results

In Lithuania wheat and barley are significant components in feed. Wet, rainy, warm, and humid weather from flowering time promotes cereals infection by fungi, resulting head blight in wheat and barley. Weather conditions weren't the same in 2003-2004 years. In summer of 2004 climate conditions were cooler with higher rainfall than in 2003 (table 1).

Table 1. Mean temperatures and precipitation in June, July, August and September in Lithuania

Years	June		July		August		September	
	Temp. (^oC)	Precip. (mm)	Temp. (^oC)	Precip. (mm)	Temp. (^oC)	Precip. (mm)	Temp. (^oC)	Precip. (mm)
2003	15.5	54.9	20.6	54.6	17.3	66.5	12.9	22.4
2004	14.2	44.2	16.8	81.6	18.1	94.5	12.9	53.2

Harvested wheat in 2004 was contaminated with fungi $14x10^3$ cfu/g ($p<0.005$) on average, compared to contamination in 2003 - 50% bigger. *Fusarium* spp. was identified in average 42% (Fig. 1). In 2004 barley were contaminated with fungi $30x10^3$ cfu/g ($p>0.005$) on average, compared to contamination in 2003 - 27% bigger. *Fusarium* spp. was identified in average 62%. *Alternaria* Nees, *Cladosporium* Link species fungi were widespread on harvested grain. *Aspergillus* Link, *Penicillum* Link was spread small.

The results of mycological tests indicate that grains contamination with fungi depend on weather conditions in summer. The bigger composition of fungi was detected in harvested grains in 2004. Detection frequency of each identified *Fusarium* species was calculated (table 2). *F. culmorum* was mostly frequently isolated from grains. In 2003 grain were contaminated by *F. equiseti*, *F. poae*. In 2004 barley was contaminated with *Fusarium* spp. more than wheat. *F. sporotrichioides, F. solani*, *F. avenaceum* were isolated from barley in 2004.

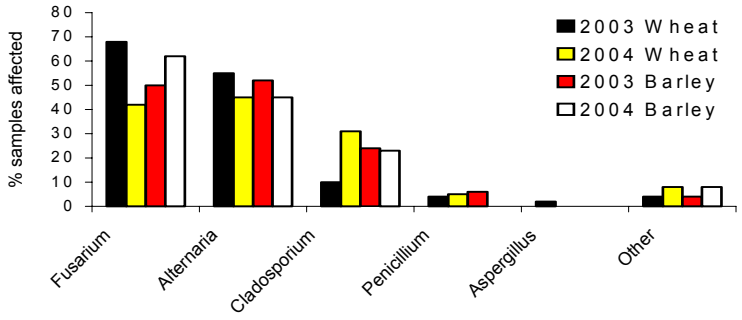

Figure 1. Dominating fungal genera (%) in wheat and barley harvested in Lithuania in 2003 and 2004

Table 2. *Fusarium* species found on harvested wheat and barley in 2003-2004

Fusarium species	Spreading frequency of species %			
	2003		2004	
	Wheat	Barley	Wheat	Barley
*F. avenaceu*m (Fr.) Sacc.	4.0	5.5	3.7	9.0
F. culmorum (W. G. Smith) Sac.	14.8	10.8	10.5	14.2
F. equiseti (Corda) Sacc.	15.4	12.7	5.4	6.4
F. graminearum Schwabe	8.2	3.0	4.8	4.2
F. moniliforme J. Sheld	-	-	0.6	-
F. oxysporium Schltdl.	0.4	1.0	0.8	0.8
F. poae (Peck) Wollenw.	11.0	7.0	5.5	4.6
F. solani (Mart.) Appel et Wollenw.		3.6	2.0	9.4
F. sporotrichioides Sherb.	5.4	0.6	6.7	10.8
Other	3.8	2.5	2.0	2.6

All identified *Fusarium* species produce mycotoxins. Results of the mycotoxins analysis are summarised in table 2. In 2004 wheat were contaminated with DON ($p>0.05$) more than in 2003. ZEN content was only 56 µg/kg ($p<0.05$). T-2 toxin in wheat was 25 µg/kg ($p>0.05$).

The barley was contaminated with mycotoxins more than wheat. DON content was 166 µg/kg ($p<0.05$), ZEN content - 300 µg/kg ($p>0.05$), T-2 - 25µg/kg ($p>0.005$) in samples of barley in 2004.

Table 3. Concentrations of deoxynivalenol (DON), T-2 toxin (T-2), zearalenone (ZEN) (µg/kg) in wheat and barley harvested in Lithuania in 2003 - 2004

Mycotoxin	2003		2004	
	Wheat	Barley	Wheat	Barley
No. of samples	10	10	13	11
DON				
Mean all	157	132	166	150
Max.	230	180	300	230
T-2				
Mean all	18	35	25	25
Max.	36	186	35	30
ZEN				
Mean all	185	308	56	300
Max.	500	450	390	632

Discussion and conclusions

Fungi are ubiquitous in nature and fulfill an essential role in the recycling of nutrients from decaying matter in soils, vegetation and water [7]. Fungi are the most important spoilage organisms in cereal grains. Mould growth leads to reduced nutritional and technical quality of cereals grains [2]. Low temperatures following infection may increase the production of mycotoxins. Previous studies have shown DON and ZEN to be the most commonly occurring mycotoxins in northern European grains [1]. The present work shows a similar distribution of DON, T-2, ZEN in Lithuanian grains.

That *Fusarium* spp. is dominating on harvested feeding grains, the problem of fusariotoxicosis is topical in Lithuania. Maximum concentrations of zearalenone in the Lithuanian grain samples were below those reported to be toxic to farm animals, especially young animals. The accumulation of mycotoxins in grain depends on many factors. In the last years the mycotoxins levels were differentiated narrowly, except ZEN in wheat and DON in barley.

Acknowledgements

This study was supported Lithuanian State Science and Study Foundation (Grant: C-03003).

References

1. *Bakutis B. 2004. Mikotoksinai gyvulių pasaruose. – "Terra Publica": 64-68 (in Lithuanian).*
2. *CAST (Council for Agricultural Science and Technology), 2003. Mycotoxins: Risk in plant, animal and human systems. Ames, Iowa, USA.*
3. *Chelkowski J., 1991. Mycological quality of mixed feeds and ingredients. In: Chelkowski J. (ed), Cereal grain, mycotoxins, fungi and quality in drying and storage. Amsterdam: Elsevier, p. 217-227.*
4. *Dänicke S., 2002. Prevention and control of mycotoxins. World's Poultry Science Journal, 58:452-475.*
5. *Fink-Gremmels J., Georgiou N.A., 1996. Risk assesment of mycotoxins for the consumer. In: Ennen G., Kuiper H. A., Valentin A. (ed.), Residues of Veterinary Drugs and Mycotoxins in Animal Products: 159-174. NL-Wageningen Press.*
6. *Jay J.M., 2000. Modern Food Microbiology. An Aspen Publication, Maryland.*
7. *Lugauskas A., Paskevicius A., Repeckienė I. 2002. Patogeniski ir toksiski mikroorganizmai žmogaus aplinkoje. Vilnius (in Lithuanian).*
8. *Nelson P.E., Toussoun T.A., Marasas W.F.O., 1983. Fusarium species. An Illustrated Manual for Identification. Pennsylvania State University Press, University Park, PA.*
9. *Samson A. Elen van Reenen-Hoekstra. 1988. Introduction to food-borne fungi. Centraalburean voor Schimmelcutures, BAARN.*
10. *Smirnova A., Kostrova O. 1989. Mikrobiologija zerna I produktov jego prererabotk Mockva, p. 6-35.*
11. *Steyn P.S., 1998. The biosynthesis of mycotoxins. Revue de Medicine Veterinaire, 149: 469-478.*