EFFECT OF STORAGE TIME OF BARLEY ON THE OCCURRENCE OF MYCOTOXINS AFTER ARTIFICIAL INOCULATION (FUSARIUM CULMORUM)

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Abstract

The samples of barley infected with *Fusarium culmorum* were included in the experiment. Control analysis was carried out after harvest in 2012. In the samples, the following mycotoxins were detected such as Deoxynivalenol; Deoxynivalenol-3-glukosid; 3-acetyl-deoxynivalenol; Zearalenon; Beta-zearalenol; Alternariol; Alternariol-methylether; Enniatin B; Enniatin B1; Enniatin A; Enniatin A1. Barley grain was stored under defined conditions. After one year, barley samples were analyzed again. From detected mycotoxins, deoxynivalenol levels were significantly increased by 29% ($P < 0.05$); DON-3-glucoside by 58% ($P < 0.05$) and zearalenone by 73% ($P < 0.05$). The results show that the stored barley leads to an increase in the concentration of mycotoxins after one year of storage, which has a direct influence on indicators such as fattening or reproductive performance of breeding pigs.

Key Words: Mycotoxin, barley, storage

Mycotoxins are low molecular organic secondary metabolites of fungi that are toxic to most organisms. These substances are produced by fungal mycelium but they can also be included in spores (Alkadri *et al.*, 2014). Mycotoxins are one of the most important groups of anti-nutritional substances found in feed. Barley is in one of the most common pig feed commodities. It is also predisposed to the occurrence of mycotoxins particularly deoxinivalenol and zearalenol causing harmfulness especially in pig reproduction. The exceeding critical levels and in combination with low housing conditions and animal hygiene, the signs of mycotoxicoses can occur in farm animals. The most frequent signs are chronic mycotoxicoses (Persi *et al.*, 2014). The dose and the duration of their action are the decisive factors for the overall toxic effects. Higher toxic effects are caused by synergistic effects of multiple mycotoxins in the feed (Basso *et al.*, 2013). The changes of the occurrence of mycotoxins in stored commodities can occur during stored feed (Sobrova *et al.*, 2010).

The aim of the experiment was to evaluate the influence of storage time of barley infected with *Fusarium culmorum* on the occurrence of each of the mycotoxins in barley biomass.

Materials and Methods

Barley samples coming from Libcany area (the Czech Republic) were put in the experiment from the harvest in 2012. The barley was artificially treated with *Fusarium culmorum* (WGSm. Sacc. Strain KM16902; DON chemotype.). The inoculation with a conidia suspension of the pathogenic isolate of *F. culmorum* (concentration 0.5 mil. conidia/1 ml of inoculum; spray dose of 200 $l.ha^{-1}$) was performed in the optimal vegetative phase according to the methodology of Tvarůžek et al. (2012). In the inoculation period, the vegetation was sprayed with clean water before the inoculation in dry and sunny weather. The barley samples were grounded on the laboratory mill with mesh size 1 mm. The samples of barley were analyzed on the individual mycotoxins after harvest in 2012. Freely laid samples were stored in specific storage conditions (temperature 20 °C, relative humidity 75%, dry matter of the samples 88%) was carried out for storage darkness) for a period of one year. After this time, the second analysis was conducted to determine the differences between mycotoxins. The samples were analyzed in three variants each year.

Determination of Mycotoxins - Extraction

A 2 g barley sample was weighed to PTFE centrifuge tubes (50 ml) followed by the addition of 10 ml of distilled water acidified (0.2% formic acid). Then the sample was shaked, closed and left for 30 minutes due to the wetting of the matrix. A 10 ml of acetonitrile was added in the sample with water followed by the extraction on the laboratory mixer for 30 minutes (240 RPM). The 4 g of $MgSO_4$ and 1 g of NaCl were put in the cuvette and shaken vigorously for 1 minute. The prepared sample was centrifuged for 5 minutes (10,000 RPM). After centrifuging, the sample was taken (approx. 1.5 ml) for purification using a microfilter with a porosity of 0.2 µm (centrifugation for 2 min, 5000 RPM). The sample was transferred to the vials and prepared for analysis. The samples were stored at –18 °C in glass vials before the analysis. For the identification and quantitative determination of the mycotoxins, Acquity UPLC® System (Waters, Milford, MS, USA) in a connection with tandem mass spectrometer QTRAP® (AB Sciex, Toronto, ON, Kanada) is used for the instrumentation of ultra-efficient liquid chromatograph Acquity UPLC ® System (Waters, Milford, MS, USA). The program Analyst ® (Thermo Fisher Scientific) is used for data processing.

Determination of Mycotoxins

The total of 57 mycotoxins of microscopic filamentous fungi of the genus *Fusarium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Claviceps* a *Stachybotrys* were set such as Fusarenon X, nivalenol, deoxynivalenol, alfa-zearalenol, beta-zearalenol, zearalenon, 3-acetyl-deoxynivalenol, patulin, alternariol, alternariol-methylether, deoxynivalenol-3-glukoside, enniatin B, enniatin B1, enniatin A, enniatin A1, ergokornin, ergokorninin, ergokristin, ergokristinin, ergokryptin, ergokryptinin, ergosin, ergosinin, ergometrin, ergotamin, ergotaminin, agroklavin, neosolaniol, diacetoxyscirpenol, fumonisin B1, fumonisin B2, fumonisin B3, 15-acetyl-deoxynivalenol, aflatoxin B1, aflatoxin B2,

aflatoxin G2, aflatoxin G1, HT-2 toxin, T-2 toxin, sterigmatocystin, ochratoxin A, citrinin, beauvericin, cyklopiazon acid, mycophenolic acid, penicillin acid, rokfortin C, tentoxin, tenuazonic acid, verrucarol, verruculogen, penitrem A, stachybotrylaktam, phomopsin A, gliotoxin, meleagrin, paxillin.

Statistica

The data were processed statistically using STATISTICA.CZ, version 10.0 (Czech Republic). Results are expressed as mean ± standard deviation (SD). Statistical significance was determined by examining the basic differences between years ANOVA and Scheffé's test (one-way analysis) Differences with P<0.05 were considered significant.

Results

It is evident that after the application of *Fusarium culmorum* were the highest concentrations of deoxynivalenol and zearalenone in the grains of barley. During the storage, the increased values of all mycotoxins except to beta-zearalenol were detected. The value of deoxynivalenol was measured higher by 29% (P <0.05) in the repeated analyzes after one year. Deoxynivalenol-3-glucoside was increased by 58% (P <0.05) in the year of storage. The concentration of zearalenone was also increased by 73% (P <0.05) in the repeated measurements in 2013. The differences were not found out significant between the first and second measurements in other detected mycotoxins. The levels of individual mycotoxins are shown in Tab. 1.

Table 1. Concentrations of detected mycotoxins in barley grains

Mycotoxin	Analysis (Ashrafi *et al.*)	
	2012	2013
Deoxynivalenol	11224 ± 1946	14500 ± 2625 *
Deoxynivalenol-3-glucoside	4362 ± 974	6951 ± 852 *
3-acetyl-deoxynivalenol	1412 ± 551	1518 ± 459
Zearalenon	2083 ± 703	3594 ± 313 *
Beta-zearalenol	127 ± 21	85 ± 27
Alternariol	19 ± 24	27 ± 34
Alternariol-methylether	2 ± 0.5	2 ± 0.4
Enniatin B	320 ± 63	428 ± 44
Enniatin B1	89 ± 16	123 ± 26
Enniatin A	4 ± 2	5 ± 1
Enniatin A1	30 ± 8	29 ± 5

* Symbol characterized statistical significance (P <0.05) compared with the analysis in 2012 and 2013.

Discussion

In our monitoring, the concentration of individual mycotoxins was increased in stored grains of barley during one year. Higher concentration of trichotacen particularly deoxynivalenol was observed at potatoes (storage) (temperature 18 ° C) (Xue *et al.*, 2014). The same results are apparent from our study, when the increased occurrence of Deoxynivalenol and 3-acetyl-deoxynivalenol was monitored at 20 °C. The highest concentrations of deoxynivalenol, zearalenone and aflatoxin B1 were measured at storing rice in similar conditions corresponding to our observation (Amadi and Adeniyi, 2009). Aflatoxin was not detected in our experiment with barley compared with the experiment of these authors at all. The risk of mycotoxins is increased in agricultural commodities for long periods of storage. The content of important nutrients is also reduced (Toth *et al.*, 2013). The application of *Fusarium culmorum* on cereal straw increased the occurrence of mycotoxins (deoxynivalenol and zearalenone) in the samples with dry matter ranging from 78-82% (Rohweder *et al.*, 2011). It is obvious that the risk of occurrence of mycotoxins can be eliminated by reducing the moisture content in the final product. This operation is not always financially advantageous. The control check points (HACCP) should be included in the control of quality. The important part is the elimination of insect disrupting plant tissues, which simplifies the penetration of pathogens(Chulze, 2010). The significant relationship between relative humidity and the presence of mycotoxins was discovered in storing corn in various conditions for seven months (Gagiu *et al.*, 2007). The relative air humidity was 75% in our observation. This value is recommended as the optimal humidity for storage of agricultural commodities resulted in the increase of the concentration in most of mycotoxins. In the artificial inoculation with *Fusarium,* the increased occurrence of

mycotoxins such as deoxynivalenol, zaearalenon and ochratoxin A was observed in our monitoring. The protective atmosphere CO_2 can be used to prevent the occurrence of pathogenic fungi (Prange *et al.*, 2005). Another way to effectively reduce the occurrence of fungal infestation is a fungicide applications in stored commodities.These substances can effectively protect against the occurrence of pathogenic fungi leading to reduction of mycotoxin production. Fungicides do not operate with the same efficiency. In the application, it is necessary to take into account the location and the crops on which they are applied (Schmidt-Heydt *et al.*, 2013).

Conclusion

The experiment was aimed at the monitoring changes in the occurrence of mycotoxins during storage of barley after artificial inoculation with *Fusarium culmorum.* The research was in the prescribed storage conditions during one year. Significant increased mycotoxin content was observed in deoxynivalenol ($P < 0.05$); DON-3-glucoside ($P < 0.05$); and zearalenone ($P < 0.05$). The above mentioned results show that the increased levels of mycotoxins during storage can be dangerous for pig breeding.

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