EFFECT OF FUNGICIDAL TREATMENT ON DIGESTIBILITY OF MYCOTOXINS IN VITRO

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ABSTRACT

In this experiment, the effect of fungicidal treatment on the release of various mycotoxins was tested in rumen fluid in vitro. The three groups of barley crop with different fungicide treatment were included in the experiment. The first group served as the control one without fungicide treatment. The second group of barley (variant A) was treated with Hutton (0.8 L/ha at BBCH 36) + Zantar (1.5 L/ha at BBCH 65). The third group of barley (variant B) was treated with the combination of Hutton (0.8 L/ha at BBCH 36) + Prosaro EC250 (0.75 L/ha at BBCH 65). In the original mass of barely, ten levels of mycotoxins were established. Subsequently, the samples were incubated in the machine Daisy II for 24 hours. The cellulase and peptic enzymes were used in the incubation. Following mycotoxins were determined in the incubation fluid such as deoxynivalenol, zearalenone, deoxynivalenol-3-glucoside and 3-acetyl-deoxynivalenol. In the variant A, the level of deoxynivalenol was higher by 36%, zearalenone by about 2%, deoxynivalenol-3-glucoside by 12%, and 3-acetyl-deoxynivalenol by 39%. Low levels of the mycotoxins were found out in the variant B. Deoxynivalenol level was lower by 19%, zearalenone by 30%, deoxynivalenol-3-glucoside by 37% (p < 0.05). The 3-acetyl-deoxynivalenol level was higher by 12% in a comparison with the control group. The obtained results showed that the fungicidal treatment and digestive enzymes could eliminate the transition of mycotoxins into incubative (rumen) liquid, and thereby to reduce the risk of the load of the organism by the mycotoxins. According to the results, it is obvious that low levels of various mycotoxins presented in the barley grains, as well as the transition of these mycotoxins in the incubation fluid were decreased. Some fungicides can play a significant role in the occurrence of mycotoxins barely grain.

Keywords: mycotoxins; barley; in vitro; digestibility

INTRODUCTION

Barley is classified as one of the most important cereals in the Czech Republic. It is used for livestock feed and food industry - especially malting (Belakova et al., 2014, Horky et al., 2012a). Mycotoxins are fungal secondary metabolites having mutagenic, carcinogenic, and cytotoxic effects. They can often contaminate agricultural commodities in spite of the various protective measures (Jancikova et al., 2012a). Recently, the attention has been focused on the so-called masked mycotoxins. The deoxynivalenol-3-glucoside and 3-acetyldeoxynivalenol metabolized from deoxynivalenol are the most common occurring. The presence of the masked mycotoxins presents no hazard as the occurrence of classic mycotoxins. In the food productive process, the production of malt, beer, and bread are back metabolized to deoxynivalenol (Horky et al., 2013, Zachariasova et al., 2012). Fusarium mycotoxins in foods are the most frequent type of contamination. Deoxynivalenon mycotoxins, zearalenone and T-toxin are responsible for the extensive damage to both feed and food. They directly threaten the health of consumers (Horky, 2014a, Maul et al., 2014). Fungicides are pesticides that are used to eliminate harmful phytopathogenic fungi on crop plants and the substances of organic origin. Fungicides have the ability to eliminate the occurrence of fungal biomass in plants and thereby to reduce the risk of mycotoxin production (Jancikova et al., 2012b, Schmidt-Heydt et al., 2013). After the fungicidal treatment, the development of mold is significantly reduced. Untreated plants can be characterized by higher levels of mold up to 260% (Pirgozliev et al., 2012). The similar effect as fungicides may have as well as the antioxidant enzymes (Horky, 2014a, Horky et al., 2012b). The susceptibility of animals to mycotoxins is different. The least susceptible animals are ruminants due to their buffering ability of the rumen (Horky, 2014b, Nevrkla et al., 2013). The aim of the experiment was to test the effect of fungicidal treatment of barley on the release of various mycotoxins in rumen fluid in vitro.

MATERIAL AND METHODOLOGY

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 ml. conidia/1 mL of inoculum; spray dose of 200 L/ha) 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. Subsequently, the chemical treatment with...
fungicides was applied in the barley. The first group was untreated and served as the control one. The second group of barley (variant A) was treated with Hutton (0.8 L/ha at BBCH 36) + Zantara (1.5 L/ha, BBCH 65). The third group of barley (variant B) was treated with the combination of Hutton (0.8 L/ha, BBCH 36) + Prosaro EC250 (0.75 L/ha, BBCH 65).

**Composition of Fungicides:**

**Hutton - active substance:**
Prothiokonazol 100 g/L 2-[2-(1-chlorcyclopropyl)-3-(2-chlorfenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazol-3-thion.

**Spioroam 250 g/L [(8-terc-butyl-1,4-dioxaspiro[4.5]dekan-2-y)methyl]ethyl(propyl)amin.**

**Tebukonazol 100 g/L 1-p-chlorofenol-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)-pentan-3-ol**

**Prosaro 250 EC - active substance:**
Prothiokonazol 125 g/L 2-[2-(1-chlorcyclopropyl)-3-(2-chlorfenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazol-3-thion.

**Zantara - active substance:**
Bixafen 50 g/L N-(3,4-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide.

**Tebukonazol 166 g/L (3-1-(4-chlorfenyl)-4,4-dimethyl-3-[(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol.**

Before the incubation (in Daisy II), all barley samples were analyzed for the content of individual mycotoxins. From each group, the three samples were collected and analyzed. The results of average concentrations of mycotoxins are listed in Table 1. The barley samples were ground on the laboratory mill with mesh size 1 mm. The machine Daisy II Incubator - ANKOM Technology, New York was used for the incubation. A 4 g milled sample was taken for the incubation divided into the incubation bags - FS7 (Ankom, Macedonia) in the amount of 0.25 g per incubation bag.

| Mycotoxins              | Control       | Variant of fungicial treatment |
|-------------------------|---------------|--------------------------------|
| Deoxynivalolen          | 12360.9 ±2045.5 | 19852.7 ±2173.8 | 11287.2 ±2718.8 |
| deoxynivalolen-3-glukosid | 6774.5 ±502.5   | 9042.7 ±678.3    | 5035.3 ±494.9    |
| 3-acetyl-deoxynivalenol | 1449.4 ±219.4   | 1969.8 ±257.4    | 1135.8 ±230.3    |
| Zearalenon              | 3737.5 ±880.3  | 4096.3 ±702.9    | 2947.2 ±704.0    |
| Beta-zearalenol         | 89.9 ±18.2     | 107.3 ±16.5      | 58.8 ±8.1        |
| Alternari               | 56.3 ±12.1     | 13.5 ±5.2        | 12.1 ±4.2        |
| Alternari-methylether   | 2.3 ±0.9       | 2.6 ±0.9         | 2.4 ±0.1         |
| Enniatin B              | 391.5 ±102.7   | 432.4 ±109.2     | 460.3 ±104.3     |
| Enniatin A              | 4.5 ±2.8       | 4.7 ±3.3         | 5.5 ±3.0         |
| Enniatin A1             | 25.8 ±12.4     | 27.7 ±18.3       | 32.4 ±13.0       |
The program Analyst® (Thermo Fisher Scientific) is used for data processing.

**Liquid Samples**

Liquid samples were purified using a microfilter with a porosity of 0.2 µm (centrifugation for 2 min, 5000 RPM) before the instrumental analysis. Furthermore, the solid samples were stored at -18 °C and measured using instrumentation consisting of ultra-efficient liquid chromatograph Acquity UPLC® System (Waters, Milford, MS, USA) in connection with tandem mass spectrometer QTRAP® (Applied Biosystems, Toronto, ON, Canada).

**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*, *alpha-zearalenol*, *beta-zearalenol*, *zearalenon*, *3-acetyl-deoxynivalenol*, *patulin*, *alternariol*, *alternariol-methylether*, *deoxynivalenol-3-glucoside*, *enniatin B*, *enniatin A*, *enniatin A1*, *ergokornin*, *ergokorninin*, *ergokristin*, *ergokristinin*, *ergokryptin*, *ergokryptinin*, *ergosin*, *ergosin-methylether*, *ergotamin*, *ergotamin-methylether*, *agroklavin*, *neosolaniol*, *diamatoxyscirpenol*, *fumonisins B1*, *fumonisins B2*, *fumonisins 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*, *tenoxicin*, *tenuazonic acid*, *verrucarol*, *verruculogen*, *penitre A*, *stachybotrylaktam*, *phomopsin A*, *gliotoxin*, *meleagrin*, *paxillin*.

**Statistics**

The data were processed statistically using STATISTICA.CZ, version 10.0 (the Czech Republic). The results were expressed as mean ± standard deviation (SD). Statistical significance was determined by examining the basic differences between groups ANOVA and Scheffé’s test (one-way analysis). The differences with *p* < 0.05 were considered to be significant.

**RESULTS**

During the analyzing of the incubated fluid, the following mycotoxins such as *deoxynivalenol*, *zearalenone*, *deoxynivalenol-3-glucoside*, *3-acetyl-deoxynivalenol* were detected. The mycotoxins that were analyzed in the original mass of barley such as *beta-zearalenol*, *alternariol*, *alternariol-methylether*, *enniatin B*, *enniatin A*, *enniatin A1* were below the detection limit indicating the fact that the mycotoxins were largely eliminated by digestive enzymes. The highest concentrations were measured in *deoxynivalenol* fungicidal treatment of the variant A (by 36%) compared with the control group of barley. Conversely, the variant B had a lower concentration of *deoxynivalenol* about 19% (Figure 1A). The identical value of the mycotoxin was observed in the control and fungicide variants A during the assessing levels of *zearalenone* in the incubation fluid. The variant B had lower levels of *zearalenone* by up 30% compared with the control group in the incubation fluid (Figure 1B). The two masked mycotoxins *deoxynivalenol-3-glucoside* and *3-acetyl-deoxynivalenol* were also detected in the incubation fluid. In the variant A, *Deoxynivalenol-3-glucoside* (Figure 1C) was increased by

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Figure 1 The concentration of mycotoxins detected in the incubation fluid (mg/kg): *deoxynivalenol* (A); *zearalenone* (B); *deoxynivalenol-3-glucoside* (C); *3-acetyl-deoxynivalenol* (D).
12% compared with the control group. The variant B had significantly lower levels of mycotoxin by 37% ($p < 0.05$). High concentrations of 3-acetyl-deoxynivalenol was measured for variant A (39%). In the variant B, the quantities of the mycotoxin in the incubation fluid was increased by 12% compared with the control group (Figure 1D).

**DISCUSSION**

In our experiment, the effects of fungicidal treatment on digestibility of the individual mycotoxins were compared with the usage of Daisy II incubator. The following mycotoxins were detected in the incubation fluid such as deoxynivalenol, zearalenone, deoxynivalenol-3-glucoside, 3-acetyl-deoxynivalenol. In the experiment, in which Fusarium toxins were added to the feed dose of ruminants in the amount of 60 and 30%, the reduction of synthesis of microbial protein was observed. In ruminal environment, the amount of Fusarium mycotoxins was significantly decreased (Hildebrand et al., 2012). In our experiment, the similar effect was observed on the release of mycotoxins in the ruminal environment during the effect of enzymes (cellulase, pepsin). During the incubation of mold feed (for 24 hours), barley base, rapecakes ced, alfalfa hay and barley straw (fungus 70%) was not affected by the degradation of dry matter of the individual components of the diet. The occurrence of mycotoxins was not monitored in the experiment. The mycotoxins such as aflatoxin 0-30%, deoxynivalenol 0-50%. T-2 toxin 0-70%, zearalenone 0-40%, deoxynivalenol 0-35%, ochratoxin A 50-100% are degraded in their derivatives with varying efficiency in the rumen. The susceptibility of animals to mycotoxins is different. The ruminants are the least susceptible category because of the buffering ability of the rumen. They are able to reduce and tolerate higher levels of mycotoxins (Undi & Wittenberg, 1996). The rumen microorganisms apparently metabolize toxines into non-toxic metabolites. We can also agree with these findings in comparison with the results of our experiment. After the incubation in a mixture of enzymes (pepsin, cellulase), the mycotoxins such as beta-zearalenol, alternariol, alternariol-methylether, enniatin B, enniatin A, enniatin A1 were degraded with high efficiency. Deynivalenol, zearalenone, deoxynivalenol-3-glucoside, 3-acetyl-deoxynivalenol were in the original mass of barley at very high concentrations. It was probably the reason why they were analyzed in the incubation fluid. From the findings of other studies, it could be suggested that the rumen environment completely eliminated the mycotoxin called ochratoxin A infected wheat straw (Abdelhamid et al., 1992). Berthiller (Belakova et al., 2014) investigated the back hydrolysis of deoxynivalenol-3-glucoside to the original mycotoxin (deoxinivalenol) in the stomach monogastry (in vitro). Deoxynivalenol-3-glucoside was resistant to the acidic environment of the stomach incubated in 0.2 M hydrochloric acid for 24 hours at 37°C. Conversely, some of the lactic acid bacteria were able to hydrolyze deoxynivalenol-3-glucoside back to deoxinivalenol.

**CONCLUSION**

In the experiment, the effect of fungicidal treatment on the release of various mycotoxins was observed in rumen fluid in vitro. The experiment included the three groups of barley using different fungicide treatment. The first control group was without fungicidal treatment. The second group of barley (variant A) was treated with Hutton (0.8 L/ha at BBCH 36) + Zantar (1.5 L/ha at BBCH 65). The third group of barley (variant B) was treated with the combination of Hutton (0.8 L/ha at BBCH 36) + Prosaro EC250 (0.75 L/ha at BBCH 65). In the mass, the level of ten mycotoxins was measured. Then the samples of barley were incubated in the incubator Daisy II for 24 hours using the cellulase enzyme and pepsin. The deoxynivalenol, zearalenone, deoxynivalenol-3-glucoside, 3-acetyl-deoxynivalenol were determined in the incubation fluid. In the variant A, deoxynivalenol level was higher by 36%, zearalenone by about 2%, deoxynivalenol-3-glucoside by 12% and 3-acetyl-deoxynivalenol by 39%. Low levels of the mycotoxins were found out in the variant B, the level of deoxynivalenol was lower by 19%, zearalenone by 30%, deoxynivalenol-3-glucoside by 37% ($p < 0.05$), and 3-acetyl-deoxynivalenol by 12%. According to the results, it is obvious that low levels of various mycotoxins presented in the barley grains, as well as the transition of these mycotoxins in the incubation fluid were decreased. Some fungicides can play a significant role in the occurrence of mycotoxins barely grain.

**REFERENCES**

Abdelhamid, A. M., El-Ayouty, S. A., El-Saadany, H. H. 1992. The influence of contamination with separate mycotoxins (aflatoxins, ochratoxin a, citrinin, patulin, penicillic acid or sterigmatocystin) on the in vitro dry matter and organic matter digestibilities of some roughages (berseem hay and wheat straw). Archiv fur Tierernahrung, vol. 42, no. 2, p. 179-185. PMid:1338408

Belakova, S., Benesova, K., Caslavsky, J., Svoboda, Z., Mikulikova, R. 2014. The occurrence of the selected fusarium mycotoxins in Czech melting barley. Food Control, vol. 37, p. 93-98. http://dx.doi.org/10.1016/j.foodcont.2013.09.033

Hildebrand, B., Boguhn, J., Danicke, S. Rodehutscheid, M. 2012. Effect of fusarium toxin-contaminated triticale and forage-to-concentrate ratio on fermentation and microbial protein synthesis in the rumen. Journal of Animal Physiology and Animal Nutrition, vol. 96, no. 2, p. 307-318. http://dx.doi.org/10.1111/j.1439-0396.2011.01143.x

Horky, P. 2014a. Effect of protein concentrate supplement on the qualitative and quantitative parameters of milk from dairy cows in organic farming. Annals of Animal Science, vol. 14, no. 2, p. 341-352. http://dx.doi.org/10.2478/aos-2014-0008

Horky, P. 2014b. Influence of increased dietary selenium on glutathione peroxidase activity and glutathione concentration in erythrocytes of lactating sows. Annals of Animal Science, vol. 14, no. 4, p. 869-882. http://dx.doi.org/10.2478/aos-2014-0056

Horky, P., Jancikova, P., Sochor, J., Hynek, D., Chavis, G. J., Ruttikay-Nedecky, B., Cernei, N., Zitka, O., Zeman, L., Adam, V., Kizek, R. 2012a. Effect of organic and inorganic form of selenium on antioxidant status of breeding boars ejaculate revealed by electrochemistry. International Journal of Electrochemical Science, vol. 7, p. 9643-9657. [cit.
Horký, P., Jancikova, P., Zeman, L. 2012. The effect of a supplement of chromium (picolinate) on the level of blood glucose, insulin activity and changes in laboratory evaluation of the ejaculate of breeding boars. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 60, no. 1, p. 49-56. [http://dx.doi.org/10.11118/actaun201260010049](http://dx.doi.org/10.11118/actaun201260010049)

Horký, P., Ruttikay-Nedecky, B., Kremplova, M., Krystofova, O., Kensova, R., Hynek, D., Babula, P., Zitka, O., Zeman, L., Adam, V., Kizek, R. 2013. Effect of different doses of organically bound selenium on antioxidant status and levels of metal ions in postpartum sows. *International Journal of Electrochemical Science*, vol. 8, p. 6162-6179. [cit. 2014-14-11] Available at: [http://www.electrochemsci.org/papers/vol7/71009643.pdf](http://www.electrochemsci.org/papers/vol7/71009643.pdf)

Jancikova, P., Horky, P., Zeman, L. 2012a. The effect of feed additive containing vitamins and trace elements on the trace elements profile in the hair, plasma and faeces and copper activity in the organism of horses. *Annals of Animal Science*, vol. 12, p. 381-391. [http://dx.doi.org/10.2478/v10220-012-0032-4](http://dx.doi.org/10.2478/v10220-012-0032-4)

Jancikova, P., Horky, P., Zeman, L. 2012b. The effect of various copper sources on the trace elements profile in the hair, plasma and faeces and copper activity in the organism of horses. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 60, p. 145-151. [cit. 2014-14-11] Available at: [http://acta.mendelu.cz/60/6/0145/](http://acta.mendelu.cz/60/6/0145/)

Maul, R., Piethau, R., Koch, M. 2014. Evaluation of an extraction method and spin column cleanup procedure for fusarium mycotoxins and their masked derivatives from grain matrix. *Food Control*, vol. 40, p. 151-156. [http://dx.doi.org/10.1016/j.foodcont.2013.12.003](http://dx.doi.org/10.1016/j.foodcont.2013.12.003)

Nevrka, P., Čechová, M., Hadaš, Z. 2013. Evaluation of selected reproductive parameters in gilts and loss of piglets after repopulation. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 61, no. 5, p. 1357-1364. [http://dx.doi.org/10.11118/actaun201361051357](http://dx.doi.org/10.11118/actaun201361051357)

Pirzgolev, S. R., Ray, R. V., Edwards, S. G., Hare, M.C., Jenkinson, P. 2012. Studies on the interactions between fungicides, *Alternaria tenuissima*, *Cladosporium herbarum* and *Microdochium* spp., on Fusarium head blight (fhb) development and deoxynivalenol (Campos et al.) concentration in grain caused by *Fusarium culmorum*. *Cereal Research Communications*, vol. 40, p. 509-517. [http://dx.doi.org/10.1556/crc.40.2012.0010](http://dx.doi.org/10.1556/crc.40.2012.0010)

Smichd-Heydt, M., Stoll, D., Geisen, R. 2013. Fungicides effectively used for growth inhibition of several fungi could induce mycotoxin biosynthesis in toxigenic species. *International Journal of Food Microbiology*, vol. 166, no. 3, p. 407-412. [http://dx.doi.org/10.1016/j.ijfoodmicro.2013.07.019](http://dx.doi.org/10.1016/j.ijfoodmicro.2013.07.019)

Undi, M., Wittenberg, K.M. 1996. Intake, rumen fermentation characteristics, and feedstuff in situ digestion kinetics as influenced by fungal biomass in alfalfa hay fed to cattle. *Animal Feed Science and Technology*, vol. 61, no. 1-4, p. 291-303. [http://dx.doi.org/10.1016/0377-8401(95)00942-6](http://dx.doi.org/10.1016/0377-8401(95)00942-6)

Zachariasova, M., Vaclavikova, M., Lacina, O., Vaclavik, L., Hajslova, J. 2012. Deoxynivalenol oligosaccharides: New “masked” *Fusarium toxins* occurring in malt, beer, and breadstuff. *Journal of Agricultural and Food Chemistry*, vol. 60, p. 9280-9291. [http://dx.doi.org/10.1021/jf302069z](http://dx.doi.org/10.1021/jf302069z)

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