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# INFLUENCE OF MYCOTOXINS IN BARLEY MONODIETS ON GROWTH PERFORMANCE AND RATS LIVER HISTOLOGY

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## Abstract

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The presence of mycotoxins in animal feed cause health disorders and can also contribute to other factors such as stress, lack of nutrition, infectious agents etc. The aim of this article was to study effect of feed ration contaminated with mycotoxins on liver histology and live weight of laboratory rats. 15 pieces of laboratory rats were divided into 3 groups (A, B, C). The rats in first group (A) fed with part of 30% mycotoxin-contaminated barley, in second group (B were fed with 60% and in control group (C) were fed without mycotoxin-contaminated barley. Content of deoxynivalenol (DON) was 9634 μg/kg and content of zearalenone (ZEN) was 2192 μg/kg. Barley monodietus were given rats in all groups to induce a nutritional imbalance. The aim of this study was to monitor the effect of mycotoxins and nutritional imbalances on weight gains and liver histology. The question is, "will the presence of mycotoxins in feed dose increase negative effect of a monodiets on health status?" No significant differences were found between the observed parameters. The presence of mycotoxins did not aggravate the negative effect of the monodiets on liver histology of the experimental rats.

Keywords: barley, deoxynivalenol, zearalenone, liver histology, growth performance

## INTRODUCTION

The presence of mycotoxins in animal feed can cause many animal diseases. Side effects may particularly affect the liver, kidney, nervous system, endocrine system, and immune system (Malhotra *et al.,* 2014). Symptoms are often bland and due to health problems may also contribute to other factors such as stress, lack of nutrition, infection by pathogenic agents etc. It is therefore very difficult to determine a direct correlation between contaminated feed and disease. Several *Fusarium* species of fungi produce toxic substances, in particular deoxynivalenol (DON), zearalenone (ZEN), T-2 toxin, HT-2 toxin and diacetoxyscirpenol. These mycotoxins contaminate cereals over the world. In this study were analysed content of DON and ZEN.

Deoxynivalenol is the most common grain contaminant worldwide and has high importance despite its low toxicity compared to other trichothecene mycotoxins (Kachlek *et al.,* 2017). Some studies already demonstrated that DON could induce liver damage remarkably through DON altering expressions of p53, caspase-3, caspase-7, caspase-8 and Bax in different cell lines and on the other hand, other articles publicised some opposite results. At present, a full explanation of the hepatic toxicity of DON has not been clarified yet (Peng *et al.,* 2017).

Adverse health effect of zearalenone is particular reproduction toxicity (Kovacs, 2012)

and hepatotoxic influence (Stadnik et Borzecki 2009). Zearalenone can induce increasing of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. Also ZEN is able to induce oxidative damage of liver (Long *et al.*, 2016).

Interactions between imbalanced nutrition and environmental factors trigger amplifying mechanism for liver disease (Larter, 2010). Diet for laboratory animals in this experiment made scraped barley. Barley monodiets is rich in energy and may cause decreasing food consumption and can change ration of nutrients, such as competition for absorption sites among certain minerals that share common active transport systems (National Research Council, 1995). The monodiets itself negatively affects liver. Mycotoxins DON and ZEN, as mentioned above, have hepatotoxic effect. Will the presence of these mycotoxins in feed ration affect findings of liver histological analysis?

## MATERIALS AND METHODS

The experiment was performed by 15 laboratory rats divided into 3 groups (A, B, C) in experimental facilities of Department of Animal Nutrition and Forage Production in Mendel University. The feed ration of rats in first group (A) contained 30% part of mycotoxin-contaminated barley, in second group (B) contained 60% and in control group (C) did not contain mycotoxin-contaminated barley. Content of DON in mycotoxins contaminated barley was 9634 µg/kg and content of ZEN in mycotoxin contaminated barley was 2192 µg/kg. Offered mycotoxin-contaminated barley was mixed equally with normal scraped barley also the rats could not prefer any feeding component. The feeding mixture and water were available *ad-libitum*. Water and feed ration were daily served and rests were removed.

The length of this experiment was 28 days. Incipient average rat's live weight was 90 ± 3 g. The rats were weighted in 0–7–14–21–28. The rats were sacrificed inhalational anaesthetic Isofuran way on 28$^{th}$ day and liver samples were removed to histological analyse. Histological analyse was performed at University of Veterinary and Pharmaceutical Sciences Brno.

### Determination of Mycotoxins

A 2 g sample of mycotoxins contaminated barley was weighed to PTFE centrifuge tubes (50 ml) followed by the addition of 10 ml of distilled water acidified (0.2% formic acid). The sample was shaken, then 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 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, Canada) was used for ultra-efficient liquid chromatograph Acquity UPLC® System (Waters, Milford, MS, USA). The program Analyst® (Thermo Fisher Scientific) was used for data processing.

### Statistical analyses

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

## RESULTS AND DISCUSSION

### Growth Performance

The results show that the weight gain of the rats among groups A, B, C had not any significant differences ($P < 0.05$). Among animals in the framework of groups, individual differences were related to the standard deviation of the detected values. Thus, we can conclude the presence of mycotoxins in the feed did not affect rats' weight gains. Thanh *et al.*, (2016) state the DON-ZEN ingestion did not affect the growth performances, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion. Similar results were found by Kachlek *et al.* (2017).

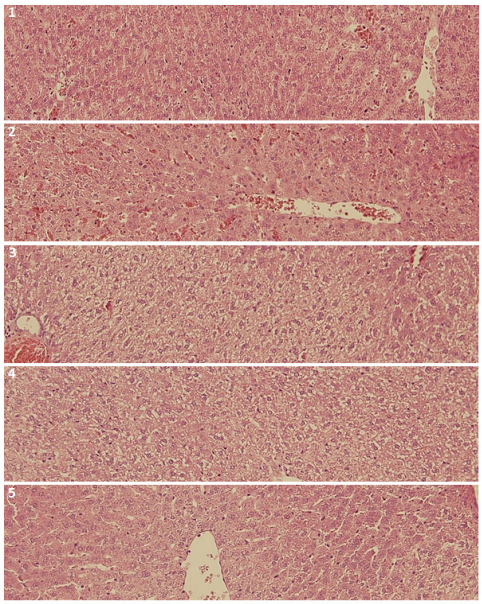
### Liver Histology

According to histological images, the animals in group C had liver dystrophy (especially LM 3, LM 4, LM 5 show wide-area dystrophy) and congestive

I: *Average daily gain (g/day)*

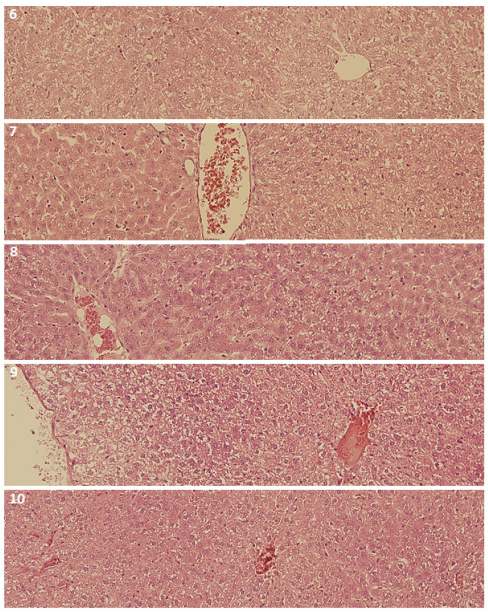
| | 0.day | 7$^{th}$.day $\bar{\alpha} \pm SD$ | 14$^{th}$.day $\bar{\alpha} \pm SD$ | 21$^{th}$.day $\bar{\alpha} \pm SD$ | 28$^{th}$.day $\bar{\alpha} \pm SD$ |
|---|---|---|---|---|---|
| **Group C (Control group)** | 0.0 | 2.3 ± 0.5 | 2.1 ± 0.4 | 1.9 ± 0.4 | 1.7± 0.5 |
| **Group A (30% part of MB)** | 0.0 | 3.0 ± 0.5 | 1.8 ± 0.3 | 3.0 ± 1.0 | 2.6 ± 0.7 |
| **Group B (60% part of MB)** | 0.0 | 3.0 ± 0.3 | 1.6 ± 0.5 | 1.8 ± 0.5 | 2.4 ± 0.4 |

MB–mouldy barley contaminated by DON and ZEN



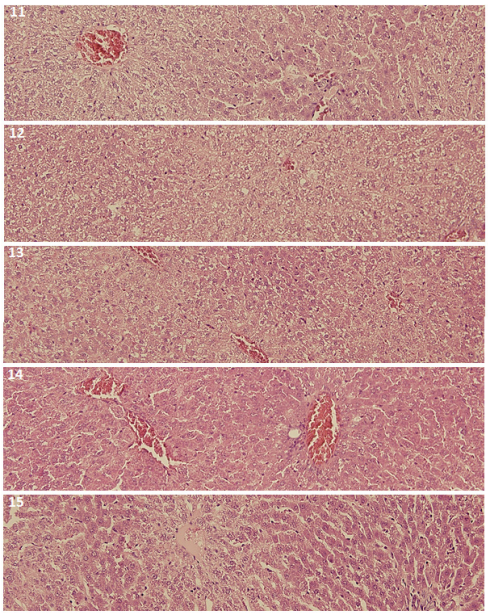
1: *Liver Mesh of rats in group C (control group). Magnification 200x*

LM 1: Parenchyma is congestive, all-dystrophic, locally with trabecular system, without necrosis, with distinct dilation of porto-biliary system.
LM 2: Locally distinct sinusoidal congestion, otherwise identical to LM 1.
LM 3: Congestion, massive wide-area hepatodystrophy with structural destruction focuses.
LM 4: Identical with LM 3.
LM 5: Sinusoidal congestion, wide-area dystrophy but less distinct compared to the previous cases, trabecular structure in good condition.



2: *Liver Mesh of rats in group A (30 % part of mycotoxins contaminated barley). Magnification 200x*

LM 6: Wide-area massive dystrophy with focuses of sinusoidal congestion.
LM 7: Sinusoidal congestion, wide-area dystrophy but less distinct compared to the previous cases, trabecular structure in good condition.
LM 8: Identical with LM 7.
LM 9: In the image occurs locally less distinct dystrophy, trabecular system in a good condition, locally massive dystrophic parenchyma with poorly visible structure.
LM 10: Wide-area dystrophy, structure of parenchyma is little distinct.




3: *Liver Mesh of rats in group B (60 % part of mycotoxins contaminated barley). Magnification 200x*
LM 11: Sinusoidal congestion, wide-area dystrophy but less distinct compared to the previous cases, trabecular structure in good condition.
LM 12: Massive wide-area hepatodystrophy with poorly visible structure of parenchyma and mononuclear cellulization, histological finding is similar to LM 10.
LM 13: Identical to LM 11.
LM 14: Significantly less dystrophic parenchyma with trabecular system in a good condition, congestive, with dilatation of porto-biliary system.
LM 15: Dystrophic focuses centrozonally, in dilated porto-biliary system of group mononuclear cellulization, locally well visible trabecular system.

parenchyma. In all figures, sinusoidal congestion can be seen.

According to histological images, the rats in group A (feed ration with content 30% part of mycotoxin contaminated barley) had liver dystrophy (LM 6, LM 7, LM 8 and LM 10 showed wide-area dystrophy, in case of LM 9 there were loci with different levels of dystrophy) and sinusoidal congestion.

Dystrophy of various extents is occurred in all figures of the group B (feed ration with content 60% part of mycotoxin contaminated barley), LM 11, LM 12, LM 13 show wide-area dystrophy, in case LM 14 and LM 15, there is less dystrophic parenchyma compared to LM 11, LM 12, LM 13. In LM 12 and LM 15, mononuclear cellulization is present. In most of the liver meshes, dilatation porto-biliary system or sinusoids is found. Trabecular structure of liver parenchyma is mostly in a good condition in LM 14 and LM 15.

In all liver histological samples, it can be seen liver dystrophy, mostly wide-area. Sinusoidal congestion occurs in the liver tissue of all rats in the group C. Moreover, the group B has also mononuclear cellulization (2 cases of 5 rats in group B) and sinusoid dilation or porto-biliary system (3 cases of 5 rats in group B). High-energy diet leads to obesity and liver damage caused by steatosis and although barley is high-energy feed, liver steatosis was not observed in the images. Provided that the experiment had taken more time, we could suppose the occurrence of liver dystrophy caused by steatosis in the animals. Diets containing surplus carbohydrate and fat evoke hepatic steatosis and steatohepatitis in mice (Pierce *et al.*, 2016). Among groups of present the significant differences in liver histology were not found. The question is: "What caused liver dystrophy?" As there were no significant differences among groups, the most probable reason is that the dystrophy had been caused by imbalanced feed (monodietus) and the presence of mycotoxins did not make the health status deteriorated (there were not found significant differences between groups fed with mycotoxin contaminated barley and control group).

Despite their high concentration, DON and ZEN did not affect body weight and body weight gain of rats in groups A and B and also histological analyse was not affected. Kachlek *et al.* (2017) observed that liver function was not affected by DON, as shown by their clinical chemistry indices. The similar results stated Renner *et al.* (2017), DON-contaminated feed did not change macroscopy and histology of the liver.

Opposite of them Bracarense *et al.* (2017) in their study observed that the ingestion of DON contaminated feed induced a significant increase in the lesional score in the liver. Liver displayed cytoplasmatic vacuolisation and hepatocelular megalocytosis and this results suggest that the ingestion of DON induced functional hepatic

impairment. The level of hepatic impairment depended on manner of a dose of DON and time interval.

Very interesting results suggest Ji *et al.* (2017), they studied metabolic profiling in liver and serum of mice for the combined toxic effects of DON and ZEN. As their results showed, consequences of the combined toxic effect of DON and ZEN have an obvious"antagonistic effect"in liver tissue metabolic profiling in mice. They observed by the metabolic pathway analysis that the combined DON and ZEN impact can introduce down-regulating of the valine, leucine and isoleucine biosynthesis, glycine, serine and threonine metabolism, and glucose metabolism in liver cells. The result, that the combinated DON and ZEN treatment have an "antagonistic effect" on liver metabolism of mice, was confirmed also by the metabolic profiling in serum.

## CONCLUSION

In this study were no differences among the observed parameters (the growth performance and liver histology). The rats' weight gains were not significantly different among the groups as individual differences among animals in the framework of one group were higher than differences among groups. All of liver mashes indicated hepatic dystrophy. However, no significant differences were observed among the groups. In conclusion, the presence of mycotoxins (DON, ZEN) did not aggravate negative effect of the monodietus on the health of the rats in our experiment. DON and ZEN have their own hepatotoxic effects. However, the results of other authors study show an antagonistic relationship between the negative effects of DON and ZEN. Thus, influence of this combination (DON + ZEN) did not have to affect parameters of our experiment significantly, as the combined effects of DON and ZEN could have eliminated each other hypothetically. Hepatic dystrophy of all animals was probably caused by an unbalanced diet.

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