Animal welfare, etológia és tartástechnológia

Animal welfare, ethology and housing systems

EFFECTS OF DIETARY FUSARIUM MYCOTOXINS ON ACTIVITY OF ANTIOXIDANT ENZYMES IN CHICKENS

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Abstract

The effect of medium dietary levels of Fusarium mycotoxins deoxynivalenol (DON) with or without zearalenone (ZEA) on the activity of glutathione peroxidase (GPx) in blood and tissues,thioredoxin reductase (TrxR) in liver tissue, superoxide dismutase (SOD) in erythrocytes, the
activity of γ-glutamyltransferase (GGT) in plasma and concentrations of malondialdehyde (MDA)in liver and kidney was investigated on Ross 308 hybrid broiler chickens of both sexes. After hatching, all chickens were fed an identical control diet for two weeks. Then chickens of Group 1 fed a diet contaminated with DON (3 mg DON/kg), while Group 2 was fed a contaminated diet containing DON and ZEA, each at a level of 3.4 mg/kg. All contaminated diets were prepared in the laboratory using maize cultivated with Fusarium graminearum. Intake of contaminated diet with the combination of mycotoxins DON and ZEA resulted in significantly increased levels of MDA in liver and kidney tissues, while the activity of TrxR in liver and GPx in both tissues did not affected. On the other hand, feeding of diet contaminated with 3 mg DON/kg and only background ZEA level (0.15 mg/kg) reduced activity of GPx in liver only and increased level of MDA in liver and kidney tissue. The activity of blood GPx and SOD in erythrocytes did not affected by dietary mycotoxins. The activity of GGT in plasma was significantly elevated in all chickens fed contaminated diets. Presented results demonstrate that diet contaminated with DON and ZEA at medium dietary levels is already able to induce oxidative stress in broilers.

Introduction

Mycotoxins as secondary metabolites of several moulds differ in their chemical structure, toxic properties and biological effects. In the mild climatic zone, maize is usually infected by members of the genus Fusarium, especially by the species Fusarium graminearum. The most important mycotoxins produced by these moulds are DON, its acetylated derivatives, ZEA and nivalenol (Binder et al., 2007). Deoxynivalenol (DON) is a member of trichothecene mycotoxin group. The mode of toxic action of DON is inhibition of protein synthesis, thus affecting rapidly dividing cells, such as those of the gastrointestinal tract and the immune system (Sergent et al., 2006). ZEA is a naturally occurring non-steroidal mycotoxin with oestrogenic properties, which is known to induce functional and morphological alteration in reproductive organs due to the activation of estrogen receptors. In addition, ZEA has been shown to be hepatotoxic, haemotoxic, immunotoxic and genotoxic (Zinedine et al., 2007). Adverse effects of mycotoxins on cells are also associated with the increased production of free radicals and reactive oxygen species resulting in oxidative damage of target tissues (Dvorska et al., 2007). The aim of our studies was to investigate the effects of diets contaminated with DON and ZEA in complete feed on parameters of tissue oxidative stress and antioxidant status in fattening chickens.

Material and methods

Experimental design, diets and analysis

One-day-old chickens of Ross 308 hybrid broilers of both sexes were randomly divided into dietary treatment groups and all were fed an identical uncontaminated starter diet for 2 weeks. After this time, control broilers continued to be fed the uncontaminated diet and chickens from experimental Group 1 being fed a diet contaminated with DON (3 mg DON/kg) and only background ZEA level (0.15 mg/kg). Group 2 was given a diet contaminated with DON and ZEA, each at a level of 3.4 mg/kg. Rearing of the chickens was done with a lighting regimen of 23 h light to 1 h dark and lasted for four

weeks. The initial room temperature of 32–33°C was reduced weekly by 18°C to a final temperature of 28°C. All birds had free access to water and feed. To provide stable dietary contents of mycotoxins during the entire experimental period, the chickens were fed only one type of diet (HYD-01). The final diets were obtained by mixing the basal diet (BD, the part of complete diet before addition of 40% portion of control or contaminated maize) with control or contaminated maize batches. Contaminated batches of maize were obtained by their cultivation with Fusarium graminearum for four weeks at the Slovak Agriculture University in Nitra (Labuda et al. 2003). The contents of deoxynivalenol (DON), zearalenone (ZEA), total aflatoxins, and ochratoxin A in BD and experimental diets are shown in *Table 1.*

Table 1

Final concentrations of mycotoxins in control and experimental diets				
Dietary treatment	DON	ZEA	AFLA total	OTA
Control group	0.1	0.005	0.0	0.0004
Group 1 (DON contaminated diet)	3.0	0.15	0.0	0.0004
Group 2 (DON+ZEA contaminated diet)	3.4	3.4	0.0	0.0005

DON = deoxynivalenol; ZEA = zearalenone; AFLA total = total aflatoxins; OTA = ochratoxin A

At the end of the 4-week experiment, eight randomly chosen chickens from each treatment were euthanised and tissue samples from liver and kidney were collected. Samples of blood were collected by intracardial punction into heparinised tubes. Mucosa from the duodenum was obtained by scraping. The blood, plasma and tissue samples were stored at −65 °C until analysis. Mycotoxin concentrations in feed were detected using the commercial competitive enzyme-linked immunosorbent assay-based Veratox 5/5 kit (Neogen, Lansing, MI., USA). The activity of blood glutathione peroxidase (GPx) was determined using the method of Paglia and Valentine (1967) with the Ransel kit (Randox, UK). Malondialdehyde concentrations (MDA) in tissue were measured by modified fluorometric methods as described by Jo and Ahn (1998). Haemoglobin (Hb) content of blood and superoxide dismutase (SOD) activity (Arthur and Boyne, 1985) in erythrocytes was analysed using kits from Randox, UK. The protein concentrations in the tissues examined were measured by the spectrophotometric method of Bradford (1976). Spectrophotometric determination of thioredoxin reductase (TrxR) activity was done with a Thioredoxin Reductase Assay Kit by the method of Holmgren and Bjornstedt (1995). The γ-glutamyltransferase (GGT) activity was measured using the kit from Randox, UK, by the method of Szasz (1969).

Statistical analysis
Statistical analysis was done by one-way analysis of variance (ANOVA) with the post hoc Tukey multiple comparison test using GraphPad Software (USA). The results are given as means ± SEM.

Result and discussion
Two weeks of feeding contaminated diets with mycotoxins resulted in increased levels of MDA in liver and kidney tissue in both experimental groups. A similar response was found in the MDA concentration in kidney of all broilers fed contaminated diets. Intake of contaminated diet with DON (3 mg DON/kg) led to significantly decreased GPx activity in liver tissue only, while no change of TrxR activity in this tissue as well as the activity of GPx in kidney did not affected in any experimental groups (*Table 2*).

Table 2

Concentration of malondialdehyde (MDA), activity of glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) in tissues of broilers fed diet contaminated with deoxynivalenol (DON, 3mg/kg) and combination DON with zearalenone (ZEA), both 3.4 mg/kg).

Parameter	Control	DON	DON+ZEA
Liver			
MDA (nmol·g -1 protein)	195.2±32.4 [a]	382.6±44.8 [b]	397.3±54.7 [b]
GPx (U·g -1 protein)	13.8±1.7 [a]	7.8±1.3 [b]	10.4±1.4 [a]
TrxR (U·g -1 protein)	18.4±1.9	28.01±5.0	24.4±3.6
Kidney			
MDA (nmol·g -1 protein)	133.2±8.4 [a]	214.4±15.4 [b]	232.0±18.3 [b]
GPx (U·g -1 protein)	15.4±3.5	16.7±2.7	17.7±3.1

a, b: Different letters in superscript within a row mean significant difference (P<0.05).

Our results demonstrate increased production of MDA with concomitant significant reduction of GPx activity in liver tissue of broilers fed on diets contaminated with DON. It has been reported that lower GPx activity in tissues is closely connected with an increase in MDA concentration (Balogh et al. 2004). DON contaminated feed has been reported as a factor increasing TBARS formation in rat and mice liver (Rizzo et al. 1994). It was found that dietary inclusion of another trichothecene mycotoxin T-2 toxin also increased lipid peroxidation in the livers of chickens, ducks and geese (Mezes et al.,1999). The kidney tissue activities of GPx did not show any differences between dietary treatments. This could be in part ascribed to a considerably smaller MDA response in kidney tissue to contaminated diets than that in the liver of chickens. Currently it is not clear if mycotoxins stimulate lipid peroxidation directly by enhancing free radicals production, or if the increased tissue susceptibility to lipid peroxidation is a result of a compromised antioxidant system (Surai 2006). It has been shown that DNA damage and apoptosis rather than plasma membrane damage and necrosis may be responsible for toxicity of DON (Minervini et al., 2004). In the liver, the gluthathione and thioredoxin systems are major players in the regulation of cell redox status (Gromer et al., 2004). The decreased activity of GPx in liver tissue together with greater MDA production indicate that combinations of DON and ZEA in concentrations 3.4 mg/kg feed are capable of inducing significant oxidative stress in chickens. Intake of contaminated diet with the combination of mycotoxins DON and ZEA, both being 3.4 mg.kg -1 resulted in significantly increased GGT activity in plasma of chickens while the activity of blood GPx and SOD in erythrocytes did not elevated (*Table 3*).

Table 3

Activity of glutathione peroxidase (GPx) in blood, superoxid dismutase (SOD) in red blood cells (RBC) and activity of γ-glutamyltransferase (GGT) in plasma of broilers fed diet contaminated with deoxynivalenol (DON, 3mg/kg) and combination DON with zearalenone (ZEA, both 3.4 mg/kg).

Parameter	Control	DON	DON+ZEA
GPx in blood (U.g [-1] Hb)	132.3±12.3	132.9±10.5	134.5±11.2
SOD in erythrocytes (U.g [-1] Hb)	1295±105	1566±113	1639±47
GGT in plasma (U.L [-1])	40.7±2.8 [a]	64.9±2.5 [b]	63.4±6.0 [b]

a, b: Different letters in superscript within a row mean significant difference (P<0.05).

The adverse effects of mycotoxins DON and ZEA on the livers of our broilers are demonstrated by findings of increased plasma activities of γ-glutamyltransferase (GGT). This enzyme is known to be involved in the transfer of amino acids across the cellular membrane and in glutathione metabolism. Serum GGT activity is widely used as a marker of liver dysfunction. Our results as well as previous studies show that serum GGT activity may be an early marker of oxidative stress (Lim et al., 2004).

Conclusion

In conclusion, the results of this experiment demonstrate the adverse effects of Fusarium mycotoxins on the antioxidative status in chickens for fattening. It is shown that diets contaminated with DON with or without ZEA at medium levels of about 3 mg/kg feed are already able to induce oxidative stress in liver and kidney tissue.

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