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EFFECTS OF FEEDING DIET CONTAMINATED WITH DEOXYNIVALENOL ON PLASMA CHEMISTRY IN GROWING BROILER CHICKENS AND THE EFFICACY OF GLUCOMANNAN MYCOTOXIN ADSORBENT

FAIXOVÁ ZITA*, FAIX Š**, LENG L'**, VÁCZI P*, SZABÓOVÁ RENÁTA* and MAKOVÁ ZUZANA*

*University of Veterinary Medicine, Košice, Slovak Republic
** Institute of Animal Physiology Slovak Academy of Sciences, Košice, Slovak Republic

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The aim of this study was to evaluate effects of modified glucomannan (Mycosorb®) on plasma chemistry of broiler chicks after deoxynivalenol (DON) inclusion in the diet from hatching to 6 weeks of age. Three groups of broiler chicks were formed with 14 birds in each group. The three diets included control (0.2 ppm deoxynivalenol), deoxynivalenol-contaminated (3 ppm deoxynivalenol) and deoxynivalenol-contaminated (3 ppm deoxynivalenol) plus Mycosorb®(2 g/kg diet). After 6 weeks of feeding all birds were sacrificed and blood samples for chemical analyses were collected. Serum calcium and alanine aminotransferase activity were significantly elevated and magnesium, total protein, triglycerides and free glycerol were decreased in chicks fed deoxynivalenol-contaminated diet compared with those fed the control diet.

Inclusion of Mycosorb®in the diet decreased plasma alkaline phosphatase and alanine aminotransferase activities and reversed plasma levels of magnesium, triglycerides, free glycerol and total protein in chicks induced by dietary deoxynivalenol. Chloride level was not affected by diets. The inclusion of Mycosorb® to DON-contaminated diet, however, did not prevent or alleviate toxic effect on calcium metabolism.

Supplementation of modified glucomannan Mycosorb® counteracted most of the plasma parameter alterations caused by deoxynivalenol-contaminated diet in chicks.

Key words: deoxynivalenol, DON, chicken, Mycosorb®, plasma chemistry

INTRODUCTION

Trichothecenes are a structurally diverse group of toxic secondary metabolites produced by *Fusarium* and related species of fungi which usually contaminate cereal grains in countries with temperate climates.

Deoxynivalenol (DON, vomitoxin) is the most prevalent trichothecene in crops used for food and feed production (Eriksen and Pettersson, 2004).

Many studies describe the adverse effects of DON on animal and human health. Indeed, in domestic or laboratory animals, large doses of DON caused feed refusal, decreased weight gain, vomiting, gastrointestinal and dermal irritation and immunological alterations. Lower doses of DON have been shown to provoke elevation of serum IgA level and are also known to affect cell- mediated and humoral immunity in several animal species (Prelusky, 1997; Pestka, 2003).

The sensitivity to DON varies considerably between species. Poultry is more sensitive to DON and to other trichothecenes than ruminants but less sensitive than pigs.

Trichothecenes are well-known inhibitors of protein synthesis (Mikami *et al.*, 2004). They also cause apoptosis both *in vitro* and *in vivo* in various organs (Maresca *et al. 2002*; Poapolathep *et al.,* 2002).

Trichothecenes are also shown to interfere with the metabolism of membrane phospholipids and to increase liver lipid peroxides *in vivo* (Rizzo *et al.,* 1994; Mezes *et al.*, 1999).

In addition, some trichothecenes are shown to alter the activity of serotonin in the central nervous system, which is known to be involved in the regulation of food intake (Rotter *et al.*, 1996).

From a regulatory point of view, different countries have enforced different thresholds to limit the passage of mycotoxins along the food chain (Whitaker *et al.,* 2005).

Basically, the best way to minimize the risk for mycotoxins to come into the food chain would be to prevent its formation during crop production and/or during storage of foodstuffs by harvesting the grain at maturity and low moisture and storing it at cool and dry conditions (Peraica *et al.*, 2002).

Unfortunatelly, a total avoidance of mycotoxin cotamination of feedstuffs can not be achieved mainly because of the major impact of climatic conditions. Therefore, nutritionists have to cope with a given level of mycotoxin contamination.

The main methods of choice are the detoxification of contaminated batches, blending with non-contaminated feedstuffs and diversion of suspect batches to animal species according to their sensitivity.

In recent years, nutritional manipulation has been actively used to improve animal selfdefence against mycotoxins or to decrease detrimental consequences of mycotoxin consumption. Many compounds have been tested for adsorptive effects on mycotoxins, but only few have proven successful.

The most effective method of neutralising mycotoxins present in feeds is by inclusion of inert adsorbents that prevent absorption of the toxin from the intestine.

Such adsorbent has to work rapidly and be uniquely structured to fit each and every mycotoxin. It needs to be stable over a wide pH range and be effective in the feed at low inclusion rates.

The objective of this study was to evaluate the efficacy of modified glucomannan (Mycosorb®) to alleviate toxicity of *Fusarium* mycotoxins in broiler chicks.

MATERIAL AND METHODS

Animals and diets

Three groups of broiler chickens were formed with 14 birds in each group. The birds were maintained on the floor for the course of the study. The three diets included control (0.2 ppm deoxynivalenol), deoxynivalenol-contaminated (3 ppm deoxynivale-nol) and deoxynivalenol-contaminated (3 ppm deoxynivalenol) plus Mycosorb® (2 g/kg diet). Chicks were fed the diets from the day of hatch to 42 d of age.

All experimental procedures with animals were in accordance with European Guidelines for care and use of animals for research purpose and they were approved by a local ethic committee.

Sampling and analyses

All birds were sacrified and blood samples for chemical analyses were collected. Plasma was separated by centrifugation at 1600 g for 10 min and stored at -20° C until analysis.

Alkaline phosphatase and alanine aminotransferase activities and concentrations of chloride, calcium, magnesium, total protein triglycerides and free glycerol were determined by the colorimetric methods using spectrophotometric kits. The mycotoxin doses were verified using HPLC method for DON.

Chemicals

Kits for alanine aminotransferase, alkaline phosphatase, chloride, calcium, magnesium and total protein assays were obtained from BIO-La-Test (Brno, Czech Republic). Kits for triglycerides and free glycerol assays were purchased from RANDOX Lab. (Crumlin, United Kingdom). Pure mycotoxin deoxynivalenol (DON) was purchased from Sigma Chemical Co. (Saint Quentin Fallavier, France). Mycosorb® was purchased from Alltech, Inc., USA.

Statistical analysis

The results are expressed as mean ± S.E.M. Statistical significance was evaluated by Student's *t*-test.

RESULTS

Plasma calcium and alanine aminotransferase activity were significantly elevated and magnesium, total proteins, triglycerides and free glycerol were decreased in animals fed the diet containing DON (3 ppm) (Table 1).

Table 1 Effect of dietary inclusion of deoxynivalenol and glucamannan Mycosorb® on plasma chemistry in growing broiler chickens

Parameter	Control diet (0.2 ppm deoxynivalenol)	Contaminated diet (3 ppm deoxynivalenol)	Contaminated diet (3 ppm deoxynivalenol) plus Mycosorb® (2g/kg feed)
Chloride (mmol/L)	104.300 ± 4.887	114.400± 4.366	103.400 ± 3.340
Calcium (mmol/L)	2.171 ± 0.078^{b}	3.360 ± 0.347^{b}	2.735 ± 0.194
Magnesium (mmol/L)	0.869 ± 0.052^{b}	0.349 ± 0.018^{ab}	0.920 ± 0.079^{a}
ALP (μkat/L)	8.060 ± 0.819	10.720 ± 0.126^{a}	6.360 ± 0.099^{a}
ALT (μkat/L)	0.251 ± 0.020^{b}	0.471 ± 0.004^{ab}	0.184 ± 0.091^{a}
Total protein (g/L)	39.110 ± 1.524^{b}	27.59 ± 1.925^{ab}	38.700 ± 1.573^{a}
Triglycerides (mmol/L)	1.021 ± 0.068^{b}	0.343 ± 0.022^{ab}	0.695 ± 0.059^{a}
Free glycerol (mmol/L)	0.911 ± 0.068^{b}	0.233 ± 0.023^{ab}	0.585 ± 0.059^{a}

Values are mean ± SEM, n = 14. Significant differences within a row are indicated by using the same superscript letter, $P<0.01$.

Supplementation of Mycosorb® to the contaminated diet decreased plasma alkaline phosphatase and alanine aminotransferase activities. Plasma concentration of magnesium, triglycerides and free glycerol were significantly increased if Mycosorb® was present in the diet. Chloride level was not affected by diets. Inclusion of glucomannan Mycosorb® to DON- contaminated diet, however, did not alleviate the toxic effects on calcium metabolism.

DISCUSSION

The deoxynivalenol treatment significantly decreased plasma level of total protein of chicks. Our results are consistent with those of Kubena *et al.* (1988) who found decreased total protein level in broiler chicks exposed to a DON (16 mg/kg) contaminated diet from 1 to 3 weeks of age.

Bergsjø *et al.* (1993) reported a significant decrease in serum protein and albumin in growing pigs fed a diet containing 3.5 mg/kg DON. They considered that these effects may be secondary to the reduced feed uptake but inhibition of protein synthesis may play a role. One of the toxicities of DON was thought to be derived from the inhibition of protein synthesis (Rotter *et al.*, 1996). Mikami *et al.* (2004) examined the toxicity of DON to porcine hepatocytes. DON was added at various concentrations to a medium of primary cultured hepatocytes. Authors reported that the concentrations of albumin in the medium of DON 100, 10 and 1 μg/mL groups were extremely low compared with the control, and were all about the same level. Comparison of the number of live hepatocytes suggests that the reduction of albumin secretion from hepatocytes into the medium was not only due to the loss of hepatocytes by apoptosis but also due to the inhibition of protein synthesis.

DON has also been reported to reduce serum albumin level in growing piglets fed a diet containing 8.6 mg/kg mycotoxin for 36 days (Doll *et al.*, 2005).

The toxicity of DON was expressed through decreased plasma triglycerides and free glycerol in broiler chickens. These findings are in agreement with the previous report of Kubena *et al*.(1987).

On the other hand, Accensi *et al*. (2006) reported that DON in low concentrations (0, 280, 560 or 840 μg/kg of feed) did not alter the performance of weanling piglets on 34 hematological, biochemical, and immune variables. As a general rule, significant biochemical changes were generally observed in animals receiving higher doses of trichothecenes. For instance, Kubena *et al.* (1985) described decreased plasma triglycerides and cholesterol in White Leghorn chicks fed a 9 and 18 mg/kg DON contaminated diet for 35 days and Huff *et al.* (1986) reported a significant decrease in serum triglycerides in chicks fed a diet containing contaminated wheat (16 mg/kg DON in feed) for 3 weeks. DON has also been reported to increase liver triglycerides and total liver lipid in White Leghorn hens fed a diet containing 0.25 or 0.70 ppm DON for 86 or 135 days (Farnworth *et al.*, 1983).

Dietary inclusion of 3 ppm DON resulted in increased plasma alanine aminotransferase, indicating liver damage. DON has also been reported to increase activities of aspartate aminotransferase, lactate dehydrogenase and gamma glutamyltransferase in broiler chicks fed DON at 15 mg/kg, indicating possible tissue damage and leakage of the enzymes into the blood (Kubena *et al.*, 1997). Similar results were observed in horses (Raymond *et al.*, 2003) and piglets (Doll *et al.*, 2005) fed *Fusarium* culture material.

In the present study, the administration of 3 ppm DON to the diet altered plasma calcium. Previous data of Bergsjø *et al.* (1993) reported a significant decerase in serum calcium and phosphorus in growing pigs fed a diet containing 3.5 mg/kg DON. DON has also been reported to induce weak hypocalcemia in rats fed 1 mg/kg DON for 6 months, suggesting that calcium metabolism disorders during chronic action of mycotoxin could be partially associated with secondary vitamin D deficiency (Sergeev *et al.*, 1990). Recently Gouze *et al.* (2006) reported that electrolytes in plasma appeared to be insensitive to a 4-week exposure to low DON in mice. The discrepancy between these results and our data could be due to a number of factors, including sensitivity to DON between species, DON concentration, DON source, animal genetics, sex and nutritional status.

The most applied method for protecting animals against mycotoxicosis is the utilization of adsorbents mixed with the feed which are supposed to bind the mycotoxins efficiently in the gastrointestinal tract.

Many compounds have been tested for adsorptive effects on mycotoxins, but only few have proven successful.

Still fewer- mainly bentonites, zeolites, aluminosilicates and a yeast-derived glucomannan- are sold commertially for this purpose. The extent to which various compounds adsorb or bind specific toxins varies considerably (Doll *et al.*, 2004; Doll *et al.*, 2005; Avantaggiato *et al.*, 2005) Some (zeolites) only bind aflatoxin, leaving other mycotoxins as T-2 unaltered (Dvorska and Surai, 2001).

In contrast, a glucomannan derived from yeast cell walls (Mycosorb®) has been shown to be able to adsorb higher levels of several important mycotoxins at lower inclusion rates than above mentioned binders.

The high adsorptive capacity of modified glucomannans for mycotoxins has been reported by many researchers.

Dvorska and Surai (2004) reported that inclusion of modified glucamannan (Mycosorb®) in aurofusarin enriched diet of quail provided a significant protective effect against changes in antioxidant composition in the egg yolk and liver. Their previous studies (Dvorska and Surai, 2001) showed that Mycosorb® in the diet at a 0.1% level was able to inhibit liver antioxidant depletion caused by T – 2 toxin (8.1 mg/kg) in growing quail. Mycosorb® was effective in diminishing the adverse effects of aflatoxin (2 mg/kg diet) on growing chicks and the higher concentration of yeast glucomannan (1g/kg feed) was more effective than the lower concentration (0.5 g/ kg) and itself had no adverse effect (Karaman *et al.*, 2005). Modified glucomannan supplementation was found to be effective in reducing the adverse effects of *Fusarium* mycotoxins in broilers (Swamy *et al.*, 2002).

Diaz *et al.* (2005), however, reported that the only feed additive capable of counteracting the adverse effects on performance caused by the dietary administration of 2 ppm T -2 toxin in broiler chickens was the additive based on the enzymatic inactivation of the 12, 13 – epoxide ring of the trichothecenes (Mycofix) while Mycosorb, Mycoad and Zeolex were not effective.

Our data show that glucomannan Mycosorb® is beneficial in reversing adverse effects of deoxynivalenol in broilers since it is able to improve the most serum chemical parameters – alanine aminotranferase, magnesium, total proteins, triglycerides and free glycerol.

CONCLUSION

It was concluded that dietary inclusion of deoxynivalenol resulted in changes of plasma indices in growing broiler chickens.

Supplementation of modified glucomannan Mycosorb® counteracted most of the plasma parameter alterations caused by dietary inclusion of deoxynivalenol in growing broiler chickens.

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Address for correspondence:
Associate Professor Zita Faixová, DVM, PhD.
Department of Pathology, Pathophysiology and Genetics
University of Veterinary Medicine
Komenského 73
041 83 Košice
Slovak Republic
e-mail: faixova@uvm.sk

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VREDNOSTI BIOHEMIJSKIH PARAMETARA U KRVNOJ PLAZMI BROJLERA HRANJENIH OBROCIMA KONTAMINIRANIM DEOKSINIVALENOLOM I PROTEKTIVNI EFEKTI ADSORBENTA NA BAZI GLUKOMANANA

FAIXOVÁ ZITA, FAIX Š, LENG L', VÁCZI P, SZABÓOVÁ RENÁTA
i MAKOVÁ ZUZANA

SADRŽAJ

Cilj ove studije je bio utvrđivanje efekata modifikovanih glukomanana (Mycosorb®) na vrednosti biohemijskih parametara krvne plazme brojlera nakom uključivanja u obrok deoksinivalenolola (DON) od izleganja do uzrasta od 6 nedelja. U ogled su bile uključene tri grupe brojlera od po 14 jedinki. Obroci su bili napravljeni prema sledećoj šemi: kontrolni (0.2 ppm deoksinivalenol), kontaminiran deoksinivalenolom (3 ppm deoxynivalenol) i kontaminiran deoksinivalenolom (3 ppm deoksinivalenol) sa dodatkom Mycosorba® (2 g/kg diet). Pilići su bili žrtvovani nakon 6 nedelja kada su prikupljeni uzorci za biohemijske analize. Koncentracija kalcijuma i aktivnost alanin aminotransferaze su bili značajno povećani

dok su koncentracije magnezijuma, ukupnih proteina, triglicerida i slobodnog glicerola bile smanjene kod brojlera hranjenih kontaminiranim obrocima u poređenju sa kontrolnom grupom.

Dodavanje Mycosorba® u obroke smanjilo je aktivnost serumske alkaline fosfataze i alanin aminotransferaze i stabilizovalo koncentraciju magnezijuma, triglicerida, slobodnog glicerola i ukupnih proteina. Različiti obroci nisu imali uticaja na koncentraciju hlorida. Dodatak Mycosorba ® u obroke kontaminirane DON-om nije umanjilo toksične efekte na metabolizam kalcijuma.

Suplementacija modifikovanim glucomananima (Mycosorb®) neutrališe većinu negativnih efekata deoksinivalenola na vrednosti biohemijskih parametara u krvnoj plazmi brojlera.