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Original article

Zearalenone and deoxynivalenol mycotoxicosis in dairy cattle herds

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Abstract

Mycotoxin contaminations pose a growing problem in animal production from the economic and toxicological point of view. Clinical symptoms of mycotoxicosis are relatively unspecific, making the disease difficult to diagnose. This study presents a clinical case of dairy cattle infected with natural mycotoxins produced by fungi of the genus *Fusarium* (zearalenone [ZEA] and deoxynivalenol [DON]) in eastern Poland. In dead and infected cows, the presence of ZEA and DON was determined in the blood serum, significant changes were observed in blood morphological and biochemical profiles, extravasations and bowel inflammations were also observed. The results reported testify to an acute autoimmune process in the intestines as well as immunosuppression.

Key words: deoxynivalenol, zearalenone, hematology, biochemistry, cows

Introduction

Dairy cattle and ruminant resistance to mycotoxins is largely determined by ruminal bacteria ability to eliminate the pathogens before they enter the bloodstream. Some mycotoxins found in silage and stored feedstuffs have antibacterial properties, they modify the ruminal microflora and minimize detoxicating effects of ruminal digesta. Contaminated digesta reach the duodenum where mycotoxins are absorbed into the bloodstream at high concentrations. Mycotoxin contaminations produce non-specific clinical symptoms such as metabolic and hormonal imbalance, inflammations and immune responses. During transition periods, cows and suckling calves are particularly sensitive to infections because a negative

energy balance is deepened by the presence of molds and/or mycotoxins in the diet (Charmley et al. 1993, Driehuis et al. 2008, Gajęcki 2010).

Mycotoxins are secondary metabolites of fungal organisms that infect growing plants, stored products and materials of plant origin. Fungi thrive in environments marked by high humidity, high temperature of plant material and air access at all stages of plant production and material storage. Fungi that infect feed materials accumulate and grow focally, which additionally obstructs the determination of fungal contaminants and their mycotoxins. In contemporary livestock feed technology, contaminated feed is often mixed or combined with the ration.

At the initial stages, mycotoxicosis may be manifested by a sudden, insignificant drop in milk yield

which deepens over time and may lead to animal death. In most cases, the disease is difficult to diagnose due to an absence of specific clinical and anatomopathological symptoms that are restricted to general inflammations of the intestinal mucosa and non-specific irritations of other mucosal membranes. The non-specific progression of individual and combined mycotoxicosis may be caused by the immunosuppressive effect of selected mycotoxins (such as DON) or their presence in very low concentrations below NOAEL thresholds. The symptoms observed do not support a full diagnosis. Every change in feeding regime should eliminate suspected contaminants, such as mycotoxins, from feed, unfortunately, such practices often introduce new contaminating agents. Lactating cows are more susceptible to infections caused by feed-borne mycotoxins than beef cattle due to their very high performance levels (Seeling et al. 2006, Gajęcki 2010).

Fusarium mycotoxins are adsorbed from the gastrointestinal lumen into the ruminant bloodstream to cause gastroenteritis, including hemorrhagic enteritis and diarrhea, ketosis, impaired rumen function, irregular heats, low conception rates, ovarian cysts, embryonic loss, mastitis and diffuse aseptic laminitis. Many of the above diseases result from the impairment of congenital immunity and a drop in local resistance to contaminants in the gastrointestinal tract, reproductive system, in the area of the hoof coronet and the hoof capsule (Keese et al. 2008).

The main symptoms of long-term exposure to low mycotoxin doses (below NOAEL thresholds) include loss of or decrease in appetite, lower body weight gains, impaired metabolism, decreased milk yield, hormonal imbalance, immune disorders and ruminal microflora dysfunction (in particular as regards saprophytic bacteria). The above symptoms are not indicative of mycotoxicosis, and additional laboratory analyses are required to identify the contaminant (Hochsteiner et al. 2000, Gajęcki, 2010).

Zearalenone, a mycoestrogen (phytoestrogen) produced by various fungi of the genus *Fusarium* spp., mainly *F. graminearum*, is one of the key lactones of fungal origin. ZEA is most often found in maize, but it also contaminates oats, rice, rye and wheat. ZEA and its metabolites, including α -zearalenol, bind to estrogen receptors to initiate an agonistic/antagonistic interaction that leads to hyperestrogenism. The most characteristic symptoms of the above include swelling and hyperemia of the vulva, vagina and udder, enlargement of undeveloped uteri, ovarian cysts and impaired oocyte development. Pigs are most susceptible to hyperestrogenism, but similar symptoms are also observed in very young ruminants with the undeveloped gastrointes-

tinal tract as well as in young heifers (Gajęcki et al. 2010).

ZEA is metabolized to a hydroxy metabolite, referred to as h-zearalenol, by ruminal protozoa, enterocytes and liver at successive stages of the mycotoxin metabolism. α -zearalenol demonstrates higher affinity to estrogen receptors, and it is more hormonally active than the original substance. Symptoms of hyperestrogenism are observed only in animals fed highly contaminated silage or exposed to low doses of feed-borne ZEA over long periods of time. The above inconsistency can be attributed to the fact that although α -zearalenol is absorbed in small quantities, this active substance is characterized by much higher polarity (Zidane et al. 2007, Gajęcki et al. 2010).

In cattle, the health effects of feed contaminated with deoxynivalenol (DON), a leading mycotoxin and type B trichothecene, are controversial (Trenholm et al. 1985). Very high levels of deoxynivalenol are found in concentrates containing ground grain. The discussed mycotoxin also contaminates straw, grain, maize silage and grass haylage. In healthy animals, DON is very quickly converted into de-epoxide in the rumen. De-epoxide is far less toxic, which explains the animals tolerance to the presence of this substance in feed. In animals with a previous history of ruminal acidosis, DON is not fully broken down, and its presence is determined in the blood (Seeling et al. 2006). Grass haylage contaminated with deoxynivalenol causes the toxic syndrome in cattle, which is characterized by more pronounced inflammations, enteritis, mastitis and laminitis (Weaver et al. 1980). The extent to which the above diseases are caused or exacerbated by DON and other trichothecenes remains unknown. DON is often accompanied by fusaric acid, a popular indicator of toxicity whose effects are sometimes attributed to DON.

Mycotoxicoses are difficult to diagnose owing to a wide variety of pathogens, their varied distribution in feed, multi-directional effects on the host (diverse clinical symptoms) and the high cost of laboratory analyses. Convincing evidence of feed contamination with mycotoxins is also very difficult to obtain. Mycotoxicosis may be suspected in all disease cases which demonstrate non-specific symptoms and are resistant to conventional treatment.

The objective of this study was to determine the effect of combined mycotoxicosis caused by long-term (about 6 months) exposure to low doses of zearalenone and deoxynivalenol on selected haematological and biochemical blood indicators in dairy cattle.

Materials and Methods

Animals

In a herd of 90 dairy cows, non-specific disease symptoms were observed in 12 animals. In the affected cows, symptoms of disease were observed only 2-3 hours prior to death. No changes in appetite were reported. In some individuals, increased salivation was observed after feeding, but only when the total mixed ration (TMR) was administered. The animals were fed grass haylage, maize silage, soybeans, cereal grains, corn-cob-mix (CCM) and mineral supplements. In addition to TMR, the cows had *ad libitum* access to a supplementary mix containing 21% protein. Locomotor dysfunctions, such as movement difficulty in closed premises (cowshed), were noted only in the animals affected. The cows assumed a calving position immediately before death. During the lactation period of 305 days, average milk yield reached 8000 to 11830 kg. Blood was sampled from cows suspected of mycotoxicosis based on untypical behavior (experimental group – E) and from clinically healthy animals (control group – C).

Experimental design

The results obtained in the experimental group (E) were divided into three sub-groups. Sub-group I (EI, n=12 cows) combines the average values of laboratory tests reported for all affected animals. Sub-group II (EII, n=5) presents the results of individuals whose serum samples revealed the presence of DON and ZEA. Sub-group III (EIII, n=7) details the results of cows whose serum samples revealed only the presence of DON.

The above findings were compared with the results of laboratory analyses of the control group of animals (C, n=5) which were free of any disease symptoms and in whose serum samples mycotoxins were not observed or were determined at <5 ng/ml, i.e. below the detection threshold.

Samples for mycotoxicological tests

After collection, blood samples for determination of ZEA and DON levels were directly transferred to chilled centrifuge tubes containing heparin and centrifuged at 3000 r.p.m. for 20 minutes at a temperature of 4°C. The resulting plasma was transferred to 3 ml Eppendorf tubes, frozen and stored at -60°C until analysis.

The plasma concentrations of ZEA and DON

were determined by combined separation techniques with the use of immunoaffinity columns (Zearala-Test™ Zearalenone Testing System, G1012, VICAM, Watertown, USA, and DON-Test™ DON Testing System, VICAM, Watertown, USA) and high-performance liquid chromatography (HPLC) with fluorescence detection (Wang et al. 2002). The analyses were performed at the Department of Veterinary Prevention and Feed Hygiene, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland.

Chromatography column: ODS Hypersil 5 µm 4 x 250 mm No. 799 260D-584, Agilent, USA; Precolumn – RP-18; Injection volume – 20 µl or 100 µl; Mobile phase – acetonitrile : methanol : water (46 : 8 : 46% or 8 : 10 : 82%), – acetonitrile (LiChrosolv™ No. 984730 109, Merck-Hitachi, Germany), – methanol (LiChrosolv™, No. 1.06007, Merck-Hitachi, Germany), – deionized water (Millipore Water Purification System, Millipore S.A., Molsheim-France); Flow rate – 1.8 ml/min or 1.5 ml/min; Detector – Hewlett Packard 1100 fluorescent detector or UV Hewlett Packard 1050 UV detector, FLD G1321A; Excitation wavelength – λ_{Ex} = 218 nm for ZEA, 242 nm for DON; Emission wavelength – λ_{Em} = 438 nm; Column temperature – 30°C; Run time – 4 min or 8 min.

The resulting data were registered and integrated using the POL-LAB Computer Integrator and processed with the CHROMAX for Windows v. 2000 application (Pol-Lab Artur Dzieniszewski). Mycotoxin concentrations were determined by the external standard method, and the resulting values were stated in terms of ppb (ng/ml).

Samples for blood morphology and biochemistry tests

Blood samples for haematological analysis were collected into test tubes containing an anticoagulant and analyzed *ex tempore*. The following haematological parameters were determined: red blood cell counts (RBC), white blood cell counts (WBC), platelet counts, hemoglobin concentrations (Hg), hematocrit levels (PCV). White blood cell percentages were determined in fresh blood smears stained according to the May-Grunwald-Giemza method (band cells [BC], segmented neutrophilic granulocytes [SNG], eosinophilic granulocytes [EG], basophilic granulocytes [BG], lymphocytes [L] and monocytes [M]). The assays were performed using the HEMOCELL 1600 haematological analyzer. Red blood cell counts were determined by conductometry, and hemoglobin concentrations – by colorimetry.

The blood preparation was the same as described above for ZEA and DON determination. The following biochemical parameters were determined: aspartate transaminase activity (AST), content of urea, total protein, albumin, globulin, Ca^{++} , inorganic P, Mg, fibrinogen and prothrombin time. Biochemical analyses were carried out using the EPOLL-20 double beam photometer and the Mindray BS-130 analyzer.

The results of blood morphology and biochemistry analyses are displayed in Tables together with reference values (Winnicka 2002). The analyses were performed at the Chair and Clinic of Internal Medicine, Department of Internal Diseases of Farm Animals and Horses, University of Life Sciences in Lublin, Poland.

Arithmetic means ($-$) and standard deviations (\pm , SD) were calculated. The results obtained were verified statistically by two-way ANOVA and the differences were verified by Duncan's test. Statistical calculations were performed using the STATISTICA® application (Statsoft).

Results

ZEA and DON plasma concentrations are presented in Table 1. High levels of DON were determined in all animals in the range of 5.10 to 5.64 ng/ml. The average values in experimental groups reached 18.48 ng/ml in EIII, 20.92 ng/ml in EI and 24.35 ng/ml in EII. Significant differences were not observed.

In many cows, ZEA concentrations (Table 1) were determined below the detection threshold (<5 ng/ml), and in the remaining animals, the values ranged from 7.34 to 24.79 ng/ml. The average values in experimental groups reached 5.94 ng/ml in EI and 14.30 ng/ml in EII. Significant differences were not observed.

The results of haematological analyses are presented in Table 2. Significant differences in WBC values were observed between groups EI, EII and group C. A highly significant difference was found between group EII and group C, which is indicative of leukocytosis. Significant differences in RBC values were reported between group EII and group C. Hb concentrations in all experimental groups were above the reference line, and no significant variations were observed. PCV levels in all experimental groups were within the reference range, and significant differences were not detected.

White blood cell percentages are shown in Table 2. The share of neutrophilic granulocytes, such

as SNG and BC, was above the reference line, excluding BC in group EIII. Highly significant differences in BC values were observed between groups EI, EIII and group C, and similar differences were reported between groups EI, EIII and group EII. As regards SNG percentages, significant variations were observed only between group EII and group C. Granulocyte percentages are indicative of a shift to the right in the blood picture, in particular in group EII.

The remaining white blood cell values (EG, BG, L, M) (Table 2) were below the reference range, except for EG in group C and lymphocyte percentages in group EIII. Significant differences were determined between group EII and group C as regards the share of L, which is indicative of lymphocytopenia. Highly significant differences were observed in EG values between EII and group C, and in L values between groups EI, EIII and group EII.

Serum biochemistry profiles for all experimental groups are presented in Table 3. AST activity, total protein content and fibrinogen concentrations were determined above the reference range. Ca^{++} levels, inorganic P concentrations (excluding in group C) and the albumin/globulin ratio (excluding in groups EII and C) were also higher than the reference values. Significant differences were reported in AST activity between group EI and group C, and highly significant differences were observed between group EII and group EIII, and between group EII and group C. Total protein levels varied significantly between group C and group EI, and highly significant differences were observed between group C and group EIII (40.51 g/l) and between group EII and group EIII. Significant variations in Ca^{++} concentrations were determined between groups EI, EII, EIII and group C. Inorganic P levels differed significantly between groups EI, EII, EIII and group C. The albumin/globulin ratio revealed significant variations between group EIII and group C and between group EIII and group EII. The total protein/fibrinogen ratio differed significantly between groups EI, EII, EIII and group C. Significant differences were also reported in fibrinogen levels between groups EII, EIII and group C.

Five animals died during the experiment, including three cows from sub-group EII and two from sub-group EIII. Postmortem examinations revealed pulmonary edema, purulent exudate in the trachea and bronchi, submucosal extravasations in the small intestine and bowel inflammation. The above material was not subjected to anatomopathological tests due to advanced decomposition.

Table 1. DON and ZEA plasma concentrations in cows (\pm , SD).

Group	Number of animals	DON ng/ml	ZEA ng/ml
EI	12 cows	20.92 \pm 17.31	5.94 \pm 5.32
EII	5 cows	24.35 \pm 16.09	14.30 \pm 5.24
EIII	7 cows	18.48 \pm 12.27	< 5
C	5 cows	< 5	< 5

Values < 5 are below the detection limit.

Table 2. Haematological values in affected cows (\pm , SD).

Group	WBC $\times 10^9$	RBC $\times 10^{12}$	Hb g/dl	PCV “1”	BC “1”	SNG “1”	EG “1”	BG “1”	L “1”	M “1”
EI	35.96* + 15.02	10.42 + 6.79	14.77 + 2.87	0.43 + 0.02	0.03*** + 0.00	0.54 + 0.14	0.01 + 0.00	0.00 0.00	0.41 + 0.12	0.02 + 0.00
EII	40.19** + 12.41	12.02* + 5.71	15.24 + 1.43	0.47 + 0.05	0.08 + 0.00	0.73* + 0.23	0.00** + 0.00	0.00 0.00	0.18** + 0.08	0.02 + 0.00
EIII	34.22* + 9.52	10.22 + 7.88	14.36 + 3.81	0.42 + 0.07	0.01*** + 0.00	0.47 + 0.08	0.01 + 0.00	0.00 0.00	0.49 + 0.15	0.03 + 0.001
C	22.80 + 4.86	8.47 + 4.75	13.72 + 2.62	0.38 + 0.06	0.07 + 0.01	0.52 + 0.10	0.02 + 0.00	0.00 0.00	0.38 + 0.18	0.02 + 0.001
Reference values	4.0–10.0	5.0–8.0	8–14	0.24–0.46	< 0.02	0.15–0.45	0.02–0.2	0.00–0.02	0.45–0.72	0.02–0.07

*, ** – statistically significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences between groups EI, EII and EIII and group C;
 •, •• – statistically significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences between groups EI, EIII and group EII

Table 3. Biochemical values in affected cows (\pm , SD).

Group	C	EIII	EII	EI	References values
AST U/l	123.54 \pm 31.96	110.02 \pm 27.58	390.34**•• \pm 42.14	181.71* \pm 87.47	30-100
Urea mg/dl	25.04 \pm 10.16	20.27 \pm 8.24	24.12 \pm 9.47	22.07 \pm 10.02	10-45
Total protein g/l	116.21 \pm 57.25	75.70** \pm 23.48	96.46** \pm 42.37	87.70* \pm 32.16	51-71
Albumin g/l	42.58 \pm 20.34	44.13 \pm 12.43	41.34 \pm 18.75	43.52 \pm 16.84	32-49
Globulin g/l	47.46 \pm 15.73	31.56 \pm 18.49	53.47 \pm 22.68	38.88 \pm 15.74	28-88
Albumin/Globulin ratio	0.98 \pm 0.03	1.26*• \pm 0.02	0.83 \pm 0.01	1.01 \pm 0.03	0.84-0.94
Ca ⁺⁺ mmol/l	1.16 \pm 0.05	1.35* \pm 0.09	1.36* \pm 0.03	1.32* \pm 0.04	1.15-1.30
Inorganic P mg/dl	8.68 \pm 3.14	10.60* \pm 4.77	10.69* \pm 5.01	10.24* \pm 4.67	4-67
Mg mg/dl	2.38 \pm 0.96	2.10 \pm 1.22	2.19 \pm 0.74	2.22 \pm 0.57	1.8-3.2
Prothrombin time sec	31.14 \pm 5.84	26.98 \pm 3.89	29.86 \pm 5.01	30.42 \pm 4.37	17-36
Fibrinogen mg/dl	829.62 \pm 495.38	962.47* \pm 501.62	957.45* \pm 659.27	872.71 \pm 251.47	510-660
Total protein/ Fibrinogen ratio	11.04 \pm 4.07	8.93* \pm 1.47	9.63* \pm 2.54	18.39* \pm 4.72	>15

*, ** – statistically significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences between groups EI, EII, EIII and group C;
 •, •• – statistically significant ($p \leq 0.05$) or highly significant ($p \leq 0.01$) differences between groups EI, EIII and group EII.

Discussion

Mycotoxins cause massive economic losses in dairy cattle production. Due to the difficulty and high cost of sampling, mycological tests are rarely performed. According to Danish research into mycotoxin contamination of feedstuffs and milk, the presence of DON and ZEA is observed in around 81% and 46% of the samples analyzed, respectively (Dänicke et al. 2005, Fink-Gremmels 2005, 2008, Gajęcki 2010). In Dutch studies involving clinical observations and evaluations of animals exposed to individual mycotoxins, clinical symptoms indicative of the disease were not reported (Fink-Gremmels 2005, 2008, Gajęcki 2010). The above findings suggest that ruminants have a physiological ability to attenuate the adverse effects of mycotoxins in feedstuffs subject to the dose applied (Calabrese 2005).

According to scant scientific data, DON causes a decrease or loss of appetite, gastrointestinal ulceration, in particular in the rumen and reticular mucosa, decreased milk yield, drop in somatic cell counts and fertility disorders. DON impairs ruminal fermentation and decreases the quantity of available protein in the duodenum (Dänicke et al. 2005). A study of mice exposed to DON revealed a significant drop in globulin levels and an increase in albumin concentrations (Rotter et al. 1996).

In this experiment, an increase in total protein levels probably resulted from dehydration or fluid sequestration, as demonstrated by high PCV and Ca^{++} values as well as leukocytosis. Total protein profiles have limited diagnostic value. The albumin/globulin ratio is a more robust indicator, and in this study, A/G ratio values were significantly lower in groups EI and EII, where the presence of ZEA was determined, than in group EIII. A drop in A/G ratio values could point to globulin overproduction which is noted in autoimmune disorders. The above was observed in animals infected with both ZEA and DON which potentiated (Boermans and Leung 2007) the effects of these mycotoxins on inflammatory bowel disease (Maresca and Fantini 2010). An increase in total protein concentrations in group EII resulted from acute or chronic inflammation of the gastrointestinal tract, as demonstrated by elevated fibrinogen levels and a decrease in the values of the total protein/fibrinogen ratio. Higher levels of AST activity and an increase in total protein concentrations point to mycotoxin-induced stimulation of the liver. PT values were found in the upper reference interval, thus ruling out the suspicion of liver damage.

It should also be noted that DON can enhance the permeability of enterocyte membranes (Sergent et al. 2006, Pinton et al. 2009). DON intoxication also sup-

presses cytotoxicity and protein synthesis, and increases the permeability of the gut wall (Kouadio et al. 2007). The above is manifested by changes in the expression of claudins, the main components of tight junctions, and it is observed in human enterocytes and porcine intestines (Pinton et al. 2009). DON also has a specific effect on the expression of claudin isoforms and their microdomains, known as rafts (Lambert et al. 2007).

The presence of DON in the gastrointestinal tract stimulates the production of mucosal antibodies and autoantibodies, as observed in IBD patients (Baumgart and Carding 2007, Briani et al. 2008). DON increases IgA secretion from Peyer's patch lymphocytes through the activation of MAP kinases and proinflammatory cytokines, and COX-2 gene expression (Pestka and Smoliński 2005). The discussed mycotoxin has nephropathic properties, and it causes dysfunctions in IgA secretion, whereas in theory, it should stimulate the production of intestinal anti-bacterial antibodies and self-antigens (Baumgart and Carding 2007, Briani et al. 2008). The above suggests that when administered in low doses to monogastric organisms, DON increases the production of IgA which interacts with many self-antigens and intestinal bacteria, as noted in IBD (Maresca and Fantini 2010). Most likely, this was also the case in this experiment.

The chemical structure of ZEA resembles that of estrogens. ZEA intoxication leads to reproductive system disorders, including symptoms of estrus in pre-pubertal calves, irregular heats, silent heats, embryonic loss, miscarriages, placental retention, metritis and mastitis. ZEA mycotoxicosis causes loss of appetite, decreased feed intake, lower milk yield, intestinal inflammations and diarrhea. Contrary to our observations, Korosteleva et al. (2007, 2009) did not report any changes in the haematological profile of the animals examined. In most cases, the cited authors observed an increase in total protein levels and globulin concentrations after 42 days of experimental intoxication with *Fusarium* mycotoxins. In our experiment, the exact date on which the combined intoxication began remains unknown, but judging from mycotoxin concentrations and post-mortem changes in the animals studied, it could have persisted for a longer period of time. Other factors that could have contributed to the above include the time required to break down the rumen defense system (microbiology) and the dose of active mycotoxins reaching successive parts of the gastrointestinal tract – threshold doses (Calabrese 2005) or maximum NOAEL values (Boermans and Leung 2007).

ZEA significance for the function of the intestinal barrier has not been determined to date, therefore, it can be assumed that its proapoptotic and cytotoxic

properties in enterocytes (Kouadio et al. 2005) cause changes in the intestinal barrier activity due to DON potentiating effects on ZEA. The data presented suggest that both mycotoxins increase intestinal permeability, facilitating the flow of antigens from the intestinal lumen into the body, which could be the prime cause of inflammatory bowel disease. The results obtained in group EII indirectly testify to the above (significantly elevated total protein levels, globulin concentrations, WBC and AST values, a high share of segmented neutrophilic granulocytes and the lowest percentage share of lymphocytes).

In addition to immunosuppressive and proinflammatory properties, selected mycotoxins affect Th1/Th2 polarization, as observed in IBD (Baumgart and Carding 2007, Briani et al. 2008). When administered to monogastric animals, DON elicits a specific immune response which is manifested by increased Th2 activity and a drop in Th1 activity (Li et al. 2005), as found in allergies (Luft et al. 2008, Maresca and Fantini 2010). A similar scenario is observed in rumen dysfunctions where DON acts as an antibacterial agent. In consequence, mycotoxins reach successive segments of the gastrointestinal tract where the course of intoxication is similar to that observed in monogastric animals (Gajęcki 2010).

DON effect on lymphocyte polarization in animals seems to suggest that DON and, probably, ZEA are capable of altering the polarization of lymphocytes, including systemic lymphocytes in Peyer's patches (Maresca and Fantini 2010). Mucosal (not systemic) lymphocytes play an important role in IBD, and the lymphocytotoxicity that accompanies mycotoxin-induced dysfunctions is the key determinant of the pathogenicity of mycotoxins (ZEA and DON) in dying animals.

The results of this study suggest that group EII animals were exposed to natural, low-dose zearalenone and deoxynivalenol mycotoxicosis which induced an acute autoimmune response, as indirectly confirmed by blood morphological and biochemical tests. Immunosuppression was also observed, as indirectly documented by blood morphological tests and post-mortem examinations.

References

- Baumgart DC, Carding SR (2007) Inflammatory bowel disease: cause and immunobiology. *Lancet* 369: 1627-1640.
- Boermans HJ, Leung MC (2007) Mycotoxins and the pet food industry: toxicological evidence and risk assessment. *Int J Food Microbiol* 119: 95-102.
- Briani C, Samaroo D, Alaedini A (2008) Celiac disease: from gluten to autoimmunity. *Autoimmun Rev* 7: 644-650.
- Calabrese EJ (2005) Paradigm lost, paradigm found: the re-emergence of hormesis as a fundamental dose response model: in the toxicological sciences. *Environ Pollut* 138: 379-411.
- Charmley E, Trenholm HL, Thompson BK, Vudathala D, Nicholson JW, Prelusky DB, Charmley LL (1993) Influence of level deoxynivalenol in the diet of dairy cows feed intake, milk production and its composition. *J Dairy Sci* 6: 3580-3587.
- Dänicke S, Matthaus K, Lebzien P, Valenta H, Stemme K, Ueberschar KH, Razzazi-Fazeli E, Bohm J, Flachowsky G (2005) Effects of Fusarium toxin-contaminated wheat grain on nutrient turnover, microbial protein synthesis and metabolism of deoxynivalenol and zearalenone in the rumen of dairy cows. *J Anim Physiol An* 89: 303-315.
- Driehuis F, Spanjer MC, Scholten M, Giffel MC (2008) Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. *J Dairy Sci* 91: 4261-4271.
- Fink-Gremmels J (2005) Mycotoxins in forages. In: Diaz, D.E. (Ed.), *The Mycotoxin Blue Book*. Nottingham University Press, Nottingham, United Kingdom, pp. 249-268.
- Fink-Gremmels J (2008) Mycotoxins in cattle feeds and carry-over to dairy milk: a review. *Food Addit Contam* 25: 172-180.
- Gajęcki M (2010) Mikotoksykozy u bydła. *Lecznica Dużych Zwierząt* 5: 108-112.
- Gajęcki M, Gajęcka M, Jakimiuk E, Zielonka Ł, Obremski K (2010) Zearalenone – undesirable substance. In: Rai Mahendra, Varma Ajit, (eds) *Mycotoxins in food, feed and bioweapons*. Springer-Verlag Berlin Heidelberg, pp 131-144.
- Hochsteiner W, Schuh M, Luger K, Baumgartner W (2000) Effect of mycotoxin contaminated feed on production parameters of dairy cows. *Berl Munch Tierarztl Wochenschr* 113: 14-21.
- Keese C, Meyer U, Rehage J, Spilke J, Boguhn J, Breves G, Danicic S (2008) Ruminant fermentation patterns and parameters acid base metabolism in the urine as influenced by the proportion concentrate in the ration of dairy cows with and without Fusarium toxin -contaminated triticale. *Arch Anim Nutr* 62: 287-302.
- Korosteleva SN, Smith TK, Boermans HJ (2007) Effect of feedborne Fusarium mycotoxins on the performance, metabolism and immunity of dairy cows. *J Dairy Sci* 90: 3867-3873.
- Korosteleva SN, Smith TK, Boermans HJ (2009) Effects of feed naturally contaminated with Fusarium mycotoxins on metabolism and immunity of dairy cows. *J Dairy Sci* 92: 1585-1593.
- Kouadio JH, Dano SD, Moukha S, Mobio TA, Creppy EE (2007) Effects of combinations of Fusarium mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. *Toxicol* 49: 306-317.
- Kouadio JH, Mobio TA, Baudrimont I, Moukha S, Dano SD, Creppy EE (2005) Comparative study of cytotoxicity and oxidative stress induced by deoxynivalenol, zearalenone or fumonisin B1 in human intestinal cell line Caco-2. *Toxicology* 213: 56-65.
- Lambert D, Padfield PJ, McLaughlin J, Cannell S, O'Neill CA (2007) Ochratoxin A displaces claudins from detergent resistant membrane microdomains. *Biochem Biophys Res Commun* 358: 632-636.

- Li M, Cuff CF, Pestka J (2005) Modulation of murine host response to enteric reovirus infection by the trichothecene deoxynivalenol. *Toxicol Sci* 87: 134-145.
- Luft P, Oostingh GJ, Gruijthuisen Y, Horejs-Hoeck J, Lehmann I, Duschl A (2008) Patulin influences the expression of Th1/Th2 cytokines by activated peripheral blood mononuclear cells and T cells through depletion of intracellular glutathione. *Environ Toxicol* 23: 84-95.
- Maresca M, Fantini J (2010) Some food-associated mycotoxins as potential risk factors in humans predisposed to chronic intestinal inflammatory diseases. *Toxicol* 56: 282-294.
- Pestka JJ, Smolinski AT (2005) Deoxynivalenol: toxicology and potential effects on humans. *J Toxicol Environ Health B Crit Rev* 8: 39-69.
- Pinton P, Nougayrède JP, Del Rio JC, Moreno C, Marin DE, Ferrier L, Bracarense AP, Kolf-Clauw M, Oswald IP (2009) The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. *Toxicol Appl Pharmacol* 237: 41-48.
- Rotter BA, Prelusky DB, Pestka JJ (1996) Toxicology of deoxynivalenol (vomitoxin). *J Toxicol Environ Health* 48: 1-34.
- Seeling K, Lebzien P, Danicke S, Spilke J, Sudekum KH, Flachowsky G (2006) Effects of level of feed intake and Fusarium toxin-contaminated wheat on rumen fermentation as well as blood and milk parameters in cows. *J Anim Physiol Anim Nutr* 90: 103-115.
- Sergent T, Parys M, Garsou S, Pussemier L, Schneider YJ, Larondelle Y (2006) Deoxynivalenol transport across human intestinal Caco-2 cells and its effects on cellular metabolism at realistic intestinal concentrations. *Toxicol Lett* 164: 167-176.
- Trenholm HL, Thompson BK, Hartin KE, Geenhalgh R, McAllister AJ (1985) Ingestion of vomitoxin (deoxynivalenol) – contaminated wheat by nonlactating cows. *J Dairy Sci* 68: 1000-1005.
- Weaver GA, Kurtz HJ, Mirocha CJ, Bates FY, Behrens JC, Robison S, Swanson SP (1980) The failure of mycotoxin to produce hemorrhaging in dairy cattle. *Can Vet J* 21: 210-213.
- Winnicka A (2002) Wartości referencyjne podstawowych badań laboratoryjnych w weterynarii. Wydawnictwo SGGW Warszawa, pp 122.
- Zinedine A, Soriano JM, Moltó JC, Mañes J (2007) Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. *Food Chem Toxicol* 45: 1-18.