POSTER PRESENTATIONS

CYTOTOXICITY OF THE MYCOTOXINS IN FEEDSTUFFS

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SUMMARY

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response when ingested by higher animals. In colder more temperature regions: Canada, USA and most European countries as Lithuania fusariotoxins: DON, ZEN and ochratoxins are found more frequently. These mycotoxins may potentially affect animal health and productivity. DON exposure leads to apoptosis both in and in vivo in several organs such lymphoid organs haematopoietic tissues, liver and intestinal crypts intoxications. ZEN has high binding affinity for the intra-cellular oestrogen receptor and can enhance the proliferation of oestrogen responsive tumour cells. Ochratoxins (ochratoxin A) have an effect on immunoglobulins and phagocytic cells. The aim of this study was to investigate the cytotoxicity of the most commonly found mycotoxins: DON, ZEN, ochratoxins in naturally contaminated grains and feedstuffs samples.

Keywords: apoptosis, deoxynivalenol, feedstuffs, K-562, MH-22A, ochratoxins, SPEV, zearalenone

MATERIALS AND METHODS

K-562 human hematopoetic and swine kidney (SPEV) cell lines were cultured RPMI Medium 1640 w/glutamine, MH-22A mice-derived hepatoma cell line was cultured in DMEM supplemented with 10% FBS and antibiotics under standard conditions.

Naturally contaminated grains and feedstuffs were purified after extraction acetonitrile/water (84/16). Samples extracts were purified using the Mycosep # 227 cleanup column for DON, ZEN – Mycosep # 226, ochratoxins – MultiSep # 212. The clear extracts were evaporated Romer-Evap^{TM} system and the residues were dissolved in cell medium and incubated in triplicates as two-fold dilution series with cell lines on a 96-well microtiter plate for 24 h. Cells were seeded at a density of $1x10^{-3}$ – $1x10^{-4}$ cells/ml. The viability of cell population was examined using the crystal violet [6] and MTT assay [3]. Cells viability was assayed spectrophotometrically using a Multiskan MS photometer (Finland).

The DON, ZEN, aflatoxins concentrations were analyzed thin layer chromatography (TLC) method. Ochratoxins, T-2 toxn were analyzed by the enzyme-linked immunosorbent assay (ELISA). Veratox test kits (Neogen Corporation, USA) were used for the analysis. The cytotoxicity of DON, ZEN, ochratoxins was determined by measuring different endpoints such as inhibition of protein and DNA synthesis, plasma membrane integrity and reduced metabolic activity. The IC_{50} value (the concentration of each sample reducing the total response to a 50% value of untreated cells) for cytotoxic compound was calculated.

RESULTS AND DISCUSSION

DON cause necrosis and haemorrhage throughout the digestive tract, depress blood regenerative processes in the bone marrow and spleen. DON has been shown to be potent inhibitors of the eucaryote protein synthesis. Cellular effects on DNR synthesis, DNR breakage and membrane integrity have been considered to be secondary effects of the inhibited protein synthesis [4]. DON exposure leads to apoptosis both in vitro [7] and in vivo in several organs such lymphoid organs haematopoietic tissues, liver and intestinal crypts intoxications [1].

A finding which according to the authors indicated that inhibition of the protein synthesis and apoptosis are the main mechanisms for DON toxicity in the cells [5].

In this study we evaluated the cytotoxic effect of grain and feedstuffs contaminated with DON (table 1). Cells lines K-562 and MH-22A was used to evaluate the effects of DON on the blood cells.

Grains and feedstuffs contaminated with DON concentration 1000–3000 ng/ml have significant effect for cell line K-562. Feedstuffs with DON caused apoptosis K-562 cells 60±15%. There were no significant differences between toxicity assay grain and feedstuffs for K-562. Significant differences of were feedstuffs for MH-22A – 56–118%. The DON-free feedstuffs samples had a cytotoxic effect on the K-562 cells equivalent to DON. A recent study has shown that less than 10 µmol/ml DON (0,296 µg/ml) selectively inhibited some intestinal transport protein in human intestinal epithelial cells when the cells were incubated with the toxin for 24 and 48 hours.

Table 1. Cytotoxicity of deoxynivalenol and concentration of mycotoxins deoxynivalenol, T-2 toxin, zearalenone, ochratoxins, aflatoxins in grain and forages (500 mg extract/ml)

Samples	[a]Toxicity assay Cell cultures		[b]IC_{50} Cell cultures		Mycotoxins concentration (ng/ml)				
	K-562	MH-22A	K-562	MH-22A	DON	T-2	ZEN	OT	AFL
Triticale	58	90	1751±264	1158±257	2000	[c]nc	50	nc	1
Barley	53	109	472±57	460±30	1000	nc	130	nc	*d*
Wheat	67	118	1951±274	1173±157	3000	nc	500	nc	nc
Farrowing sows composite forage	53	73	1180±115	856±67	1250	nc	400	nc	*d*
Fattening pigs composite forage	68	56	1112±136	1339±339	1500	20	300	nc	1

[a] % apoptosis of cell cultures exposed to duplicate samples at 500 mg extract/ml compared to cells.
[b] IC_{50} (mg extract/ml cell culture medium). Data as mean ± standard deviation of at least three experiments.
[c] Not calculated.
[d] Not detected (concentration below the limit of detection).

Zearalenone is usually non-lethal to animals, but it is important to animal producers because its hyperestrogenic effects adversely influence the reproductive performance of animals. ZEN has high binding affinity for the intra-cellular estrogens receptor and can enhance the proliferation of oestrogen responsive tumour cells. There have been suggestions of the involvement of ZEN in human cervical cancer and premature initial breast development [2].

The cytotoxic effect of grain contaminated with ZEN was 394–563 ng/ml (table 2). Toxicity effect the grain samples were 46±6% for K-652, MH-22A – 52±23%. Feedstuffs with ZEN had

toxicity effect for MH-22A bigger. According to our data, K-562 cells and MH-22A proved to be resistant to grain samples contaminated with ZEN.

Table 2. Cytotoxicity of zearalenone and concentrations of mycotoxins deoxynivalenol, T-2 toxin, zearalenone, ochratoxins, aflatoxins in grain and forages (562 mg extract/ml)

Samples	[α]Toxicity assay Cell cultures		[b]IC_{50} Cell cultures		Mycotoxin concentration (ng/ml)				
	K-562	MH-22A	K-562	MH-22A	DON	T-2	ZEN	OT	AFL
Triticale	53	41	388±37	489±54	8,4	[c]nc	394	nc	0.4
Barley	37	46	782±143	634±148	8.4	nc	563	nc	0.4
Pease	47	69	627±162	424±93	0.56	nc	563	nc	*d*
Gestating sows composite forage	87	56	396±69	613±119	0.56	nc	675	nc	nc
Farrowing sows composite forage	99	73	285±191	387±109	0.56	nc	563	nc	nc

[α] % apoptosis of cell cultures exposed to duplicate samples at 500 mg extract/ml compared to cells.
[b] IC_{50} (mg extract/ml cell culture medium). Data as mean ± standard deviation of at least three experiments.
[c] Not calculated.
[d] Not detected (concentration below the limit of detection).

According to our data, K-562 cells and MH-22A proved to be resistant to grain samples contaminated with ZEN.

Ochratoxins (ochratoxin A) damages the kidneys of many types of animals. High concentrations of dietary ochratoxins can cause liver damage as well intestinal necrosis and haemorrhage. Ochratoxin A has been shown suppress immunity and to be carcinogenic. General indicators of immunosuppression following ochratoxin A ingestion include lymphocytopenia and depletion of lymphoid cells. This mycotoxin has an effect on immunoglobulins and phagocytic cells [1].

The cytotoxic effect of grain and feedstuffs contaminated with ochratoxins were 49±16% swine kidney SPEV cells, K-562 cells. Insignificant toxic effect was for MH-22A.

Table 3. Cytotoxicity of ochratoxins and concentrations of mycotoxins deoxynivalenol, T-2 toxin, zearalenone, ochratoxins, aflatoxins in grain and forages (875 mg extract/ml)

Samples	[α]Toxicity assay Cell cultures			[b]IC_{50} Cell cultures			Mycotoxin concentration (ng/ml)				
	SPEV	K-562	MH-22A	SPEV	K-562	MH-22A	DON	T-2	ZEN	OT	AFL
Barley	48	48	80	123±12	129±28	73±2	175	17	189	118	*d*
Barley	61	54	94	17±5	19±2.5	11±4	*d*	39	*d*	20	*d*
Wheat	45	55	89	39±1	32±3	20±3	*d*	[c]nc	*d*	35	nc
Wheat	48	51	101	22±5	20±3	10±0.5	nc	nc	nc	20	nc
Piglets composite forage	45	39	119	23±2	23±2	10±4	*d*	0.9	*d*	23	*d*

[α] % apoptosis of cell cultures exposed to duplicate samples at 500 mg extract/ml compared to cells.
[b] IC_{50} (mg extract/ml cell culture medium). Data as mean ± standard deviation of at least three experiments.
[c] Not calculated.
[d] Not detected (concentration below the limit of detection).

About 50% of the SPEV and K-265 cells were obviously dead after exposure ochratoxins concentrations 20–23 ng/ml in samples extracts.

The fact, that higher mycotoxins concentrations have toxic effect for different cell cultures. Despite of using different cell cultures and cytotoxicity endpoints than other authors, the our results comparable to literature data.

CONCLUSIONS

Cell culture systems can be more sensitive and more reproducible than tests involving intact animals. These cell culture assays can be used for the screening of toxicity of mycotoxins. The comparison of toxic responses obtained with each bioassay may orient to its toxicological mechanism.

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