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Short communication

In vitro evaluation of zearalenone toxicity by comet assay

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Abstract

The aim of this study was to reveal the potentially genotoxic effect of zearalenone on bovine lymphocytes by comet assay *in vitro*. The bovine lymphocytes were exposed to various zearalenone concetrations (50; 10; 2; 0.4 and 0.08 ppm). The viability and DNA damage of lymphocytes was monitored after 2 h, 24 h, 48 h and 72 h. After 2 hours of zearalenone exposure, statistically significant DNA damage occurred at all tested concentrations of 0.08 ppm (12.2±1.25; $p<0.05$), 0.4 ppm (12.7±0.88; $p<0.01$), 2 ppm (12.0±0.51; $p<0.01$), 10 ppm (11.2±0.47; $p<0.01$) and at 50 ppm (14.2±0 61; $p<0.001$). Significantly greater DNA damage was also found after 24 h, 48 h and 72 h. The obtained results showed that zearalenone may induce DNA damage of the bovine lymphocytes.

Key words: mycotoxins, toxicity, comet assay

Introduction

The most important secondary metabolite of the *Fusarium* fungi is zearalenone (ZEN). ZEN is most commonly found in cereals (corn, wheat, oats and barley), which are the main bovine feed components (Reddy et al. 2018). This mycoestrogen causes fertility problems, especially in pigs and cattle. Zearalenone negatively affects conception, ovulation, implantation, fetal development and the viability of the newborn. Its hepatoxic and genotoxic effects, which also lead to immunosuppression, are also known (Thapa et al. 2021). A favorable genotoxic analysis is comet assay, because it is cheap, simple, and can be used on many organisms and different tissues (Szabó et al. 2019). *In vitro* alkaline single cell gel electrophoresis is a sensitive method for detecting reparable DNA damage such as single- and double-strand breaks or alkaline labile sites (Klarić et al. 2010). The aim of this study was *in vitro* evaluation of the genotoxicity of ZEN at various concentrations on bovine lymphocytes by comet assay.

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Table 1. DNA damage (%) and viability (%) after 2, 24, 48 and 72 hours of zearalenone treatment at various concentrations on bovine lymphocytes.

Z	2 h		24 h		48 h		72 h	
	DNA damage x±SD	V (%)	DNA damage x±SD	V (%)	DNA damage x±SD	V (%)	DNA damage x±SD	V (%)
0	9.9±0.19	94.8	10.3±0.74	94.1	8.8±1.55	91.7	12.0±1.00	87.5
0.08	12.2±1.25 *	94.7	11.7±1.76	92.9	10.9±0.76	87.5	13.3±1.52	83.3
0.4	12.7±0.88 **	95.8	13.7±0.72 **	92.3	13.6±1.95 *	91.0	12.5±1.52	85.7
2	12.0±0.51 **	94.4	15.5±0.60 ***	91.0	12.4±0.84 *	87.5	14.3±0.77 *	80.0
10	11.2±0.47 **	94.7	14.6±0.34 ***	91.7	13.8±0.82 **	85.7	14.4±0.93 *	75.0
50	14.2±0.61***	90.0	15.9±0.60 ***	87.5	15.6±1.05 **	83.3	15.7±0.83 **	66.7

Z – zearalenone (mg/kg), x – average value, SD – standard deviation, V (%) – viability of lymphocytes, * $p<0.05$; ** $p<0.01$; *** $p<0.001$ – statistical significance compared to control sample (ANOVA).

Materials and Methods

For comet assay, bovine peripheral lymphocytes were isolated using Histopaque 1077 separation medium (Sigma-Aldrich, St. Louis, MO, USA). The lymphocyte isolation procedure was performed according to the Human PBMC isolation protocol (Sigma Aldrich, St. Louis, MO, USA). Lymphocytes were incubated in culture medium (Sigma Aldrich, St. Louis, MO, USA). Cells were treated with ZEN at concentrations of 50, 10, 2, 0.4 and 0.08 ppm (Sigma Aldrich, Schnelldorf, Germany) for 2 h, 24 h, 48 h, 72 h and with distilled water (negative control) and 3% hydrogen peroxide solution (positive control) (Mikrochem, Slovak Republic) after their mixing with low melting point agarose (LMP) on microscope slides before lysis. Using Trypan blue, the relative cell viability (V) was determined, which was calculated according to the formula:

$$V = 100 - \left(\frac{non\ viable\ cells}{total\ number\ of\ cells} \right) \times 100$$

The detailed procedure was previously described by Koleničová et. al (2021). A total of 100 comets were analyzed on each slide using a NIKON Labophot 2A fluorescence microscope, equipped with a single band pass filter, Texas Red. The comets were evaluated according to the intensity of DNA damage in the tail (Końca et al. 2003). The results were evaluated using GraphPad Prism 5.0 (GraphPad software Inc. CA, USA) using a one-way ANOVA test, Student´s T test. The results are presented as mean ±SD, where n= 3.

Results and Discussion

The results of the comet analysis of DNA damage and the viability of bovine peripheral blood lymphocytes after zearalenone exposure are summarized in Table 1. After 2 hours of exposure, statistically significant DNA damage occurred at all tested concentrations of ZEN. Kachlek et al. (2017) in a similar study monitored the viability of porcine lymphocytes after exposure to zearalenone at a concentration of 50 mg/kg and the viability of lymphocytes after 24 hours exposure was 19.55%, after 48 hours 20% and after 72 hours it had decreased to 16.6%. These different results confirmed the well-known fact that pigs are the most sensitive species of all animals to zearalenone (Marin et al. 2011) and the bovine lymphocytes we tested were less sensitive to toxic ZEN effects. The principle of alkaline comet assay is based on the disruption of non-bonding interactions between nitrogen bases and the division of the twin-screw into two fibers. Single-strand breaks are manifested by the release of single-stranded DNA fragments, which are detectable by comet analysis and determine the percentage of DNA damage (Collins 2004). However, single-strand breaks can be repaired quickly and are not very serious in violating genomic integrity (Jackson 2002). The results of our study indicate that zearalenone may induce DNA damage. Kachlek et al. (2017) reached similar conclusions; they confirmed the genotoxic effect of ZEN also in combination with deoxynivalenol and fumonisin B_1, especially after long-term exposure (72 h). According to Bouaziz et al. (2013), ZEN induces DNA damage and apoptosis through oxidative stress. However, Gao et al. (2013) reported that the lysosome is the main determining factor for ZEN-induced DNA strand breaks.

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