Assessment of genotoxicity and intestinal microbiome in sexually immature gilts exposed to low doses of zearalenone

Abstract

Zearalenone (ZEN) is a toxic low-molecular-weight molecule, produced naturally by molds in crop plants as a secondary metabolite. The aim of this study was to determine the activity of ZEN in the intestines based on an analysis of time-dependent changes in the genotoxicity of *cecal water*, intestinal microbiote and mycobiote in pre-pubertal gilts exposed *per os* to low ZEN doses (MABEL, NOAEL and low LOAEL dose).

An *in vivo* experiment was performed on 60 clinically healthy pre-pubertal gilts with initial body weight of 14.5 ± 2 kg. The animals were randomly divided into three experimental groups (group ZEN5, group ZEN10 and group ZEN15; n=15) and a control group (group C, n=15). Group ZEN5 gilts were *per os* administered 5 μg ZEN/kg BW (MABEL), group ZEN10 – 10 μg ZEN/kg BW (NOAEL) and group ZEN15 – 15 μg ZEN/kg BW (low LOAEL). Five gilts from each group were euthanized on analytical dates 1 (exposure day 7), 2 (exposure day 21) and 3 (exposure day 42). Samples for *in vitro* analyses were collected from an intestinal segment resected from the following regions: duodenum – the first part of the duodenum, i.e. duodenal bulb (*bulbus duodeni*) and the third (horizontal) part of the duodenum; jejunum; cecum; descending colon – from the middle part. Samples for genotoxic analyses were collected from the ileocecal region and the colon. Intestinal segments were tied at both ends before resection to avoid tissue damage. The experimental material was collected under sterile conditions, and it was transported to specialist laboratories where samples for further analyses were obtained. The genotoxicity of *cecal water* was determined by the comet assay. The intestinal microbiota and mycobiota of gilts were analyzed using microbiological tests.

Genotoxic analyses revealed increasing strength of correlations on the first analytical date in group C and group ZEN5 (weak correlation) with a strong falling trend, and an absence of a linear correlation in group ZEN5 on two successive dates**.** A moderate correlation was observed in groups ZEN10 and ZEN15, with a slightly falling trend on successive days of exposure. The electrophoresis of exposed cells revealed an absence of comet tails in groups C and ZEN5, whereas groups ZEN10 and ZEN15 were characterized by less numerous comets with long and very long tails, respectively. The distribution of LLC-PK1 cells based on the percentage of their DNA in the comet tail was as follows: 15 to 20% in groups C and ZEN5, 30 to 60% in groups ZEN10 and ZEN15; the percentage of cells with damaged DNA was up to 20% in groups C and ZEN5, and from 5 to 15% in the remaining groups. Analyses of the intestinal microbiota revealed static (determined on a given day of the experiment) and dynamic (determined throughout the experiment) differences in the logarithmic (log) values between the proximal and distal intestinal segments, mostly with regard to *Escherichia coli* and *Enterococcus faecalis*. Zearalenone affected the colony counts of intestinal bacteria rather than microbiota diversity, and its effect was greatest in groups ZEN10 and ZEN15. Microbial colony counts were similar in groups ZEN5 and C. In the intestinal mycobiote, ZEN exerted a stimulatory effect on the log values of yeast and mould counts in all intestinal segments, in particular in the colon, and the greatest increase was noted on the first analytical date.

The results of this experiment and laboratory analyses indicate that very low doses of ZEN (MABEL, NOAEL and low LOAEL dose) at different exposure dates contribute to DNA damage proportional to the applied dose of the analyzed mycotoxin. Analyses of the intestinal microbiote and mycobiote show that ZEN induces a counter response and a compensatory effect in gilts. Therefore, it could be hypothesized that the proposed MABEL dose may be used for preventive purposes in young growing gilts.

Key words: zearalenone, low doses, *cecal water*, genotoxicity, intestinal microbiota intestinal mycobiota, pre-pubertal gilts.