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Short communication

## Phytic acid protects porcine intestinal epithelial cells from deoxynivalenol (DON) cytotoxicity

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## ABSTRACT

The purpose of this study was to evaluate the effects of phytic acid (IP<sub>6</sub>) as a possible inhibitor of cellular damage induced by toxic substances such as mycotoxins on a porcine intestinal epithelial cell line (IPEC-1). We first observed that a dose of 5 mM phytic acid decreases cell viability and transepithelial electrical resistance (TEER) of cell monolayer. We next investigate the effect of non-cytotoxic dose of phytic acid on the deoxynivalenol (DON) induced decreased TEER. We showed that treatment with 0.5 mM or 1.0 mM phytic acid restores the decrease in TEER caused by 25 μM DON. In conclusion this study demonstrates that phytic acid decreased the negative effects of deoxynivalenol on the membrane integrity of the IPEC-1 intestinal epithelial cell line.

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## 1. Introduction

Phytic acid is a highly phosphorylated molecule present in cereal grains and seeds. It serves as the major form of storage of phosphorus in the seed as well as being a natural antioxidant (Graf & Eaton, 1993). It is found in most mammalian cells, wherein it is important in regulating vital cellular functions such as signal transduction, cell proliferation and differentiation (Szwergold et al., 1987; Shamsuddin et al., 1997).

Mycotoxins are toxic secondary metabolites produced by fungi that may contaminate animal and human food at all stages of the food chain (Oswald et al., 2005). DON is a type of β-trichothecene that is mainly produced by *Fusarium graminearum* and *Fusarium culmorum*, which are found naturally worldwide, and their cytotoxicity has been demonstrated in animals and various kinds of cells (Zhang et al., 2009). It is known that DON disrupts the functions of cellular membranes (Bunner & Morris, 1988) and alters the intercellular communication (Khera et al., 1982). A decrease in trans-epithelial electrical resistance was observed by Pinton et al. (2009) and Van De Walle et al. (2010).

In fact, studies have demonstrated that trichothecenes stimulate lipid peroxidation, however the dietary use of antioxidants can pro-

vide protection against cell damage. In this study, we used a porcine intestinal epithelial cell line to determine the effects of phytic acid and DON on intestinal epithelial integrity.

## 2. Materials and methods

## 2.1. Cell culture and reagents

The Intestinal Porcine Epithelial Cells (IPEC-1) cell line is a newborn swine intestinal epithelial cell line that was derived from the small intestine of a newborn unsuckled piglet. The cell lines were maintained by serial passages as previously described (Bouhet et al., 2004). Purified DON (Sigma, St Quentin Fallavier, France) was dissolved in DMSO and stored at –20 °C before dilution in cell culture media. Inositol hexaphosphate (phytic acid sodium salt hydrate purchased from Sigma–Aldrich Co.) was dissolved in sterilized water (0.4 g/mL) and stored at –20 °C before dilution in cell culture media.

## 2.2. MTS bioassay

The effects of phytic acid on the viability of IPEC-1 cells were studied in a colorimetric assay using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay Kit (Promega Charbonnières, France). IPEC-1 cells were seeded at a concentration of 5 × 10<sup>3</sup> cells/well in 100 μL of adequate medium in flat-bottomed 96-well plates (Polylabo-Nunc). After culturing for 24 h, various concentrations of phytic acid (0;

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0.5; 1.0; 2.5 and 5.0 mM) were prepared, pH-adjusted with NaOH to 7.0 and sterilized by passing through a 0.22- $\mu$ m membrane filter. Subsequently, the solutions were added to the cells. After 48 h, 20  $\mu$ L of a freshly prepared MTS/PMS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium/phenazine methosulfate] solution was added to the wells, and the cells were further incubated for 2–4 h. The amount of soluble formazan produced by cellular reduction of the MTS was measured by the absorbance at 492 nm on an ELISA plate reader (Spectra thermo, Tecan, Trappes, France).

2.3. Measurement of transepithelial electrical resistance (TEER)

Differentiated IPEC-1 cells were then treated with 0; 0.5, 1.0 and 5.0 mM of phytic acid. After further culturing for 24 h, the TEER was measured. After measuring, the cells were treated with 25  $\mu$ M of DON. After 24 h of culture, the TEER was measured again. Experimental TEER values were expressed as kohms  $\times$  cm<sup>2</sup>.

2.4. Statistical analysis

Data were analyzed with Statview software, version 5.0 (SAS Institute Inc., Cary, NC), using ANOVA and PLSD Fisher test. *P* values < 0.05 were considered significant.

3. Results

3.1. Phytic acid decreases the cell viability and TEER in a dose-dependent manner

The effect of phytic acid on cell viability was investigated via MTS assay. The conversion of tetrazolium salt to colored formazan by succinate dehydrogenase, the main mitochondrial enzyme, is proportional to the number of living cells. In the present study, we searched for a non-cytotoxic dose of phytic acid. The results presented in Fig. 1 demonstrate that phytic acid decreased the viability of the IPEC-1 cell line in a dose-dependent manner. A significant decrease in cell viability was noted at a concentration of 5 mM phytic acid. The effect of phytic acid treatment was also determined on differentiated cells. IPEC-1 cells were seeding in trans well system and allow to differentiate for 14 days before phytic acid was added on the apical part of the monolayer. Changes in TEER are indicative of alterations in epithelial barrier function or in the tran-

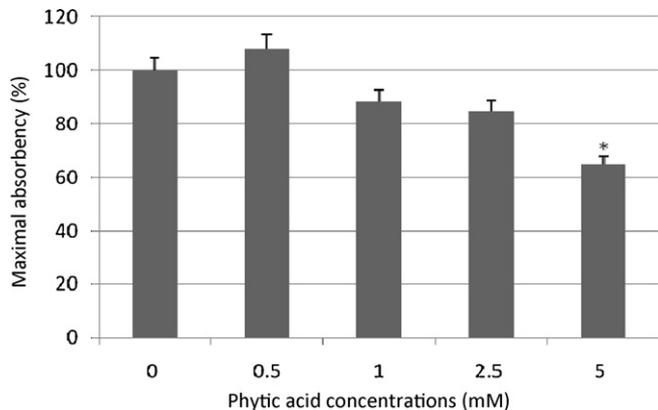


Fig. 1. Effects of phytic acid on cell viability of IPEC-1 cell line as measured by the MTS assay. IPEC-1 cells were seeded at low density in 96-multiwell plates, allowed to adhere, and then treated for 48 h with increasing concentrations of phytic acid. The amount of soluble formazan produced by cellular reduction of the MTS was then measured by absorbance at 492 nm. Values are expressed as the percentage of the maximal absorbance observed in untreated cultures, mean  $\pm$  SD of seven independent wells. Statistical significance between treated culture and control is shown with \**p* < 0.05.

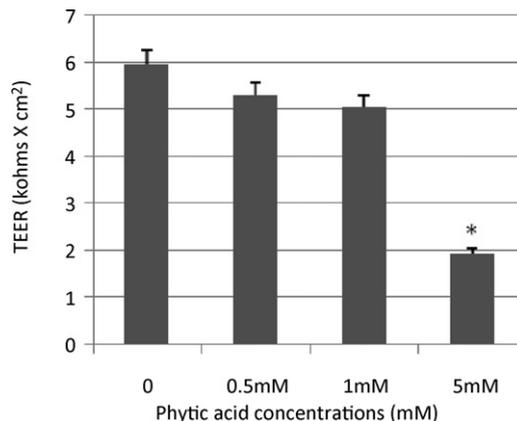


Fig. 2. Effect of phytic acid on trans-epithelial electrical resistance (TEER) in polarized intestinal epithelial cell monolayers. IPEC-1 cells were grown and differentiated on inserts then at day 0 concentrations of phytic acid (0.5, 1.0 and 5.0 mM) were added in the apical compartment. After 24 h the TEER was measured. The values are expressed in kohms  $\times$  cm<sup>2</sup> as the mean  $\pm$  SD of 10 independent experiments. \*\*\**p* < 0.001.

scellular permeability of ions. After 24 h of treatment (Fig. 2), we observed that the TEER values in the control and the treatments with 0.5 mM and 1 mM phytic acid did not differ. On the other hand, IPEC-1 cells treated with 5 mM of phytic acid exhibited a significantly (*P* < 0.001) lower TEER value when compared with the other treatments. This confirms that the phytic acid at this concentration affected the integrity of intestinal epithelial cell monolayers, a finding also observed in the cell viability test.

3.2. Phytic acid restores the negative effects of DON on the membrane integrity of IPEC-1

Previous studies demonstrated that the IPEC-1 cells treated with DON (25  $\mu$ M) for 24 h exhibited a significant decrease in the TEER values (Pinton et al., 2009; Van De Walle et al., 2010). The pretreatments of cell monolayer for 24 h with 0.5 mM or 1.0 mM of phytic acid were able to partially restore (*P* < 0.001) the decreased TEER values induced by DON (Fig. 3). This result indicates that phytic acid may prevent the toxic effect induced by DON.

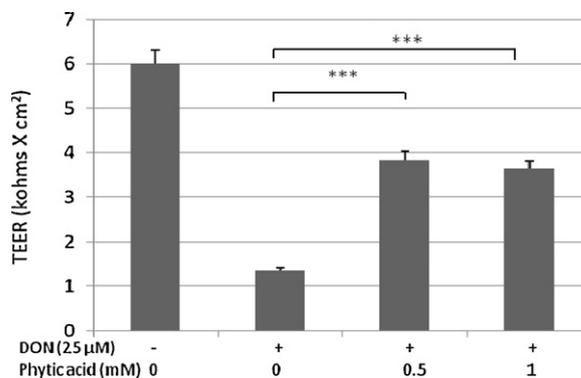


Fig. 3. Protective effect of phytic acid on DON-induced decreased trans-epithelial electrical resistance (TEER) in polarized intestinal epithelial cell monolayers. IPEC-1 cells were grown and differentiated on inserts then at day 0 various concentrations of phytic acid (0.5 and 1.0 mM) were added in the apical compartment. After 24 h, 25  $\mu$ M of DON was added in the apical compartment and the TEER was measured again after others 24 h. The values are expressed in kohms  $\times$  cm<sup>2</sup> as the mean  $\pm$  SD of 10 independent experiments. \*\*\**p* < 0.001.

#### 4. Discussion

An important function of gastrointestinal epithelia is to provide a barrier against the penetration of food contaminants and pathogens present in the intestinal lumen (Arrieta et al., 1996; Oswald, 2006). Several components present in plants are capable of interfering with both bacterial ecology and the immune response of the gut to food antigens. Beneficial nonnutrients, such as antioxidants, occur in foods of plant origin (Weglarz et al., 2007). Thus, the intake of foods high in phytic acid may indirectly influence health. In the present study, phytic acid decreased the viability of the IPEC-1 cell line in a dose-dependent manner. A significant decrease in cell viability was noticed at a concentration of 5 mM phytic acid. This result agrees with Weglarz et al. (2007), who evaluated the effect of phytic acid on the growth of Caco-2 cells.

In order to evaluate the effect of a non-cytotoxic dose of phytic acid on DON exposed cells, we used a concentration of 25  $\mu$ M DON. We found that DON decreased the TEER values, which is a good indicator of the epithelium integrity. Similar result was observed by Pinton et al. (2009) who used various concentrations of DON ranging between 5.0 and 50  $\mu$ M in a polarized intestinal epithelial cell monolayer. The protective effect of IP<sub>6</sub> was suggested based on its antioxidant effect and its ability to alter cell signaling pathways or antioxidant enzymes that detoxify the reactive oxygen species (ROS) (Shamsuddin et al., 1997). Phytic acid and its hydrolysis products (IP<sub>5</sub>, IP<sub>4</sub>, IP<sub>3</sub>, IP<sub>2</sub> e IP<sub>1</sub>) are still able to protect the biological membranes against lipid peroxidation (Miyamoto et al., 2000).

In the present study, phytic acid at a concentration of 0.5 mM or 1.0 mM protected the membranes of the IPEC-1 intestinal epithelial cell line against cell damage induced by the mycotoxin DON. The administration of products rich in phytic acid in food or feed, such as co-products derived from maize and rice, may act endogenously, protecting cellular systems against the effects caused by substances that induce cellular damages, such as mycotoxins, reducing the negative effects caused by toxins. Thus, losses in animal production and health problems will decrease considerably. However, further studies are needed to confirm the effects of cellular oxidative stress produced by DON in the intestinal IPEC-1 cells and how phytic acid can effectively minimize these effects.

In conclusion, our results indicate that phytic acid affects cell viability at a concentration of 5 mM, but 0.5 mM and 1 mM phytic acid decreased the negative effects of deoxynivalenol on the membrane integrity of the IPEC-1 intestinal epithelial cell line.

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