Modulation of intestinal transport of nutrients following dietary exposure to *Fusarium* mycotoxins in broiler chickens

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*Fusarium* mycotoxins affect nutrient transport

**Summary**

Deoxynivalenol (DON) and fumonisins are secondary metabolites produced by *Fusarium* fungi which frequently contaminate animal feed. Results of a global survey indicate that DON and fumonisins contaminate 55% and 54% of feed and feed ingredients, respectively (Streit et al., 2013). Following oral intake, the gastro-intestinal epithelium is exposed to these mycotoxins. Recently we showed that DON contamination, when present below the EU maximum guidance level of 5 mg/kg feed, increased the intestinal protein content and decreased the plasma concentration of 21 different free amino acids in broilers (Antonissen et al., 2013).

The aim of this study was to investigate the impact of DON and fumonisins contaminated diets on the nutrient uptake in the small intestine of broilers. The transport of nutrients into, and out of intestinal epithelia is mediated by digestive enzymes and membrane bound transporter proteins located at the brushborder and basolateral membranes of intestinal epithelial cells.

Ross 308 broiler chickens were fed either a control diet, a DON contaminated diet (~ 5 mg/kg feed), a fumonisins contaminated diet (~ 20 mg/kg feed) or a DON and fumonisins contaminated diet (respectively ~ 5 mg/kg feed and ~ 20 mg/kg feed) for 15 days. Subsequently, chickens were euthanized and samples of the jejunum were collected. Gene expression of a panel of 2 digestive enzymes, 10 amino acid, 1 peptide, 3 sugar transporters and 2 mineral transporters from jejunal RNA was measured by qRT-PCR (Paris and Wong, 2013).

Chickens fed a diet contaminated with DON, fumonisins or a combination of both mycotoxins had a decreased expression of the basolateral zinc transporter-1 and brushborder sucrase isomaltase. In addition, in chickens fed a fumonisins contaminated diet the brush border neutral and dibasic amino acid transporter \(b^0^{\text{AT}}\) and the anionic amino acid transporter EAAT3 were upregulated; while the basolateral cationic amino acid transporter-1 \(\text{CAT}1\) was...
downregulated. These results indicate an impact of *Fusarium* mycotoxins on intestinal nutrient transport.

**INTRODUCTION**

Contamination of food and feed with mycotoxins is a worldwide problem. Mycotoxins of importance for the poultry industry are mainly produced by fungi of the genera *Aspergillus, Fusarium* and *Penicillium*. The most commonly found and toxicologically important mycotoxins are the *Fusarium* mycotoxins, such as deoxynivalenol (DON) and fumonisins. A global mycotoxin survey indicates that DON and fumonisins contaminated 55% and 54% of feed and feed ingredients, respectively, in the period 2004-2011. The majority of the feed samples was however found to comply with the most stringent European Union regulations or recommendations on the maximal tolerable concentrations (Streit et al., 2013). The maximum European guidance level for poultry feed is indeed set at 5 mg DON/kg feed and 20 mg fumonisins/kg feed (European Commission, 2006). High concentrations of mycotoxins negatively affect animal growth. Limited information is, however, available on the impact of low to moderate mycotoxin contaminations on the activity of digestive enzymes and nutrient transporters (Grenier et al., 2013). Recently we showed that exposure of broilers to feed contaminated with DON, below the EU guidance levels, increased the intestinal protein content and decreased the plasma concentration of 21 different free amino acids (Antonissen et al., 2013). The transport of nutrients into and out of intestinal epithelia is mediated by digestive enzymes and membrane bound transporter proteins located at the brushborder and basolateral membranes of intestinal epithelial cells (Paris and Wong, 2013).

**OBJECTIVE**

The aim of this study was to investigate the impact of DON and fumonisins contaminated diets on the expression of genes involved in the nutrient uptake in the jejunum of broiler chickens.

**MATERIALS and METHODS**

A trial was performed with Ross 308 broiler chicks of both sexes (50/50), which were obtained as one-day-old chicks from a commercial hatchery. Two hundred twenty-four chicks were randomly divided into four experimental groups. Each group consisted of 8 cages of 7 chicks. All birds were fed a starter diet during the first eight days of the experiment, and subsequently a grower diet until the end of the trial (day 15). The diet was wheat/rye (43%/7.5%) based, with soybean meal as the main protein source. The experimental groups were respectively fed a control diet, a DON contaminated diet, a fumonisins contaminated diet and a diet contaminated with both DON and fumonisins. All diets were analysed by a validated multi-mycotoxin liquid chromatography-tandem mass spectrometry method (LC-MS/MS) (Monbaliu et al., 2010) (Table 1). After 15 days, chickens were euthanized and samples of the mid-jejunum were collected and stored in RNAlater® until qRT-PCR analysis. The expression of a panel of genes encoding digestive enzymes, amino acid, peptide, sugar and mineral transporters (Table 2), of eight chickens per experimental group, was measured by qRT-PCR of RNA extracted from the jejunum, following the protocol as described by Paris and Wong (2013).
Table 1: Mycotoxin contamination in experimental diets

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>DON</th>
<th>FB1</th>
<th>FB2</th>
<th>FB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>starter diet</td>
<td>µg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 control diet</td>
<td>223 ± 60</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>2 DON diet</td>
<td>4,601 ± 1,344</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>3 fumonisins diet</td>
<td>nd</td>
<td>19,271 ± 2,264</td>
<td>7,125 ± 571</td>
<td>1,754 ± 638</td>
</tr>
<tr>
<td>4 DON + fumonisins diet</td>
<td>4,221 ± 1,232</td>
<td>17,873 ± 2,100</td>
<td>6,483 ± 519</td>
<td>1,651 ± 600</td>
</tr>
</tbody>
</table>

FB1=fumonisin B1, FB2=fumonisin B2, FB3=fumonisin B3, nd=not detected

Table 2: Investigated panel of digestive enzymes, amino acid, peptide, sugar and mineral transporters

- b^{0, +}AT: Na^{+}-independent neutral and dibasic amino acid transporter
- rBAT: protein related to b^{0, +}AT
- B^{0, AT}: Na^{+}-dependent neutral amino acid transporter
- EAAT3: excitatory amino acid transporter-3
- ASCT1: alanine, serine, cysteine and threonine transporter-1
- CAT1: cationic amino acid transporter-1
- CAT2: cationic amino acid transporter-2
- LAT1: L type amino acid transporter
- y^{+}LAT1: y^{+} L amino acid transporter-1
- y^{+}LAT2: y^{+} L amino acid transporter-2
- PepT1: peptide transporter-1
- APN: aminopeptidase N
- SI: sucrase isomaltase
- GLUT2: glucose transporter-2
- GLUT5: glucose transporter-5
- SGLT1: sodium glucose transporter-1
- NPT2b: type II sodium-dependent phosphate cotransporter
- ZNT1: zinc transporter-1

RESULTS and DISCUSSION

In *Fusarium* mycotoxin-challenged chickens the expression of the zinc transporter-1 (ZNT1), which is located at the basolateral membrane and acts as an exporter of zinc into the portal
circulation, was significantly reduced (Table 3). Zinc is an essential micronutrient for several cellular processes, including enzyme activities and DNA and protein synthesis, and has been implicated as an inhibitor of apoptosis and oxidative stress (Liuzzi et al., 2000). The decrease in expression of ZNT1 may represent a compensatory response to maintain intracellular zinc concentration. Additionally, the expression of sucrase isomaltase was significantly reduced in chickens fed a Fusarium mycotoxin contaminated diet (Table 3). This decrease may be reflecting intestinal epithelial cell damage caused by DON and fumonisins. However, the brush border neutral and dibasic amino acid transporter b^{0,+}AT and the anionic amino acid transporter EAAT3, were upregulated in chickens fed a fumonisins contaminated diet (Table 3). Upregulation of b^{0,+}AT would increase the uptake of key amino acids such as lysine (Lys) and methionine (Met), while upregulating EAAT3 would increase the uptake of glutamic acid (Glu) and aspartic acid (Asp), with Glu being especially important because it is the main energy source for intestinal epithelial cells helping them to survive or proliferate. Furthermore, the basolateral cationic amino acid transporter CAT1 was downregulated in chickens fed a fumonisins contaminated diet, leading to a reduced efflux of cationic amino acids like Lys and arginine (Arg). This decreased expression suggests a mechanism for the cell to try to cope with the mycotoxins by building up its intracellular pools of key amino acids. In conclusion, these results indicate an impact of relevant contamination levels of Fusarium mycotoxins on the intestinal nutrient transport.

Table 3: Relative gene expression of zinc transporter-1, Na^{+}-independent neutral and dibasic amino acid transporter, excitatory amino acid transporter-3, cationic amino acid transporter-1 and sucrase isomaltase in jejunum of Fusarium mycotoxin challenged chickens.

<table>
<thead>
<tr>
<th></th>
<th>ZNT-1</th>
<th>b^{0,+}AT</th>
<th>EAAT3</th>
<th>CAT1</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.14 ± 0.22</td>
<td>1.08 ± 0.18</td>
<td>1.07 ± 0.15</td>
<td>1.27 ± 0.28</td>
<td>1.24 ± 0.28</td>
</tr>
<tr>
<td>2</td>
<td>0.54 ± 0.10*</td>
<td>2.19 ± 0.82</td>
<td>2.41 ± 1.29</td>
<td>1.42 ± 0.17</td>
<td>0.64 ± 0.12*</td>
</tr>
<tr>
<td>3</td>
<td>0.37 ± 0.15*</td>
<td>22.39 ± 12.91*</td>
<td>11.02 ± 5.81*</td>
<td>0.56 ± 0.17*</td>
<td>0.32 ± 0.11*</td>
</tr>
<tr>
<td>4</td>
<td>0.62 ± 0.13*</td>
<td>0.84 ± 0.17</td>
<td>0.69 ± 0.09</td>
<td>0.92 ± 0.21</td>
<td>0.52 ± 0.11*</td>
</tr>
</tbody>
</table>

ZNT1 = zinc transporter-1, b^{0,+}AT = Na^{+}-independent neutral and dibasic amino acid transporter, EAAT3 = excitatory amino acid transporter-3, CAT1 = cationic amino acid transporter-1 and SI = sucrase isomaltase

* indicates statistical significance from control at P<0.05 (Dunnett’s method)

REFERENCES


