In Vitro And *In Vivo* Safety Testing Of Mycotoxin Detoxifying Agents

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Adding mycotoxin detoxifying agents to feed contaminated with mycotoxins is commonly used and seems to be the most promising way of counteracting the harmful effects of mycotoxins. The European Food Safety Authority (EFSA) stipulates that the interaction of these products with oral absorption of drugs and nutrients should be investigated. The binding of drugs by these products in the gastro-intestinal tract could lead to subtherapeutic plasma and tissue concentrations and enhanced microbial resistance. Nevertheless, only few literature is available and no models are present to evaluate these possible interactions. Therefore, the goal of this study was to develop an *in vitro* and *in vivo* model for safety testing of these products. The interaction between bentonite and tylosin was investigated as a possible interaction was described previously. In addition, a commercially available detoxifying agent (gluco-mannan, GMA) was tested.

First, *in vitro* trials were carried out. Intestinal porcine epithelial cells (IPEC-J2) were cultivated on Transwell® cell culture inserts until differentiation (21 days). Tylosin (Tylan® Soluble, Elanco, Belgium) (20 µg/mL) was added to the basolateral compartment with or without addition of a mycotoxin detoxifying agent (bentonite or gluco-mannan (GMA), 1 mg/mL) for 48 h. To determine the safety of bentonite and GMA towards adsorption of tylosin, several parameters were evaluated: cellular viability by means of a neutral red assay, cellular integrity based on the trans-epithelial electrical resistance (TEER) and passage through the monolayer by quantification of tylosin in the apical compartment using LC-MS/MS. No differences were found in viability or TEER between groups, indicating that the tylosin, bentonite or GMA concentration applied was not cytotoxic. Quantification of tylosin in the apical compartment showed a significant lower passage of tylosin due to binding to bentonite. Apical tylosin concentrations of GMA treated inserts did not differ from controls.

Next, these findings were evaluated by an *in vivo* model. Tylosin (24 mg/kg BW) was administered as a single intra-crop bolus to broiler chickens (n=8), with or without a mycotoxin detoxifying agent (bentonite or GMA, 1 mg/kg BW). Blood was withdrawn at several time points after bolus administration and plasma concentrations of tylosin were determined. Plasma concentration-time profiles were evaluated and several pharmacokinetic parameters were compared to the control group in order to evaluate the interaction between tylosin and bentonite/GMA. Plasma concentrations of tylosin were significantly lower after bolus administration of tylosin and bentonite, compared to the control group. No interaction between tylosin and GMA was found. These *in vivo* results confirm our *in vitro* findings.

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