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POTENTIAL EFFECT OF DIETARY FUMONISINS ON THE COLONIZATION OF LACTOBACILLI IN THE GUT AND FECAL MICROBIOTA OF WEANED PIGS

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ABSTRACT

Research on the capacity of certain microbial species to breakdown mycotoxins and reduce their toxicity is expanding. To identify prospective strains that may be isolated to break down and reduce fumonisins (FUMs) toxicity, the study examined the trend of Lactobacillus sp. colonization in weaned pigs' gut and faecal microbiota. After 2 weeks adaptation period, eighteen weaned pigs of 7 weeks old (n = 6) were administered a diet that contained either 0 (G1), 15 (G2) or 30 (G3) mg FUMs/ kg diet for 21 days. The growth rate and composition of intestinal and faecal Lactobacillus sp. were examined. The bacterial composition analysis of duodenal, jejunal, ileal, and faecal samples of the three experimental groups was analyzed based on taxonomic tables containing relative abundance data per sample prepared by LGC Genomics GmbH using the QIIME 1.9.0 pipeline application of the IBM SPSS software package. The results showed no significant differences ($p > 0.05$) between the individual treatments during sample analysis with either the SPSS Kruskal-Wallis or the Wilcoxon Signed-Rank test. However, by observation, the proportion of Lactobacillus sp. increased in the duodenum, ileum, and jejunum of the intestine in a trend-like fashion in the order G1–G2–G3. In the faeces, the abundance was exceedingly low but the rising fashion was still discernable. In conclusion, the presence of dietary FUMs at concentrations of 15 or 30 mg/kg diet has the potential to alter the abundance of Lactobacillus sp. in weaned pigs. The detoxification of dietary FUM by specific Lactobacilli strains is advised for further research.

ÖSSZEFOGLALÁS

Egyre nő azoknak a kutatásoknak a száma, amelyek egyes mikroba fajok mikotoxinok lebontására és toxicitásuk csökkentésére irányulnak. A fumonizin (FUM) toxicitásának csökkentése érdekében vizsgálatunkban a Lactobacillus kolonizációját értélkeltük választott malacok tápcsatornájából és bélsarából vett mikrobiotában. A választott malacokat 2 hetes adaptációt követően, 7 hetes korban három kísérlei csoportba osztottuk (n=6), alaptakarmányuk azonos volt, azonban 0 (G1), 15 (G2) vagy 30 (G3) mg/kg mennyiségben FUM-t tartalmazott. A vizsgálat 21 napig tartott, melynek végén a malacokat leöltük és béltartalom mintát vettünk a vékonybél duodenum, jejunum és ileum szakaszából, valamint a végbélből bélsárat gyűjtöttünk. A minták bakteriális összetételének elemzését mintánkénti relatív abundancia adatokat tartalmazó taxonómiai táblázatok alapján értékeltük IBM SPSS szoftvercsomag, LGC Genomics GmbH által QIIME 1.9.0 pipeline alkalmazásával. A kísérlet során a malacok egészségi állapota megfelelő, növekedésük a fajtára jellemző volt. A Lactobacillus sp. dózisfüggően, nem szignifikánsan növekedett a duodenumban, az ileumban és a jejunumban, G1-G2-G3 sorrendben. A bélsárban az abundancia rendkívül alacsony volt, de a növekvő tendencia itt is érzékelhető volt. Összefoglalva, a takarmánnyal bevitt FUM 15 vagy 30 mg/kg koncentrációban megváltoztathatta a Lactobacillus sp. jelenlétét a választott malacok belében. További vizsgálatora van szükség olyan Lactobacillus törzsek kiválasztása céljából, amelyek hatékonyak lehetnek a takarmánnyal felvett FUM méregtelenítésében.

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Introduction

Mycotoxins are categorized as harmful secondary metabolites of moulds that may negatively affect both human and animal health, either directly or indirectly, as a result of biological alteration (Beardall and Miller, 1994). *Fusarium verticillioides* and *F. proliferatum* are the two principal plant pathogens that make fumonisins (FUMs). Fumonisins frequently contaminate maize and maize-based products and when consumed, they can negatively affect health. Among the commonly existing FUMs – B1 (FB_1), B2 (FB_2), B3 (FB_3) and B4 (FB_4) – FB_1 is the most abundant in nature and toxic (Lerda et al., 2017). The structural resemblance of FB_1 to the sphingoid bases – sphingosine and sphinganine – has been established as the principal pathway of FB_1-induced toxicity. This similarity disrupts the function of the enzyme ceramide synthase by interfering with sphingolipid metabolism and subsequently harming biological processes such as cell proliferation, differentiation, morphogenesis, permeability, and apoptosis. Fumonisin B1 promotes varying clinical disorders in certain animal species examined. Oesophageal cancer and neural tube anomalies in humans have been connected to eating foods contaminated with FB_1 in some parts of the world as well (Chilaka et al., 2017). The International Agency for Research on Cancer has since categorized FB_1 as a category 2B carcinogen (IARC, 2002).

There are numerous economic risks associated with mycotoxin contamination of food and feed products. As a result, several approaches are being utilized to help remove mycotoxins from contaminated commodities. These approaches include physical, chemical, and biological techniques to eradicate, inactivate, or lower the mycotoxin concentration in food and feed commodities (Temba et al., 2016). However, due to concerns about safety, potential reductions in the nutritional value of the treated commodities, a lack of efficacy, and economic considerations, and the use of physical or chemical techniques on food products that are contaminated with mycotoxins can occasionally be problematic. Because of this, a lot of attention has been paid to biological detoxification techniques (Kabak et al., 2006). Among the biological approaches, the Lactobacilli-mediated decontamination technique is one of the methods that has gained recognition for its ability to reduce mycotoxins' toxicity without compromising safety or cost.

Thus, in the present study, we studied the colonization pattern of Lactobacilli in the gut and faeces of weaned pigs exposed to either a no-contaminated FUM diet (control), a 15 or a 30 mg/kg total-FUMs-contaminated diet for 21 days.

Materials and methods

Animals, Feeding and Housing

The research protocol was reviewed and authorized by the Animal Use and Care Administrative Advisory Committee and approved by the Agricultural Administrative Authority, Hungary (Protocol SOI/31/00308-10/2017).

Eighteen male Danbred weaned pigs weighing an average of 13.5 ± 1.3 kg and aged 5 weeks were used in the study. After a 2-week physiological acclimation phase and at precisely 7 weeks of age the pigs were split into 3 groups (n = 6). Group 1 (G1) served as the

control group fed a diet free of FUMs (FB_1 + FB_2 + FB_3), Group 2 (G2) was fed a diet containing 15 mg/kg FUMs, and Group 3 (G3) received a diet contaminated with 30 mg/kg FUMs, for 21 days. Feed was a corn-soybean meal diet containing 175 g crude protein, 11.1 g Lys, and 14.1 MJ/kg ME per kg of feed offered as an amount that covers 2.5 times the maintenance energy requirement, and was provided twice a day in two equal portions. Drinking water was made available *ad libitum*.

Pigs were kept in individual metabolic cages (80 x 80 cm) located in the Experimental Animal Unit of the Department of Farm Animal Nutrition of the Hungarian University of Agriculture and Life Sciences, Kaposvár Campus. Pigs were weighed individually at the start of the trial and at weekly intervals and, their health status and diarrhea score were monitored every day. At the end of the experiment period, pigs were euthanized by exsanguination after sedation (Euthanyl-Pentobarbital Sodium, 400 mg/mL, Dechra Veterinary Products, Shrewsbury, UK).

The finely ground culture of *Fusarium verticillioides* RL 596 was carefully mixed thoroughly into the diets of the experimental animals to provide a daily FUMs feed concentration of 15 and 30 mg/kg diet. The mycotoxin concentration of the control and the experimental feed was then determined with the LC-MS method which utilizes a Shimadzu Prominence Ultra-Fast Liquid Chromatography separation system equipped with an LC-MS-2020 single quadrupole (ultra-fast) liquid chromatograph-mass spectrometer (Shimadzu, Kyoto, Japan) with electrospray source (Bartok et al., 2010). The limit of detection (LOD) for FB_1 and FB_2 were 0.031 and 0.051 mg/kg respectively; LOD for FB_3 was not measured. Diet fed to the control group did not contain detectable amounts of FUMs as well as deoxynivalenol, zearalenone and T-2 toxin while the two contaminated diets contained 15.40 and 29.75 mg FUMs/kg diet.

Gut and Faecal Microbiota Analysis and Statistical Analysis

Bands were firmly tied at predetermined points throughout the length of the small intestine in order to collect samples from the duodenum, jejunum, and ileum for the examination of the intestinal flora. Samples collected were weighed and stored appropriately for the analysis. The bacterial composition analysis of samples from duodenum, jejunum, ileum, and faeces of the three treatment groups was done based on taxonomic tables containing relative abundance data per sample prepared by LGC Genomics GmbH using the Quantitative Insights into Microbial Ecology (QIIME) 1.9.0 pipeline application (Caporaso et al., 2010). The path of the relative abundance tables and bar graphs used in the analysis was normalized by the rarefaction method described by Ju and Zhang (2015). The relative abundance data of G1, G2 and G3 were compared using the Kruskal-Wallis test of the IBM SPSS Statistics software. And the bacterial composition of faecal samples in G1, G2 and G3 was examined using the SPSS Wilcoxon Signed-Rank test (IBM Corp., Armonk, NY, USA). A p value < 0.05 was described as significant.

Results and discussion

At the end of the experiment, i.e. after exposure to dietary FUM for 21 days, pigs did not show any signs of growth retardation or any health problems. Although there was some

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transitional diarrhea during the adaption phase, the pigs had fully recovered by the time the intoxication started. The effects of stress exposure to host microbiota *Lactobacillus* presence and abundance trend showed that neither the SPSS Kruskal Wallis nor the Wilcoxon Signed-Rank test were statistically significant ($p > 0.05$) between the treatment groups in all intestinal sections and faeces. However, a dose-response increment pattern of *Lactobacillus sp*. present in the duodenum, ileum, and jejunum and albeit occurrence in the faecal samples was extremely low, a pattern could be seen (Figure 1).

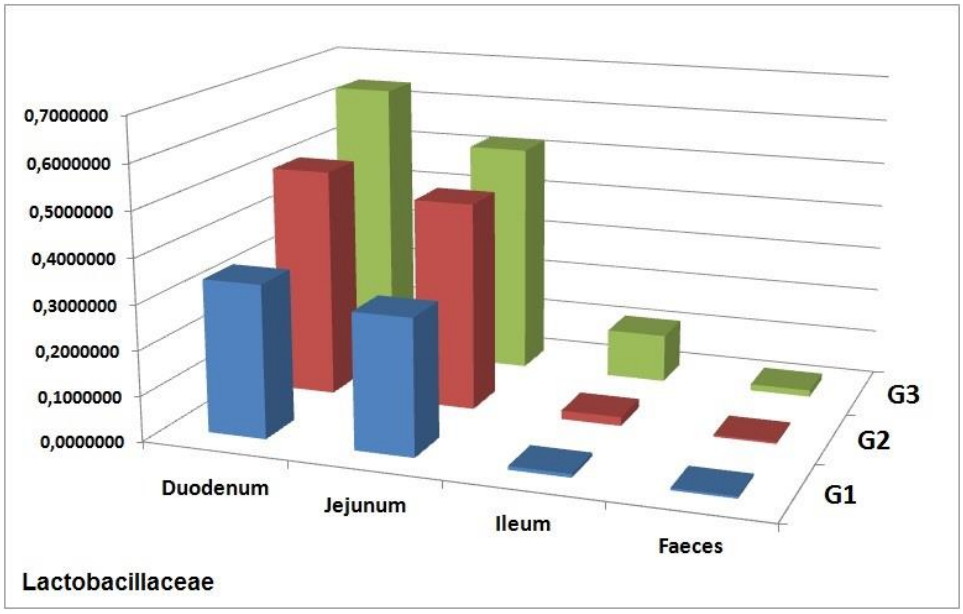

Fig. 1. Abundance trend of Lactobacillus in all sections of the intestinal tract examined. G1= Control group, G2= 15 mg/kg FUMs- fed group, G3= 30 mg/kg FUMs- fed group.

Animals benefit from the nutritional and protective properties of microbiota by way of increased host immunity, fermentation outputs, and protection from pathogen colonization (Piotrowska et al., 2014). For a very long time, the lactic acid bacteria (LAB) subgroup Lactobacilli have been utilized in food and are regarded as innocuous. Additionally, they have been reported to have a remarkable effect on a variety of fungi that produce mycotoxins (Magnusson et al., 2003). Agreeing with the present finding, Becker and colleagues (1997) used culture techniques and observed no inhibition of bacterial growth including *Lactobacillus acidophilus*, *Lactobacillus johnsoni*, *Lactobacillus plantarum* and *Lactobacillus reuteri* in the presence of 50 to 1000 µM FB_1 *in vitro*.

A substantial number of studies have demonstrated a modification in *Lactobacillus sp*. after mycotoxin exposure. According to reports, certain LAB, propionic acid bacteria, and bifidobacteria have the ability to attach mycotoxins to their cell wall polysaccharides and peptidoglycans (Wambacq et al., 2015). According to one study, trichothecenes and aflatoxins can both be sequestered from liquid media by LAB and propionic acid bacteria (El-Nezami et al., 2002). The addition of FB_1 to *in vitro* incubation of cecal chyme from pigs decreased the anaerobic bacteria, whereas *Lactobacillus* and total bacteria increased (Dang et al., 2017). Later, Mateos and colleagues (2018) found more Lactobacillus in the

faeces of piglets that received 12 mg/kg FB_1- contaminated feed and attributed this to the competitive advantage associated with their ability to metabolize FB_1.

Conclusion and recommendation

In conclusion, a dietary dose of 15 or 30 mg/kg FUM fed to weaned pigs increased the proportion of *Lactobacillus sp*. in the duodenum, ileum, and jejunum of the intestine and in the faeces as well, in a trend-like fashion in the order G1–G2–G3. The detoxification of dietary FUM by specific Lactobacilli strains is advised for further research.

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