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< Short Communication>

Exposure to low concentrations of mycotoxins triggers unique responses from the pig gut microbiome

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Abstract

The aim of this study is to investigate how the gut microbiome shifts when pigs were exposed with low concentrations of mycotoxins, deoxynivalenol (DON) and zearalenone (ZEN) in feed. Fifteen of pigs, 15 kg in weight which were negative for PRRSV and PCV2 were purchased, acclimatized until 20 kg in weight, and randomly divided into 3 groups; the DON group (DON treated), the ZEN group (ZEN treated) and the CTL (untreated negative control). DON and ZEN administered to each group for 30 days at 0.8 mg/kg (800 ppb) and 0.20 mg/kg (200 ppb) in feed, respectively. After extraction of microbial DNA from intestine and fecal samples, sequencing procedures were performed in the Ion PGM using an Ion 316 V2 chip and Ion PGM sequencing 400 kit. The results suggested that the bacterial communities in duodenum, jejunum and ileum of the DON and ZEN groups presented low-abundant OTUs compared with the CTL group. OTUs in cecum, colon and feces were determined more than in small intestine of all three groups. However, the CTL group yielded more OTUs than other two groups in inter-group comparison. It is not fully clarified how the richness and abundance in microbiome functions in the health condition of animals, however, the exposure to DON and ZEN has caused microbial population shifts representing microbial succession and changes following the diversity and abundance of porcine gut microbiome. The metabolomic analysis correlate with microbiome analysis is needed for further study.

Key words : Deoxynivalenol, Feed, Gut microbiome, Mycotoxin, Pig, Zearalenone

INTRODUCTION

Mycotoxins have been increasingly mentioned worldwide as an important issue because of their acute and chronic toxicity to humans and animals (Sforza et al, 2006). Among the *Fusarium* mycotoxins, deoxynivalenol (DON) and zearalenone (ZEN) are of special importance as they are formed at the field prior to harvest. Usually, the formation of DON and ZEN cannot be completely prevented depending on the impact of weather conditions. Animals reacts variably on mycotoxins. Especially, pigs react sensitively at the exposure to mycotoxins even in low concentrations by decreasing feed intake at the exposure to DON and by presenting hyperestrogenism and infertility at the exposure to ZEN (Döll and Dänicke, 2011).

Although there are hundreds of known mycotoxins, guidelines for regulatory detection limits or tolerated maximum levels in feed have been established for a few (Bennett and Klich, 2003). Recognition that mycotoxins affect health and productivity in pig farm has led to legislated maximum tolerated levels (directives) for aflatox-

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ins, and regulatory guidelines (recommended tolerance levels) for ochratoxins and a small number of fusariotoxins. The limits vary with not only type of mycotoxin, animal species, the intended use, raw materials, feed, and diet but also with the regulatory organization or country (Guerre, 2016). So far, the levels of Aflatoxin (AFT) and Ochratoxin (OCT) in animal feeds have been controlled according to the guidelines for livestock control and fish feed acts in South Korea; recently, the guidelines for *Fusarium* mycotoxins were set based on the monitoring results and guidelines of the European Union (EU) (Chang et al, 2017).

With the close links between gut microbiome and animal health, an impaired balance could have deleterious effects on general health condition. Mycotoxins are known to give an effect like antimicrobial properties to gut microbes and their communities, favoring a shift toward intestinal aerobic bacteria as observed in enteritis (Pierron et al, 2016).

Therefore, the aim of this study is to investigate how the gut microbiome shifts when pigs were exposed with low concentrations of DON and ZEN in feed.

MATERIALS AND METHODS

Fifteen of pigs, 15 kg in weight which were negative for PRRSV and PCV2 were purchased, acclimatized until 20 kg in weight, and randomly divided into 3 groups; DON group (DON treated), ZEN group (ZEN treated) and CTL (untreated negative control). DON and ZEN was purchased from Biomin (GmbH, Austria) and administered to each group for 30 days at 0.8 mg/kg (800 ppb) and 0.20 mg/kg (200 ppb) in feed, respectively.

Fecal samples of each group were collected and the intestinal samples (duodenum, jejunum, ileum, cecum and colon) were opened longitudinally and the mucosal layer scraped with a microscope slide to remove loosely associated contents and most of the mucosal tissue. The fibrous serosal tissue was discarded. The mucosal samples were weighed and 1 gram of each was used.

Microbial DNA was extracted by the Fast DNA Isolation Kit for Soil (MP Bio, USA) from gut samples to be used as a template for PCR. The primer pairs for the amplification of the V4-V5 region were used; 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CGTCAATTCMTTTRAGTTT-3').

Sequencing was performed on Ion Torrent™ Personal Genome Machine® (PGM) System (Thermo Fisher Scientific, USA) using an Ion 316 V2 chip and Ion PGM sequencing 400 kit. After sequencing, sequence reads were filtered using the PGM software to remove low quality and polyclonal sequences.

Operational Taxonomic Units (OTUs) were clustered using the QIIME (V.1.9.0; Caporaso et al, 2010) and MicroSEQ 16S Reference Library v2013.1 (Thermo Fisher Scientific, USA), Greengenes v13.5 (McDonald et al, 2012) database. The alpha diversity metrics included; OTUs, Evenness and Shannon diversity while the beta diversity distance matrices included unweighted UniFrac, weighted UniFrac and Bray-Curtis dissimilarity. The estimated beta diversity between communities was then visualised using principal coordinate analysis (PCoA) plots (Hamady et al, 2010).

RESULTS AND DISCUSSION

The DON and ZEN group did not present variable pattern of the gut microbiome compared with the CTL group. The intestinal microbiome of the DON and ZEN group did not present a variable and abundant pattern compared with that of the CTL group. The colon microbiome of the CTL group showed 2,981 OTUs, which consisted of 5 phyla (Fig. 1A) and 18 families including Rhodospirillaceae (Fig. 1B). However, the DON group had 321 OTUs and 2 phyla (Fig. 1A) and 3 families, which were Pasteurellaceae, Clostridiaceae and Lactobacillaceae (Fig. 1B).

The ZEN group had 890 OTUs and 5 phyla (Fig. 1A), which were Firmicutes, Bacteroidetes, Tenericutes and Proteobacteria and 12 families including Lactobacillaceae (Fig. 1B).

Distribution of bacterial phyla and family in pig intestinal tissues (duodenum, jejunum, ileum, cecum and colon) and feces with the lower dose of DON and ZEN group were shown in Fig. 2. Compare to normal feed group (Fig. 2A), bacterial communities of duodenum, je-

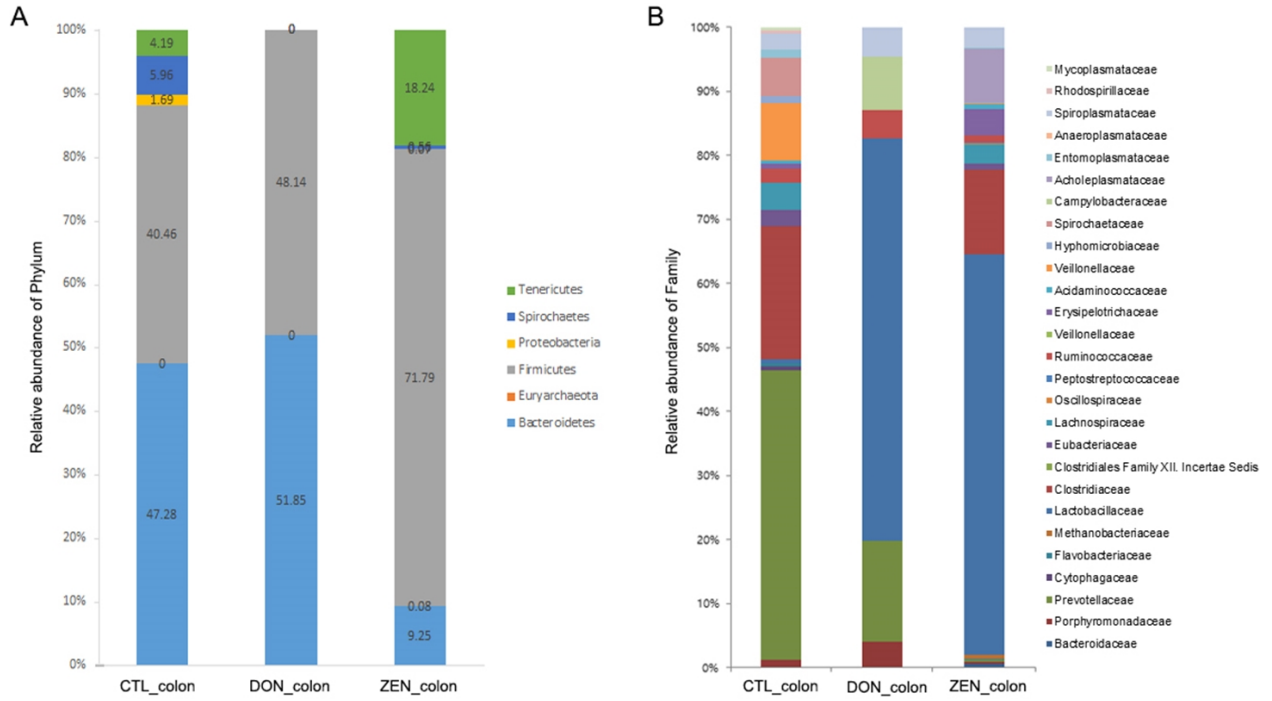

Fig. 1. Stacked bar graphs showing the phylogenetic composition of the colon microbiome in pigs with low dose of deoxynivalenol (DON) and zearalenone (ZEN) contaminated feed. The comparison is made in (A) at phylum level and (B) at family level.

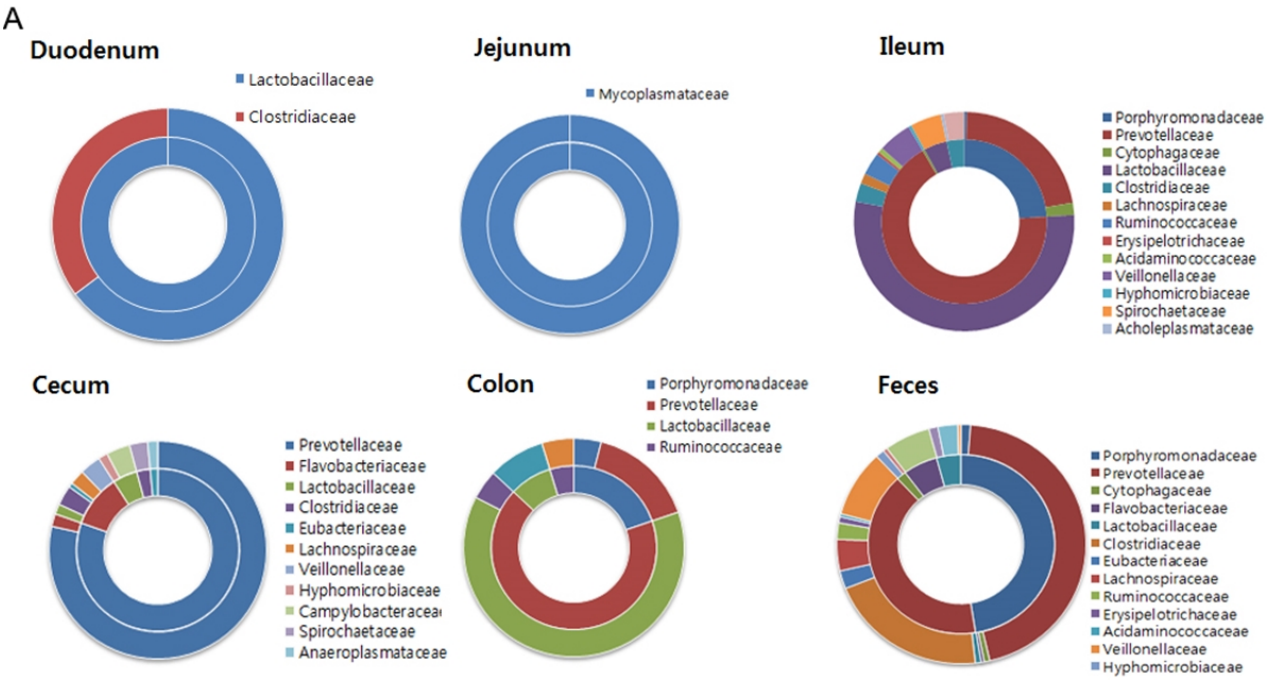

Fig. 2. Distribution of bacterial phyla and family in pig intestinal tissues and feces. Graph of the distribution of different phyla (inner circle) and family (outer circle) in pig tissues (duodenu, jejunum, ileum, cecum and colon) and feces with normal feed group (A), low dose of deoxynivalenol (DON) (B) and zearalenone (ZEN) (C) contaminated feed.

junum and ileum represented by low-abundant OTUs in DON (Fig. 2A) and ZEN (Fig. 2C) treated groups. Although in the cecum, colon and feces samples represented more abundant OTUs, composition of bacterial communities were different those of control group (Fig. 2).

Microbial diversity of DON and ZEN treated group in

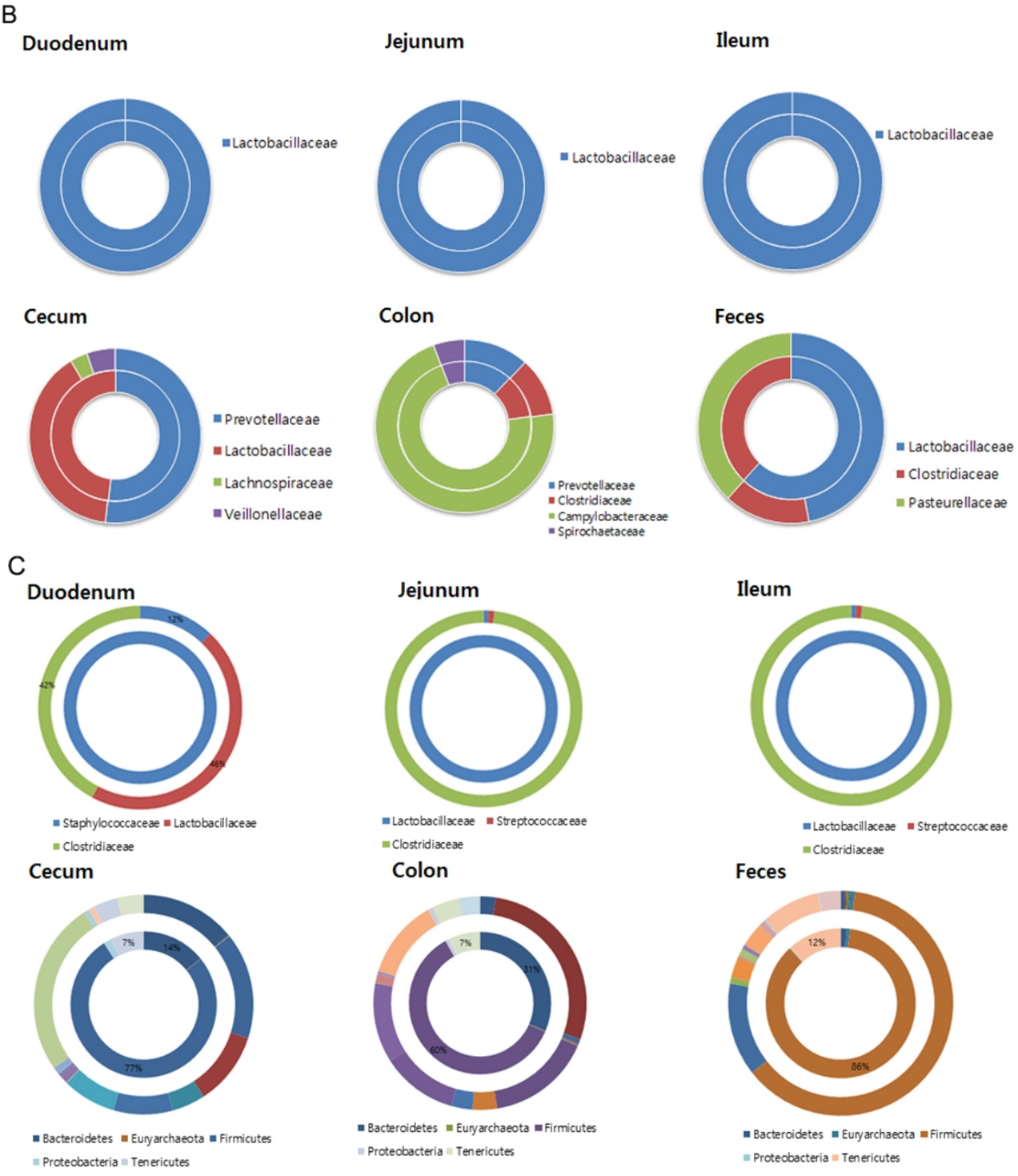

Fig. 2. Continued.

the phyla and family level was significantly decreased in all tissues and feces compared to control (data not shown).

Fast UniFrac was used to perform principal coordinate analysis (PCoA) to compare the overall compositions of the microbiomes in the DON and ZEN treatment groups (Fig. 3). Each groups showed distinct bacterial taxa. Based on membership, bacterial communities clustered together and separated from those from DON and ZEN contaminated feed which explained the largest

Fig. 3. Principal coordinate analysis (PCoA) was showed that the colon microbiome of pigs harbor distinct bacterial taxa. Based on membership, bacterial communities clustered together and separated from those of DON and ZEN contaminated feed which explained the largest amount of variation.

amount of variation (Fig. 3).

The research postulated that the exposure to low concentration of DON and ZEN reduce the abundance and the variability in porcine gut microbiome. It is difficult to say how diverse and rich the microbiome of healthy individuals should be, but the control group was statistically significantly different in diversity and abundance. It is still not clear how variable and abundant intestinal microbiome functions but the CTL group presented obviously more abundant and variable gut microbiome pattern than the DON and ZEN groups. The data showed microbial population shifts representing microbial succession and changes in response to DON and ZEN administration. The metabolomic analysis correlate with microbiome analysis is needed for further study.

Healthy microbiome protect against other pathogenic bacteria. However, if the composition of gut microbiota is altered (dysbiosis), these mechanisms may be disrupted, leading to several inflammatory and other diseases and infections (Karuppannan and Opriessnig, 2018; Toor et al, 2019). In this study, we confirmed that administration of lower dose DON and ZEN contaminated feed to pigs showed dysbiosis of microbiome even though it does not develop any typical clinical signs. Therefore, various defense trial against mycotoxins should be using all practical efforts to reduce pig exposure to mycotoxins. Because mycotoxins are often multi-contaminated in pig farms, the effect on dysbiosis of intestinal microbes is estimated to be greater, and further research is needed.

It has been reported that consumption of feed contaminated with a moderate level of DON had an effect on cultivable bacteria in pig intestines, and the composition of intestinal microbiota was changed in DON-exposed animals (Burel et al, 2013). Similarly, chronic exposure to low concentrations of DON caused an increase in the number of intestinal aerobic bacteria and modified the dynamics of intestinal bacteria communities in pigs (Wache et al, 2009). For further study, microbiome changes and its effects on porcine health condition when being exposed by DON and ZEN through feed at a concentration below the regulated concentration.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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