Canadian Journal of Animal Science
Revue canadienne de science animale

**Conventional solid fermentation alters mycotoxin contents
and microbial diversity analyzed by high-throughput
sequencing of a Fusarium mycotoxin-contaminated diet**

Journal:	<i>Canadian Journal of Animal Science</i>
Manuscript ID	CJAS-2017-0093.R3
Manuscript Type:	Article
Date Submitted by the Author:	26-Oct-2017
Complete List of Authors:	<p>Yang, Lijie Yang, Zaibin; Department of Animal Science and Technology, Shandong Agricultural University, Tai'an, Shandong, 271018, PR China Yang, Weiren; Department of Animal Science and Technology, Shandong Agricultural University, Tai'an, Shandong, 271018, PR China Li, Huirong; Shandong Feed Quality Inspection, Jinan, 250022, PR China Zhang, Chongyu; Department of Animal Science and Technology, Shandong Agricultural University, Tai'an, Shandong, 271018, PR China Jiang, Shuzhen; Department of Animal Science and Technology, Shandong Agricultural University, Tai'an, Shandong, 271018, PR China Li, Xiangming; Shandong Feed Quality Inspection, Jinan, 250022, PR China</p>
Keywords:	Solid fermentation, pH, Fusarium toxin, Microbial community diversity, High-throughput sequencing

SCHOLARONE™
Manuscripts

**Conventional solid fermentation alters mycotoxin contents and microbial
diversity analyzed by high-throughput sequencing of a *Fusarium*
mycotoxin-contaminated diet**

L. J. Yang^a, Z. B. Yang^a, W. R. Yang^a, H. R. Li^b, C. Y. Zhang^a, S. Z. Jiang^{a,*} and X. M.
Li^{b,*}

^aDepartment of Animal Science and Technology, Shandong Agricultural University,
Tai'an, Shandong, 271018, PR China

^bShandong Provincial Feed Quality Inspection Station, Jinan, 250022, PR China

*Correspondence: Professor Shu Zhen Jiang: shuzhen305@163.com; Xiang Ming Li:

lidsjs@163.com

Abstract

Fusarium mycotoxins, commonly present in corn and its derived products for animals, has caused significant economic impact on swine reproduction in China. The objective of the present study therefore was to evaluate changes in mycotoxin contents and microbial diversity by conventional solid fermentation of a contaminated diet. Three diets were evaluated, as follows: control group, basal diet; test group 1 (25Toxin), basal diet in which corn and corn gluten meal were replaced with 25% mycotoxin-contaminated corn and corn gluten meal, respectively; test group 2 (50Toxin), basal diet in which corn and corn gluten meal were replaced with 50% mycotoxin-contaminated corn and corn gluten meal, respectively. Compound strains were used for solid-state fermentation of the compound feed. Under the present experimental conditions, conventional solid fermentation altered microbial diversity, as demonstrated by high-throughput sequencing of a *Fusarium* mycotoxin-contaminated diet, and the content of ZEA was reduced significantly. However, deleterious effects were also observed with regard to the contents of DON and FUMB1. These results may have implications for animals consuming *Fusarium* mycotoxin-contaminated food or feed by conventional solid fermentation.

Key words: Solid fermentation; pH; *Fusarium* toxin; Microbial community diversity; High-throughput sequencing

Abbreviations: **ZEA**, zearalenone; **DON**, deoxynivalenol; **FUMB₁**, fumonisin B₁; **ACE**, abundance-based coverage estimator; **OTUs**, operational taxonomic units; **HPLC**, high-performance liquid chromatography; **PCR**, polymerase chain reaction; **ANOVA**, analysis of variance.

INTRODUCTION

Feed and raw materials can easily grow mildew during processing, storage, and transportation (Ren et al. 2016; Davide et al. 2017). Previous investigations have revealed that feedstuffs for animals may be severely contaminated with mycotoxins (Moltó 2007; Palacio et al. 2016; Juan et al. 2017), which can result in adverse reactions owing to the presence of exotoxin and endotoxin (Moretti et al. 2017). In addition to that, the generation of mycotoxins consumes the nutrients of the feed, leading to reduced nutritional quality (Abrunhosa et al. 2016). Affected livestock and poultry usually exhibit a series of symptoms, such as poisoning, diarrhea, and impaired growth and reproductive performance, after ingesting mycotoxin-polluted feeds; death is also observed in severe cases (Gashaw 2016; Magnin et al. 2016). Therefore, preventing mycotoxin contamination is an important issue in the feed industry.

A number of methods have been developed for detoxification, including physical detoxification, chemical detoxification, and adsorbent detoxification (He et al. 2010; Bata et al. 1999; Jiang et al. 2010; Aiko et al, 2015). However, most of these methods play roles in detoxification of mycotoxins in the laboratory or for the adsorption of a single toxin, and sufficient evidence is not available to determine whether the

adsorbent takes up the nutrients and trace elements from the feed. Alternatively, microbial fermentation may be used as a novel detoxification method; however, little information is available regarding the effects of conventional solid fermentation on mycotoxin content and microbial diversity in a contaminated diet.

Therefore, in this study, we hypothesized that conventional solid fermentation could alter mycotoxin contents and microbial diversity of a *Fusarium* mycotoxin-contaminated diet.

MATERIALS AND METHODS

Strains and mycotoxin source

Lactobacillus (5×10^9 cfu/g), yeast (4.6×10^9 cfu/g), and *Bacillus subtilis* (1.9×10^{10} cfu/g) were obtained purchased from Beijing CRVAB Biological Technology Co. Ltd. (China). Fermentative strains were homogenously mixed into the diets. All other chemicals used were of analytical grade. A 3-year survey program was initiated by our research group in order to evaluate the incidence of mycotoxins in feed and feed raw materials in Shandong Province, China. A total of 293 analyses were performed by the Inspection and Quarantine Center of Shandong Entry & Exit Inspection and Quarantine Bureau (Shandong, China). One *Fusarium* mycotoxin-contaminated corn

sample and one *Fusarium* mycotoxin-contaminated corn gluten powder were selected as the source of the *Fusarium* mycotoxins.

Diet preparation, experimental design, and mycotoxin quantification

As shown in Table 1. The basic diet served as control treatment. The 100% *Fusarium* mycotoxin-contaminated diet was formulated by complete replacement of corn and corn gluten meal in basic diet with mycotoxin-contaminated corn and corn gluten meal. The 25% *Fusarium* mycotoxin-contaminated diet (25Toxin) and 50% *Fusarium* mycotoxin-contaminated diet (50 Toxin) were obtained from a mixture of the 100% *Fusarium* mycotoxin-contaminated diet and the basic diet at proportions of 1:3 and 1:1, respectively. Next, all test diets were prepared by blending with a certain proportion of the fermentation strains (*Lactobacillus*: 6.33×10^6 cfu/g, yeast: 1.05×10^6 cfu/g, and *Bacillus subtilis*: 8.94×10^{10} cfu/g). All diets were prepared in one batch and then stored in covered containers prior to testing. Representative feed samples were taken before and at the end of the experiment and analyzed for dry matter, crude protein, Ca, and P (AOAC 2012). Additionally, mycotoxin analyses were performed by the Inspection and Quarantine Center of Shandong Entry & Exit Inspection and Quarantine Bureau (Shandong, China). The concentrations of the mycotoxins deoxynivalenol (DON) and aflatoxin B₁ (AFLB₁) were analyzed using

high-performance liquid chromatography (HPLC) tandem mass spectrometry (HPLC-MS/MS) and fluorometry. HPLC and fluorometry were used to measure zearalenone (ZEA) and fumonisin B₁ (FUMB₁) levels. The detection limits for these mycotoxins were 1.0 µg/kg for AFLB₁, 0.1 mg/kg for ZEA, 0.1 mg/kg for DON, and 0.25 mg/kg for FUMB₁. The concentrations of the analyzed mycotoxins are shown in Table 1.

Fermentation management, sample collection, and processing

All diets were tempered with water, and the final moisture content of the three experimental diets was 50%. Diets were encased in plastic barrels (435 mm diameter × 460 mm height) after mixing evenly, and the barrels were then sealed for fermentation at a temperature of 20–25°C. There were three treatments with eight replicates of six subunits each, and the total number of barrels was 144.

Samples on day 0 were collected immediately from one barrel for each replicate before the barrels were sealed. Sample collection was then carried out from different subunits (barrels) of each replicate on days 3, 6, 9, 12, 15, and 18 of diet fermentation. Specific sampling methods are as follows. Six parallel samples were obtained from the upper (20 cm below the top), central, and lower (20 cm above the bottom) section of each barrel in duplicate. The pH was determined immediately using a pH meter

(PHB – 10; Shanghai Shuangxu Electronic Co., Shanghai, China), and sensory evaluations were conducted. Two samples were collected from the mixture of the six parallel samples for each barrel in accordance with the quartering method. One sample was used for mycotoxin measurements, and the second sample was stored at -80°C immediately until the microbial diversity and composition analysis.

Microbial diversity analysis

DNA extraction and polymerase chain reaction (PCR)

DNAs in the fermented feed on day 18 were extracted using a Bacterial Genome DNA Kit (DN 11; Aidlab Biotech, Beijing, China). The extracted DNA was subsequently purified with AMPure XP beads (Beckman Coulter, California, America). A library was then prepared using PCR products with a TruSeq Nano DNA LT Library Prep Kit (Illumina, USA), and the libraries were quantified using a Quant-iT PicoGreen dsDNA Assay Kit (P7589; Biological Technology Co., Ltd, Shanghai, China). The 16S universal primers 520F (5'-barcode+GCACCTAAYTGGGYDTAAAGNG-3') and 802R (5'-TACNVGGGTATCTAATCC-3') were used for amplifying the 16S rRNA genes. A Fast Start High Fidelity PCR System was used for PCR under the following conditions: 98°C for 30 s; 26 cycles of 98°C for 15 s, 50°C for 30 s, and 72°C for 30 s;

and a final elongation step at 72°C for 5 min.

High-throughput pyrosequencing

The PCR products were purified by AMPure XP beads (Beckman Coulter). After the products were quantified, sequencing was performed using a Miseq Reagent Kit V3 (600 cycles) according to the manufacturer's instructions by a commercial sequencing facility (Illumina). After sequencing, operational taxonomic units (OTUs) were generated using the uclust method (Macias et al. 2011), and the taxonomic assignment of OTUs was performed by BLAST (Altschul et al. 1990) classifier.

Statistical analyses

Data were analyzed by one-way analysis of variance (ANOVA) using SAS 9.2. The data were first analyzed as a completely randomized design with the content of mycotoxins as a random factor to examine the overall effects of treatments. Orthogonal polynomial contrasts were applied to determine the linear responses to the fermentation time of each treatment. The significance of differences among treatments was tested using Duncan's multiple range tests, and results with P values of less than 0.05 were considered significant.

RESULTS

Fermentation pH

Changes in pH are shown in Table 2. The pH (day 0) decreased significantly as the levels of *Fusarium* toxins increased ($p < 0.001$). However, compared with the control, the pH values of the two *Fusarium* toxins treatments were significant increased ($p < 0.001$) on days 9, 12, 15, and 18; there were no significant differences between the 25Toxin and 50Toxin treatments on these days. The pH decreased linearly ($p < 0.001$) during the fermentation process, until the fermentation pH reached the equilibrium point at 12 day.

Changes in mycotoxin content

Effects of fermentation on mycotoxins content among different contaminated diets are shown in Table 3. At all the measured time points, the levels of ZEA, DON, and FUMB1 were significantly different across different treatments with highest in 50 Toxin treatment and lowest in control treatment ($p < 0.001$). In the presence of lactic acid bacteria (6.33×10^6 cfu/g), yeast (1.05×10^6 cfu/g), and *Bacillus subtilis* (8.94×10^6 cfu/g) and with a fermentation temperature of 20–25°C and a dietary moisture content of 50%, the contents of ZEA decreased linearly ($p < 0.05$) during the fermentation process, whereas those of DON and FUMB₁ increased linearly ($p < 0.05$).

Microbial community overall diversity

Alpha diversity indexes were determined to evaluate the richness levels of bacterial and fungal diversity during the fermentation process (Table 4). Compared with the control, no significant difference of the Chao 1, abundance-based coverage estimator (ACE), and Shannon indexes in both the 25Toxin and 50Toxin treatments were observed ($p>0.05$). However, the Chao 1 and ACE decreased linearly ($p<0.05$) as the dietary toxin contents increased.

Composition of the dominant bacterial communities

The sequences of bacteria were assigned to genera to determine the relative microbial abundance for each treatment (Table 5). *Lactobacillus*, *Acetobacter*, *Bacteroides*, *Enterococcus*, and *Zea* were the core microbiome genera, accounting for 49.00%, 13.85%, 2.10%, 2.44%, and 2.26% of the total number of sequences, respectively. Compared with the control treatment, the 25Toxin and 50Toxin treatments showed significantly decreased percentages of *Lactobacillus*, *Acetobacter*, and *Bacteroides* ($p<0.05$), and the percentages of *Acetobacter* and *Bacteroides* in the 50 Toxin treatment were significantly lower ($p<0.05$) than those in the 25 Toxin treatment. However, compared with the control and 25 Toxin treatments, the 50 Toxin treatment showed a significant increase of the percentage of *Enterococcus* ($p<0.05$). As the dietary toxin content increased, the percentages of *Lactobacillus*, *Acetobacter*,

and *Bacteroides* decreased linearly ($p < 0.05$), whereas the percentages of *Enterococcus* and *Zea* increased linearly ($p < 0.05$).

Heatmap of observed species

The 50 most abundant OTUs were selected and used to create a heatmap (Fig. 1). The heatmap showed that several OTUs were present in all stages of the fermentation process. Consistent with the results shown in Table 5, *Lactobacillus*, *Acetobacter*, and *Bacteroides* were commonly observed in the control treatment, whereas *Enterococcus* and *Zea* were abundant in the 25Toxin and 50Toxin treatments.

DISCUSSION

Changes in pH and mycotoxin content

pH is one of the most basic and important indexes reflecting the degree of fermentation. The present pH result decreased slowly as the toxin content increased. This could be related to the presence of moniliformin, which is usually present in the form of sodium and potassium ($pK_a = 0 + 0.05 - 1.7$), yielding a type of anionic acid, similar to inorganic acid (Gao et al. 2012; Kandler et al. 2002). Thus, it was reasonable that the pH was significantly higher on day 18 in the 50Toxin and 25Toxin treatments than in the control treatments Li et al. (2009) found that lactic acid bacteria

were capable of generating large amounts of lactic acid by fermentation of carbohydrates. Increased lactic acid contents in the fermentation feed and decreased pH have been also observed previously (Li et al. 2010). The current study demonstrated that the pH (< 4.5) of the control met the standard of qualified feed at the end of fermentation (Canibe et al. 2012). The growth of *Fusarium* consumed a large amount of nutrients, resulting in decreased acid production during the fermentation process (Seefelder et al. 2002) and elevating the pH in the 50Toxin and 25Toxin treatments.

Fusarium toxins can lead to plant diseases and economic losses, and the contaminated food and feed can also affect the health of animals and humans. Previous studies have shown that *Bacillus subtilis* ANSB01G can effectively degrade 89% of ZEA; the mechanism was thought to involve the binding of the phenolic hydroxyl treatment of the ZEA molecule to the γ -carboxyl treatment of glutamic acid, followed by hydrolysis of the lactone ring in the molecular structure, decarboxylation and reduction of carbonyl treatments, and ultimately removal of the water (Lei 2014). Many proteases can be produced by *Bacillus* ZZ, and these enzymes are capable of DON degradation (Cheng 2010). The degradation rates of FUMB₁ by *Lactobacillus plantarum* B7 and *Lactobacillus pentosus* X8 at 37°C were 52.9% and 58%,

respectively (Wang et al. 2015). In the present study, conventional solid fermentation could significantly reduce ZEA contents while enhance DON and FUMB₁ contents. Previous studies have shown that the optimum humidity and temperature for DON and FUMB₁ production by *Fusarium* were 40–50% and 5–25°C for DON and 40–50% and 15–25°C for FUMB₁ (Samapundo et al. 2005). Our findings were consistent with this previous study. However, further studies are needed to assess the mechanisms involved in these processes.

Microbial communities

The alpha diversity index was used to compare the richness and diversity of bacterial communities under each treatment. The species observed from the exponential dilution curve with testing depth increased gradually, but did not reach saturation. Although most bacteria were covered, some bacteria were not detected. A previous study showed that the relative abundances of the phyla *Actinobacteria* and *Proteobacteria* increased as the pH increased (Yun et al. 2017). Another important study revealed that *Alcaligenes* (hydrolic bacteria) and *Erysipelothrix* (acidogenic bacteria) were enriched when the pH was equal to 10, while less acidogenic bacteria existed when the pH was equal to 4 (Yuan et al. 2015). Previous studies indicated that *Bacillus subtilis* (Cho et al. 2010; Lei et al. 2014) and *Bacillus licheniformis* (Yi et al.

2011; Fu et al. 2016) could degrade ZEA. Lactonohydrolase from *Gliocladium roseum* can convert ZEA into nonestrogenic compounds and promote the secretion of harmless metabolites (Takahashi-Ando et al. 2002). Moreover, *Clostridiales*, *Anaerofilum*, *Collinsella*, and *Bacillus* could degrade DON, and these microbes could also be screened from chicken intestines using PCR-denaturing gradient gel electrophoresis (Yu et al. 2010). Consistent with the percentages of shared core microbiota, the heatmap results for *Lactobacillus*, *Acetobacter*, and *Bacteroides* OTUs were characteristic in the control, and OTUs for *Enterococcus* and *Zea* were abundant in the 25Toxin and 50Toxin treatments. Our heatmap analysis results indicated that the number of beneficial microorganisms decreased linearly, whereas the number of harmful microorganisms increased linearly as the dietary toxin content increased. Although ZEA biotransformation (Cho et al. 2010; Lei et al. 2014; Yi et al. 2011; Fu et al. 2016), DON biotransformation (Yu et al. 2010), and *Lactobacillus* detoxification (Revillaguarinos et al. 2013) have been reported, further studies are needed to determine which bacteria degrade ZEA and facilitate accumulation of DON and FUMB₁.

CONCLUSION

Under the present experimental conditions, conventional solid fermentation altered microbial diversity by high-throughput sequencing in the *Fusarium* mycotoxin-contaminated diet, and the content of ZEA could be reduced significantly. However, these conditions also caused deleterious effects with regard to the contents of DON and FUMB₁. Further studies are needed to determine the cellular and molecular mechanisms underlying the changes in mycotoxin contents and microbial diversity during the fermentation of mycotoxin-contaminated diets.

ACKNOWLEDGMENTS

Financial support was provided in part by the special funds of the construction of the modern agricultural industry technology system for the pig industry of Shandong Province (SDAIT-08-04), Research and development plan project of shandong province (2016GNC111018) and Funds of shandong "Double Tops" program.

Conflict of interest: The authors declare that there are no conflicts of interest.

REFERENCES

- Abrunhosa L., Moraes H., Soares C., Calado T., Vila-Chã A. S., Pereira M., Benâncio A. 2016.** A Review of Mycotoxins in Food and Feed Products in Portugal and Estimation of Probable Daily Intakes. *Crit Rev Food Sci.* **56**: 249-265.
- Aiko V., Mehta A. 2015.** Occurrence, detection and detoxification of mycotoxins. *J Biosci.* **40**: 943-954.
- Altschul M., Ignatz G., Currie W. B., Ball B. A. 1990.** Synthesis and release of peptides produced by equine trophoblastic vesicles. *Theriogenology.* **33**: 187-187.
- AOAC Int. 2012.** Rockville. Official methods of analysis of the AOAC, 19ed, USA.
- Bata A., Lasztity R. 1999.** Detoxification of mycotoxin-contaminated food and feed by microorganisms. *Trends Food Sci Tech.* **10**: 223-228.
- Canibe N., Jensen B. B. 2012.** Fermented liquid feed-Microbial and nutritional aspects and impact on enteric diseases in pigs. *Anim Feed Sci Tech.* **173**: 17-40.
- Cheng B. C. 2010.** Screening and Mechanism Analysis of Probiotics for the Inhibition of Fusarium and Degradation of Two Mycotoxins. Central South University.
- Cho K. J., Kang J. S., Cho W. T., Lee C. H., Ha J. K., Song K. B. 2010.** In vitro degradation of zearalenone by *Bacillus subtilis*. *Biotechnol Lett.* **32**: 1921-1924.
- Davide S., Angelo G. 2017.** Containment of Mycotoxins in the Food Chain by Using Decontamination and Detoxification Techniques. *Practical Tools for Plant and Food Biosecurity.* **7**: 163-177.

Fu G., Ma J., Wang L., Yang X., Liu J., Zhao X. 2016. Effect of Degradation of Zearalenone-Contaminated Feed by *Bacillus licheniformis* CK1 on Postweaning Female Piglets. *Toxins*. **8**: 300.

Gao S., Lei M. Y., Qi D. S., Zhang N. Y., Zhang J. F. 2012. The toxic effect and mechanism of moniliformin and the overview of poison animals. *Feed Industry*. **8**: 53-57.

Gashaw M. 2016. Review on Mycotoxins in Feeds: Implications to Livestock and human health. *J Agr Res*. **5**: 137-144.

He J. W., Zhou T. 2010. Patented techniques for detoxification of mycotoxins in feeds and food matrice. *Recent Patents Food Nutri Agr*. **2**: 96-104.

Jiang S. Z., Yang Z. B., Yang W. R., Gao J., Liu F. X., Chen C., Chi F. 2010. Physiopathological effects of zearalenone in post-weaning female piglets with or without montmorillonite clay adsorbent. *Livest Sci*. **131**: 130-136.

Juan C., Berrada H., Mañes J., Oueslati S. 2017. Multi-mycotoxin determination in barley and derived products from Tunisia and estimation of their dietary intake. *Food Chem Toxicol*. **5**: 148-156.

Kandler W., Nadubinska M., Parich A., Krska R. 2002. Determination of moniliformin in maize by ion chromatography. *Anal bioanal chem*. **374**: 1086-1090.

Lei Y. P. 2014. Mechanism of Degrading ZEA by ANSB01G strain and Its Application Effect in Animal Production. Doctoral dissertation, Beijing: China Agricultural University Dissertation.

Lei Y. P., Zhao L. H., Ma Q. G., Zhang J. Y., Zhou T., Gao C. Q., Ji C. 2014. Degradation of zearalenone in swine feed and feed ingredients by *Bacillus subtilis* ANSB01G. Duxbury Press. **7:** 1473-151.

Li S. Z., Zhao N., Yang X. H., Wei J. T., Yan N. D., Guo W. Z., Zhang W., Huang S, W. 2010. Research on the effect of sensory analysis,pH and weight wasting of lactobacillus plantarum fermentated feed technology. Feed Industry. **31:** 31-34.

Li Y. K., Mao S. Y., Zhu W. Y. 2009. Current situation in research and application of fermented feed with probiotics. Anim Husbandry Vet Med. **3:** 90-93.

Macias E. S., Pereira F. A., Rietkerk W., Safai B. 2011. Superantigens in dermatology. J Am Acad Dermatol. **64:** 455-472.

Magnin M., Travel A., Bailly J. D., Guerre P. 2016. Effects of mycotoxins on health and performance in poultry. Prod Anim. **29:** 217-231.

Moltó J. C. 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: an oestrogenic mycotoxin. Food Chem Toxicol. **45:** 1-18.

Moretti A., Logrieco A. F., Susca A. 2017. Mycotoxins: An Underhand Food Problem. Mycotoxigenic Fungi.

Palacio A. D., Bettucci L., Pan D. 2016. Fusarium, and Aspergillus, mycotoxins contaminating wheat silage for dairy cattle feeding in Uruguay. *Braz J Microbiol.* **47**: 1000-1005.

Ren Z. H., Deng H. D., Wang Y. C., Deng J. L., Zuo Z. C., Wang Y., Peng X., Cui H. M., Fang J., Yu S. M., Shen L. M., Hu Y. C. 2016. The Fusarium toxin zearalenone and deoxynivalenol affect murine splenic antioxidant functions, interferon levels, and T-cell subsets. *Environ Toxicol Phar.* **41**: 195-200.

Revillaguarinos A., Gebhard S., Alcántara C., Starón A., Mascher T., Zúñiga M. 2013. Characterization of a Regulatory Network of Peptide Antibiotic Detoxification Modules in *Lactobacillus casei* BL23. *Appl Environ Microb.* **79**: 3160-3170.

Samapundo S., Devlieghere F., De M. B., Debevere J. 2005. Effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on corn. *J Food Protect.* **68**: 1054-1059.

Seefelder W., Gossmann M., Humpf H. U. 2002. Analysis of Fumonisin B1 in *Fusarium proliferatum*-Infected Asparagus Spears and Garlic Bulbs from Germany by Liquid Chromatography-Electrospray Ionization Mass Spectrometry. *J Agric Food Chem.* **50**: 2778-2781.

Takahashi-Ando N., Kimura M., Kakeya H., Osada H., Yamaguchi I. 2002. A novel lactonohydrolase responsible for the detoxification of zearalenone' enzyme purification and gene cloning. *Biochem J.* **365**: 1-6.

Wang X., Zheng J. Y., Peng X. L., Qiao Y. F., Zhou F., Zhao L. L., Zhang B. L. 2015. Study on the removal of fumonisins by *Lactobacillus* strains. *SCI Tech Food Industry.* **36**: 162-165.

Yi P. J., Pai C. K., Liu J. R. 2011. Isolation and characterization of a *Bacillus licheniformis* strain capable of degrading zearalenone. *World J Microb Biot.* **27**: 1035-1043.

Yu H., Zhou T., Gong J. H., Young C., Su X. J., Li X. Z., Zhu H. H., Tsao R., Yang R. 2010. Isolation of deoxynivalenol-transforming bacteria from the chicken intestines using the approach of PCR-DGGE guided microbial selection. *BMC Microbiol.* **10**: 182-190.

Yuan Y., Wang S., Liu Y., Baikun L., Bo W., Yong Z. P. 2015. Long-term effect of pH on short-chain fatty acids accumulation and microbial community in sludge fermentation systems. *Bioresource Techno.* **197**: 56-63.

Yun C., Xie J., Keke X., Nan S., Raymond J. Z., Yan Z. 2017. Enhanced volatile fatty acids (VFAs) production in a thermophilic fermenter with stepwise pH

increase-Investigation on dissolved organic matter transformation and microbial community shift. Water Res. **112**: 261-268.

FIGURE CAPTIONS

Figure 1. Effects of fermentation on heat-map of observed species of mycotoxin-contaminated diet (n=8). The 50 most abundant OTUs were selected and used to create a heat-map. The results showed that several OTUs were present in all stages of fermentation processes. Data are means for 8 replicates per treatment.

Table 1 Composition and nutrient levels of diets (air-dry basis, %)

Item	Control	25 Toxin	50 Toxin
Ingredients			
Corn	61.70	46.28	30.85
Contaminated-corn	—	15.42	30.85
Corn gluten meal	5.00	3.75	2.50
Contaminated- corn gluten meal	—	1.25	2.50
Soybean meal	24.00	24.00	24.00
Whey powder	3.00	3.00	3.00

Soybean oil	2.50	2.50	2.50
CaHPO ₄	1.30	1.30	1.30
Limestone	1.10	1.10	1.10
NaCl	0.40	0.40	0.40
1% Premix ^a	1.00	1.00	1.00
Total	100	100	100
Nutrient levels^b			
DE/(MJ/kg)	14.25	14.25	14.25
CP/%	19.26	19.11	19.06
Ca/%	0.80	0.82	0.83
Total P/%	0.58	0.54	0.54
NaCl/%	0.42	0.42	0.42
Lys/%	1.11	1.11	1.11
Met/%	0.41	0.41	0.41
Threonine/%	0.78	0.78	0.78
Mycotoxins content (µg/kg)			
Zearalenone, ZEA	146.44±8.48	295.67±2.66	571.75±1.12
Deoxynivalenol, DON	290.13±1.56	412.58±2.84	644.11±4.33

Fumonisin B ₁ , FUMB ₁	----	1014.25±10.24	2020.55±13.14
--	------	---------------	---------------

Note: Contrpol was the basal diet. 25Toxin and 50Toxin were the basal diet in which 25% and 50% corn and corn gluten meal were replaced by mycotoxin-contaminated corn and corn gluten meal, respectively.

^aThe premix provided the following per kg of diets: Vitamin A 3300 IU, Vitamin D₃ 330 IU, Vitamin E 24 IU, Vitamin K₃ 0.75 mg, Vitamin B₁ 1.50 mg, Vitamin B₂ 5.25 mg, Vitamin B₆ 2.25 mg, Vitamin B₁₂ 0.026 mg, Pantothenic acid 15.00 mg, Niacin 22.5 mg, Biotin 0.075 mg, Folic acid 0.45 mg, Mn 6.00 mg, Fe 150 mg, Zn 150 mg, Cu 9.00 mg, I 0.21 mg and Se 0.45 mg.

^bProtein, calcium and total phosphorus were analyzed values, and the other nutrient levels were calculated values.

Table 2 Effects of fermentation on pH at different time-point of mycotoxin-contaminated diet¹⁾ (n=8)

Treatment								SEM	P	
	0 d	3 d	6 d	9 d	12 d	15 d	18 d		Treatm	Linear
									ent	
Control	6.65Aa	5.10Bc	4.91 C	4.56Db	4.22Eb	4.12Eb	4.13E b	0.007	<0.001	<0.001
25Toxin	6.01Ab	5.26B b	4.87 C	4.79Da	4.56Ea	4.61Ea	4.57E a	0.009	<0.001	<0.001

		5.55 <i>B</i>	4.92				4.63 <i>E</i>			
50Toxin	5.64 <i>Ac</i>			4.80 <i>Da</i>	4.63 <i>Ea</i>	4.67 <i>Ea</i>		0.007	<0.001	<0.001
		<i>a</i>	<i>C</i>				<i>a</i>			
SEM	0.010	0.010	0.010	0.010	0.007	0.007	0.006	/	/	/
P	<0.001	<0.001	0.138	<0.001	<0.001	<0.001	<0.00	/	/	/
							1			

Note: Contrpol was the basal diet. 25Toxin and 50Toxin were the basal diet in which 25% and 50% corn and corn gluten meal were replaced by mycotoxin-contaminated corn and corn gluten meal, respectively.

^{A, B, C} Means within a row with different letters differ significantly ($p<0.05$). The same as below.

^{a, b, c} Means within a column with different letters differ significantly ($p<0.05$). The same as below.

Table 3 Effects of fermentation on mycotoxins contents of mycotoxin-contaminated diet¹⁾ (n=8)

Treatment ¹⁾	0 d	3 d	6 d	9 d	12 d	15 d	18 d	SEM	P	
									Treatment	Linear
ZEA (µg/kg)										
Control	143.92 <i>c</i>	135.39 <i>c</i>	126.86 <i>c</i>	105.61 <i>c</i>	0.00 <i>c</i>	0.00 <i>c</i>	0.00 <i>c</i>	0.91	<0.001	<0.001
25Toxin	292.98 <i>b</i>	279.62 <i>b</i>	266.27 <i>b</i>	239.57 <i>b</i>	231.58 <i>b</i>	223.58 <i>b</i>	207.60 <i>b</i>	0.26	<0.001	<0.001
50Toxin	568.12 <i>a</i>	527.70 <i>a</i>	487.27 <i>a</i>	406.43 <i>a</i>	384.72 <i>a</i>	363.01 <i>a</i>	319.59 <i>a</i>	0.86	<0.001	<0.001
SEM	1.251	1.055	1.104	1.404	0.838	0.510	0.830	/	/	/
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	/	/	/
DON (mg/kg)										
Control	0.28 <i>c</i>	0.30 <i>c</i>	0.31 <i>c</i>	0.34 <i>c</i>	0.37 <i>c</i>	0.41 <i>c</i>	0.48 <i>c</i>	0.001	<0.001	<0.001

25Toxin	0.39 <i>b</i>	0.43 <i>b</i>	0.48 <i>b</i>	0.58 <i>b</i>	0.64 <i>b</i>	0.70 <i>b</i>	0.83 <i>b</i>	0.005	<0.001	<0.001
50Toxin	0.60 <i>a</i>	0.60 <i>a</i>	0.60 <i>a</i>	0.61 <i>a</i>	0.68 <i>a</i>	0.75 <i>a</i>	0.88 <i>a</i>	0.012	<0.001	<0.001
SEM	0.011	0.007	0.004	0.009	0.006	0.005	0.008	/	/	/
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	/	/	/
FUMB₁ (mg/kg)										
Control	0.00 <i>c</i>	0.69 <i>c</i>	1.33 <i>c</i>	1.98 <i>c</i>	2.05 <i>c</i>	2.13 <i>c</i>	2.21 <i>c</i>	0.013	<0.001	<0.001
25Toxin	1.00 <i>b</i>	1.38 <i>b</i>	1.78 <i>b</i>	2.16 <i>b</i>	2.55 <i>b</i>	2.94 <i>b</i>	3.33 <i>b</i>	0.021	<0.001	<0.001
50Toxin	1.99 <i>a</i>	2.14 <i>a</i>	2.34 <i>a</i>	2.51 <i>a</i>	3.07 <i>a</i>	3.64 <i>a</i>	4.20 <i>a</i>	0.007	<0.001	<0.001
SEM	0.010	0.007	0.007	0.015	0.010	0.017	0.029	/	/	/
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	/	/	/

Note: Control was the basal diet. 25Toxin and 50Toxin were the basal diet in which 25% and 50% corn and corn gluten meal were replaced by mycotoxin-contaminated corn and corn gluten meal, respectively.

Table 4 Effects of fermentation on alpha diversity indices of mycotoxin-contaminated diet (n=8)

Treatment ¹⁾	Chao1 ^a	ACE ^b	Simpson ^c	Shannon ⁴⁾
Control	701.65	702.27	0.82	3.54
25Toxin	687.55	695.32	0.82	3.53
50Toxin	650.37	663.54	0.82	3.37
SEM	25.737	22.140	0.034	0.285
P				
Treatment	0.677	0.755	0.971	0.641
Linear	0.014	0.021	0.988	0.735

Note: Control was the basal diet. 25Toxin and 50Toxin were the basal diet in which 25% and 50% corn and corn gluten meal were replaced by mycotoxin-contaminated corn and corn gluten meal, respectively.

^aChao1, by calculating the communities detected the singleton and doubleton of OTUs, estimated actual community species. The greater Chao 1, the more richness the communities, initiated by Chao.

^bACE (abundance-based coverage estimator), the sequence within 10 OTUs are counted to estimate the number of species in community. The greater ACE, the more richness the communities, initiated by Chao.

^cSimpson and Shannon index are also used to evaluate the microbial community diversity. Simpson index was more sensitive to evenness and dominance of OTU in community, the greater Simpson, the less richness the communities, initiated by Simpson. While Shannon index was more sensitive to community richness and rarity of OTU, the greater Shannon index, the more richness the communities, initiated by Shannon.

Table 5 Effects of fermentation on the percentage of shared core microbiota of mycotoxin-contaminated diet (%) (n=8)

Treatment ^a	Lactobacillus	Acetobacter	Bacteroides	Enterococcus	Zea
Control	59.43a	29.81a	2.66a	2.11b	1.70
25Toxin	49.17b	10.36b	2.37b	2.27b	2.28
50Toxin	43.78b	1.39c	1.26c	2.94a	2.80
SEM	7.591	0.985	0.537	0.493	0.788
Treatment	0.047	0.017	0.032	0.026	0.198
P					
Linear	0.038	0.013	0.026	0.041	0.022

Note: Select the content of more than two percent of the microorganism as the core microbiome to carry out statistics.

^aControl was the basal diet. 25Toxin and 50Toxin were the basal diet in which 25% and 50% corn and corn gluten meal were replaced by mycotoxin-contaminated corn and corn gluten meal,

respectively.

For Review Only

Figure 1