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Author	Jin Young Jeong[1,*], Junsik Kim[1], Minji Kim[1], and Sungkwon Park[1]
Affiliation	[1]Animal Nutrition and Physiology Division, National Institute of Animal Science, Wanju 55365, Republic of Korea [2]Department of Food Science and Biotechnology, Sejong University, Seoul 05006, Republic of Korea
Special remarks – if authors have additional information to inform the editorial office	[*]Corresponding Author
ORCID (All authors must have ORCID) https://orcid.org	Jin Young Jeong (https://orcid.org/0000-0002-8670-7036) Junsik Kim (https://orcid.org/0000-0001-9692-757X) Minji Kim (https://orcid.org/0000-0003-2106-1921) Sungkwon Park (https://orcid.org/0000-0002-7684-9719)
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Jin Young Jeong
Email address – this is where your proofs will be sent	jeong73@korea.kr
Secondary Email address	jeongjinyoung73@gmail.com
Postal address	Wanju 55365, Republic of Korea

Cell phone number	+82-10-9754-5880
Office phone number	+82-63-238-7487
Fax number	+82-63-238-7497

Abstract

Deoxynivalenol (DON) toxicity causes oxidative stress, immunological disorders, and gastrointestinal injury, which reduce animal survival and productivity. Pigs are particularly susceptible to DON; therefore, clear standards for DON levels in animal feed are essential. Therefore, we investigated growth characteristics, biochemistry, histology, and metabolite profiles of growing pigs fed dietary DON levels. Twelve pigs were randomized to one of four diets for 28 d: 1) CON, control group fed conventional diets; 2) T1, 1 mg; 3) T2, 3 mg; and 4) T3, 10 mg DON/kg conventional diet. The results revealed that the final body weight of the growing pigs in the T3 group was the lowest of all DON-treated groups ($p < 0.05$). Additionally, the T3 group demonstrated the highest blood alkaline phosphate levels, whereas the T2 and T3 treatment groups exhibited reduced lipase levels compared to the other groups ($p < 0.01$). Histological analysis showed that fibrosis increased in the muscle, liver, and various tissues, while apoptosis increased in the liver and ileum with increasing DON levels. Metabolomic profiling revealed that several metabolic pathways, such as purine metabolism, were involved in the weight loss induced by DON toxicity. In conclusion, our study suggests that DON levels above the maximum residue limits have adverse effects on growing pigs and that these effects are caused by altered metabolites.

Keywords: growing pig, deoxynivalenol, histology, metabolite

Introduction

Mycotoxins are fungal secondary metabolites that frequently contaminate agricultural crops worldwide and adversely affect farm animals (Abdallah et al., 2015; Holanda and Kim, 2021). Deoxynivalenol (DON) is a type B trichothecene. It is produced by *Fusarium* species and is the most frequently detected mycotoxin in feed samples (Kwon et al., 2023). DON toxicity can lead to impairment of the immune system, oxidative stress, and damage to the gastrointestinal tract, which can affect the survival and productivity of livestock (Holanda and Kim, 2020; Chen et al., 2023). According to the European Food Safety Authority (EFSA), 75.2% of EU feed samples were contaminated with DON (EFSA, 2013). Furthermore, Gruber-Dorninger et al. (2019) reported DON contamination in 64.1% of feed samples from 2008 to 2017. In response, the maximum residue level for DON has been established by the US Food and Drug Administration (FDA) in grain and grain byproducts for swine at < 5 mg/kg (FDA, 2010). In contrast, the European Commission has set strict limits for capping DON levels in compound feeds for pigs at 0.9 ppm (European Commission, 2016).

Pigs, followed by mice, rats, poultry, and ruminants, are most susceptible to DON toxicity (Zhao et al., 2016). This is likely because pigs consume cereal-rich diets and lack the rumen microorganisms required to break down mycotoxins (Pierron et al., 2016; Jia et al., 2023). Consequently, pigs exhibit a higher bioavailability of DON and a prolonged elimination period of the toxin from the body compared to other animals (Schelstraete et al., 2020; Sun et al., 2022). A notable consequence of DON toxicity in pigs is growth retardation (Pestka and Smolinski, 2005). Symptoms such as diarrhea, vomiting, and anorexia result from ingestion of feed containing high levels of DON, which reduces feed intake and efficiency (Pinton et al., 2009; Pestka et al., 2017). Furthermore, DON causes oxidative stress through the generation of reactive oxygen species

(ROS), further compromising the immune system and causing histological alterations, including fibrosis and apoptosis (Chaytor et al., 2011; Kang et al., 2022). DON toxicity disrupts several metabolic processes, including glycolysis, protein biosynthesis, and cellular metabolism (Wang et al., 2019; Saenz et al., 2021).

Despite extensive research, the effects of DON may be highly variable and depend on several factors: the amount of toxin in the animal, its origin, the animal's age, duration of exposure, and its simultaneous interaction with other substances (Serviento et al., 2018). Weaned piglets are more vulnerable to DON toxicity because their intestines are less adapted to sudden changes in feed. Many studies have focused on DON toxicity in piglets. However, as slow growth in pigs results in reduced profitability, knowledge of the harmful effects of feed to pigs is important to enable farmers to manage their diets effectively (López-Vergé et al., 2018). Determining the amount of DON in the diets of growing pigs is essential to reduce the risk of DON in pig production. However, studies on DON toxicity in growing pigs are limited. The toxicity of DON in this study was evaluated at concentrations higher than the maximum residue level during the growing period. We investigated the effects of different DON levels on histological alterations, growth characteristics, and blood biochemistry of growing pigs. Additionally, this study explored metabolites and their correlation with growth performance.

Materials and Methods

Ethics statement

The Committee on the Institutional Care and Use of Animals of the National Institute of Animal Science of the Republic of Korea reviewed and approved all experimental procedures (NIAS-2020-0479).

Animals and study design

Castrated pigs were sourced from Taeheung (Yeonggwang, Republic of Korea). Twelve pigs (Landrace × Yorkshire) were housed in individual pens measuring 130 × 240 cm. Housing conditions were maintained throughout the study, including acclimation, according to the following specifications: a light-dark cycle of 12:12 h, a room temperature of 25 ± 2 ℃, and a relative humidity of 60 ± 5%. The pigs were divided into four groups as follows: the control group received a basal diet, the T1 group received a basal diet with 1 mg/kg added DON, the T2 group with 3 mg/kg, and the T3 group with 10 mg/kg. Pigs had ad libitum access to water and food for 4 weeks. Diets were supplemented with DON (TripleBond, Guelph, Ontario, Canada) according to established experimental concentrations. Mycotoxins were dissolved with 1-5% ethanol in an autoclaved sterilized beaker and stirred until completely dissolved. The solvent amount was tested to ensure no impact on feed fluidity despite moisture content. At the end of the experimental period, blood samples were taken 1 d before tissue sampling. T61 was used to anesthetize all animals. Samples were taken from the feces, ileum, liver, muscle, rectum, and urine immediately after exsanguination. Blood and debris were removed using phosphate-buffered saline (PBS) and sterile disposable wipes. Samples were rapidly frozen in liquid nitrogen for storage at -80 ℃. Additionally, tissue fixation for histologic analysis was performed with 10% neutral buffered formalin (NBF; Sigma-Aldrich, St. Louis, MO, USA). The average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated as follows:

ADG = (final weight - initial weight)/age(d)

ADFI = feed supplied - feed remaining

FCR = feed consumed/ADG

Deoxynivalenol content analysis

Ultra-performance liquid chromatography (UPLC) mass spectrometry was used to analyze DON in diets, as described previously (Jeong et al., 2024). Briefly, a 1 g homogenized DON sample was extracted with water and diluted in PBS, then applied to the appropriate columns. The diets contained 0.73, 2.61, and 9.52 mg/kg DON. The same diet was used as previously described. The control sample had no DON contamination.

Blood biochemical analysis

Pig blood samples were taken in tubes from each growing pig via the jugular vein. Briefly, serum was centrifuged at 700 x g for 15 minutes at 4 ℃, then stored at -80 ℃ (Jeong et al., 2024). Blood parameters, including glucose, creatine, blood urea nitrogen, phosphate, calcium, total protein, albumin, globulin, alanine aminotransferase, alkaline phosphatase, total bilirubin, cholesterol, amylase, and lipase levels, were analyzed using a VetTest chemistry analyzer (IDEXX, Westbrook, ME, USA).

Histological analysis

Analysis of DON-induced fibrosis and apoptosis may improve our understanding of tissue damage and repair. Samples (5 × 5 mm) of liver, muscle, duodenal, ileal, rectal, jejunal, cecal, and colonic (ascending, transverse, and descending) tissues were collected as previously described (Jeong et al., 2024). Each sample was fixed in 10% NBF, dehydrated, embedded in paraffin, and heated. Slides were deparaffinized, rehydrated, and stained. They were then observed under a microscope at 200× and 400× magnifications.

Metabolite preparation and analysis of blood, liver, cecum, urine, and feces

UPLC-Q-TOF MS was used to analyze changes in pig metabolites following DON-contaminated diets. Experimental pretreatment and analytical methods were carried out as previously described (Jeong et al., 2024). Briefly, 100 µL serum was mixed with 400 µL acetone, shaken, and the 400 µL supernatant collected, lyophilized, then dissolved in 100 µL 20% methanol containing an internal standard. . Urine was treated the same way, while liver, cecum, and feces samples were dissolved in 80% methanol with an internal standard. The resulting solutions were analyzed by UPLC-Q-TOF MS. After metabolomic analysis, samples were pooled. Samples were injected into an Acquity UPLC C18 column with a mobile phase of water and acetonitrile. Blood, liver, cecum, feces, and urine took 12 mins; blood and urine at 40 ℃ took 16 min. The eluted compounds were analyzed by MS in ESI mode. TOF-MS data were scanned between 100 and 1500 m/z with 0.2 s scan time. Capillary and sample cones set at 3 and 40 V, 800 L/h desolvation flow, 300 ℃ desolvation temperature, and 100 ℃ source temperature. Leu-Enk was used as the reference compound due to its low mass and was analyzed every 10 s. QC samples were analyzed every 10 runs. MS/MS spectra were obtained at m/z 50–1500 using a ramped collision energy. MS data were processed using MarkerLynx 4.1, including m/z, RT, and intensity calculations. LC-MS data were acquired using MarkerLynx. Peak data were identified using various parameters and normalized. Metabolites were identified using multiple databases and relevant literature.

Statistical analysis

Metabolite data were analyzed with SIMCA-P+. PLS-DA was used to visualize the results. PLS-DA using R2, Q2, and permutation tests. R2X/Y assessed the model fit; Q2, future data. The PLS-DA results were validated using a permutation test. One-way ANOVA with Duncan test was used to analyze metabolite abundances ($p < 0.05$). Heatmaps of identified compounds were

created in R using a color scale based on z-scores. Prism 9.5.1 was used to perform a one-way ANOVA and Tukey's tests. The results are expressed as the mean ± SEM. Statistical significance was set at $p < 0.05$.

Results

Growth performance

Table 2 shows the impact of DON intake on 10-week-old pigs over 28 days. The control and DON treatment groups had similar initial body weights (BW) (34.5 ± 0.53 kg). The T3 group had the lowest final BW (46.4 ± 0.84 kg). ADFI, ADG, and FCR were not significantly different among the four diet groups.

Blood biochemistry

The effects of DON treatment on the biochemical parameters of the blood of growing pigs over a 28-day period are presented in Table 3. Blood parameters that did not differ significantly among dietary treatments were not reported. The levels of alkaline phosphate (ALKP) in the blood of growing pigs in the T3 group were the highest among the diet treatment groups ($p = 0.003$). However, in the T3 group, the level of lipase (LIPA) was significantly lower than that of the other treatments ($p = 0.007$).

Histological analysis

Masson's trichrome staining was used to observe histological changes, including fibrosis, in the liver, muscle, duodenum, ileum, rectum, jejunum, cecum, and colon (ascending, transverse, and descending) (Fig. 1). Increased fibrosis was observed in the portal areas of the liver lobules formed by an envelope of fibrous connective tissue. In skeletal muscle, fibrosis was caused by

DON in the endomysium and blood vessels. In the duodenum, fibrosis was observed in the muscularis mucosa and the submucosa. Blue staining was observed in the ascending colonic mucosa. Fibrosis was also observed in other tissues. However, these differences were minimal and difficult to detect. The TUNEL staining results, performed to observe apoptosis in the liver and ileum, are shown in Fig. 2. The figures represent 200 × and 400 × magnified images. The DON group showed more TUNEL-positive staining than the control group, suggesting severe apoptosis. DON increased fibrosis and apoptosis; however, the effects were minimal or insignificant in some tissues.

Metabolomic profiling

To understand the metabolic impact of DON toxicity at different levels, liquid chromatography-mass spectrometry was used to characterize the metabolites in the blood, cecum, feces, liver, and urine of growing pigs. PLS-DA indicated that metabolites in the DON and control groups were significantly separated in the cecum, urine, and feces compared to those in the control but not in the blood or liver (Fig. 3A–E). Additional analyses were performed on DON-contaminated pigs to identify biomarkers. Based on VIP values > 1.0 and $p < 0.05$, the metabolites in tissues including blood, liver, cecum, urine, and feces were as follows. In blood, levels of N-Boc-L-2-aminoadipic acid, phenylalanine, N-retinoylleucine, tetracosaheptaenoic acid, nisinic acid, benzoic acid, ethyl docosahexaenoate, LPC(P-18:0), LPC(16:0) 2M, and LPC(17:0) were significantly altered. The levels of several compounds in the liver were also significantly altered. In the cecum, L-alpha-glycerylphosphorylcholine, creatine, 7H-purin-8-ol, tyrosine, phenylalanine, butyrylcarnitine, tryptophan fragment, glycolic acid, glycoursodeoxycholic acid, 3-hydroxy-5-cholenoylglycine, 7-ketoglycolithocholic acid, 5,6-benzoarachidonic acid, ethyl docosahexaenoate, LPC(14:0), LPC(14:1), LPC(15:0), LPC(16:0),

LPC(16:0, 17:0, 18:0, and 18:1 were all significantly altered. Urine showed significant changes in 4-aminobenzoic acid, Gly-Pro-Glu, chrysin-7-O-β-D-glucuronide, oroxindin, chrysin-7-O-glucuronide, baicalin, and 5-hydroxy-2-(3-methoxystyryl)-1-benzofuran-3-carbaldehyde. In feces, levels of threonic acid, phenylalanine, N-{[1-(L-alanyl)-4-piperidinyl]carbonyl}-L-isoleucine, and tert-butyl 2-(2-butoxy ginkgolic acid, tetracosaheptaenoic acid, and tetracosapentaenoic acid were significantly changed. Figure 3G shows changes in purine metabolism, phenylalanine-tyrosine, tryptophan biosynthesis, and phenylalanine metabolism in the DON-treated group. Most candidate metabolites increased at 3 mg/kg (T2) and decreased at higher concentrations (10 mg/kg; T3), but urinary levels only increased at higher concentrations.

Correlation analysis between final body weight and metabolites

A linear regression model was used to analyze the correlation between final BW and blood and tissue metabolites (Fig. 4). Phenylalanine, tyrosine, and creatine levels were significantly correlated with the final BW. Phenylalanine in both cecum ($R^2 = 0.4771$, $p = 0.0137$) and feces ($R^2 = 0.3266$, $p = 0.0428$) metabolites, and tyrosine ($R^2 = 0.3820$, $p = 0.0322$) in cecum metabolites were negatively correlated with the mean final body weights (Fig. 4A–C). Among the cecal metabolites, creatine ($R^2 = 0.3509$, $p = 0.0424$) was positively correlated with mean final body weight (Fig. 4D). However, correlations with blood biochemical variables did not differ.

Discussion

The U.S. Food and Drug Administration (FDA) has recommended a maximum residue level of DON of <5 mg/kg for grain and grain by-products within 20% of the total swine diet, which is equivalent to 1 mg/kg when converted to complete feed (FDA, 2010). The Canadian Food

Inspection Agency (CFIA) also recommends the same standards as the FDA (CFIA, 2024). European Commission's minimum residue level for DON in pig feed of 0.9 mg/kg (European Commission, 2016). China's Feed Safety Standard sets the standard for deoxynivalenol in complete feed for pigs at 1 ppm (Zhao et al., 2021). The Korean Feed Standards and Specifications recommend a deoxynivalenol management level of 0.9 mg/kg in pig feed. Based on these recommendations and regulatory standards from these different countries, the minimum standard for DON treatment was set at 1 mg/kg in this study. Furthermore, we anticipated that DON levels above 1 mg/kg would adversely affect the health of growing pigs and thus focused on evaluating the effects of graded levels of DON. Although Wu et al. (2015) found that a DON concentration of 3 mg/kg had no significant effects on the health of growing pigs, Wellington et al. (2021) reported serious effects at the same concentration. Therefore, 3 mg/kg was used as the intermediate level to clarify the effects of DON toxicity in growing pigs. Additionally, to assess the effect of high levels of DON toxicity during the growth period, the maximum treatment was set at 10 mg/kg (Jeong et al., 2024). Thus, the effects of graded levels of DON (1, 3, and 10 mg/kg feed) on the growth performance, blood biochemistry, histology, and metabolite levels were examined in growing pigs over a 4-week period.

DON adversely affects the growth performance of pigs (Wu et al., 2015; Reddy et al., 2018). The main symptoms of DON toxicity include vomiting, diarrhea, and anorexia (Pinton et al., 2009; Pestka et al., 2017), possibly resulting in intestinal damage, reducing overall nutrient absorption and utilization, which leads to decreased growth performance (Ghareeb et al., 2015). In this study, high levels of DON (10 mg/kg) significantly decreased growth performance, with an 11.6% decrease in final BW compared with the control group. However, our results did not show any effect of DON toxicity on ADFI or FCR. Similar to our results, Reddy et al. (2018) reported that the final BW of growing pigs fed 8 mg/kg DON decreased by 17% compared with

that of the control group, whereas DON supplementation had no significant effect on the ADFI of growing pigs compared with the control group. Sayyari et al. (2018) also administered high doses of 5 mg/kg DON during the growth phase, but neither ADFI nor FCR showed significant differences compared to the control group. In contrast, Wu et al. (2015) reported that ADFI in growing pigs fed a high level of DON (12 mg/kg) was reduced by 41.6% compared with the control group. Although approximately 85% of weight loss due to mycotoxicosis is attributed to reduced feed intake, various factors, such as the contamination level, pig health status, and feeding period, can also have an effect (Pastorelli et al., 2012; Weaver et al., 2013). Therefore, further research that considers internal and external factors is required to determine the exact causes of weight loss.

In the present study, ALKP and LIPA levels were affected by high DON levels. We observed increased serum ALKP levels in growing pigs in the high-level DON treatment groups, which is consistent with the findings of Wu et al. (2015), who administered 12 mg/kg DON to 60–88-d-old pigs. Serum ALKP is secreted by mucosal cells lining the biliary tract of the liver and can leak into the blood when the liver cells are damaged (Wu et al., 2013; Ji et al., 2023). Therefore, the increase in serum ALKP levels in the high DON treatment groups may indicate liver damage owing to DON-induced systemic toxicity, which may be explained by the abnormal excretion of hepatic metabolites (Chaytor et al., 2011). Our results also showed reduced blood LIPA levels in growing pigs fed 3 mg/kg DON. LIPA is a hydrolytic enzyme secreted by the pancreas that breaks down fatty acids, and its activity is an important indicator of intestinal digestive function (Long et al., 2021; Qin et al., 2023). To date, no study has reported a direct relationship between deoxynivalenol and blood lipid levels in growing pigs. However, DON intake damages the intestinal mucosa and increases intestinal permeability, reducing intestinal absorption and impairing digestive organ function (Pierron et al., 2016). This may result in the suppression of

digestive enzyme secretion. Additionally, abnormalities in the biliary tract tissue may impair bile flow from hepatocytes during cholestasis, leading to the accumulation of bile acids in the liver, which may cause abnormal secretion of ALKP (Tannergen et al., 2006; Reyer et al., 2019). In all mammals, the hepatopancreatic biliary system consists of branching ducts linking the liver and pancreas to the duodenum (Zhang et al., 2023). Thus, abnormalities in these biliary tracts can cause pancreatitis and inhibit the secretion of digestive enzymes, including LIPA, from the pancreas (Tsomidis et al., 2008; Yin et al., 2023). Consequently, our results suggest that DON negatively affects the digestive processes of growing pigs, which may explain the decline in growth performance observed in the DON treatment group.

The liver is the primary organ affected by DON exposure, as it is crucial in detoxifying and metabolizing mycotoxins following the ingestion of DON-contaminated feed (Hasuda et al., 2022). Mycotoxins and their metabolites are primarily absorbed in the small intestine, with 51% of ingested DON absorbed in the small intestine (Lewczuk et al., 2016). Additionally, DON may be more susceptible to break down by microorganisms in the large intestine of pigs than by those residing in the initial segments of the intestine (Kollarczik et al., 1994; Lewczuk et al., 2016). In the present study, histological alterations, including fibrosis and apoptosis, were observed in liver and intestinal tissues in a dose-dependent manner. Our previous studies and several others have also observed fibrosis and apoptosis in porcine liver and intestinal tissues due to DON toxicity (Skeiepko et al., 2020; Jeong et al., 2024). Histological liver and small intestine damage may explain the abnormal secretion of ALKP and LIPA from the blood in this study. These changes may be closely related to DON-induced oxidative stress. Several studies have shown that DON induces oxidative stress by increasing the accumulation of reactive oxygen species (ROS), impairing the function of key antioxidant enzymes such as superoxide dismutase (SOD), GSH-Px and catalase (CAT), and increasing the levels of malondialdehyde (MDA) and 8-OHdG, a marker

of oxidative damage (Ji et al., 2023; Xu et al., 2020). Ji et al. (2023) found that increased levels of ROS, MDA and 8-OHdG were strongly correlated with an increase in the number of apoptotic cells in pig liver, demonstrating that hepatocyte apoptosis is induced by DON-mediated oxidative damage. Furthermore, DON-induced oxidative stress increased the expression of apoptosis-related genes and proteins, such as interleukin-1 beta (IL-1β), cyclooxgenase-2 (COX-2), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), caspase-3, caspase-8 and caspase-9 in porcine intestinal epithelial cells (IPEC-J2 cells) (Kang et al., 2019). DON-induced oxidative stress can lead to fibrosis (Lan et al., 2015). Fibrosis refers to the excessive accumulation of fibrous connective tissue in the extracellular matrix (ECM) of a damaged tissue (Antar et al., 2023). Oxidative stress boosts fibrotic factors like TGF-β1, leading to fibrosis by amassing extracellular matter (Yao et al., 2021; Antar et al., 2023). In addition, oxidative stress can trigger the release of inflammatory cytokines such as TNF-α, monocyte chemoattractant protein (MCP-1), IL-6 and IL-8, which can lead to tissue fibrosis (Ranneh et al., 2017; Yao et al., 2021; Antar et al., 2023). Our results show the risk of increased DON concentrations in pig diets due to tissue damage. However, as this study did not analyse DON-induced oxidative stress, future research investigating histological changes and DON-induced oxidative stress will be necessary.

Metabolomic analysis was conducted to explore the biological processes related to DON toxicity. Metabolites are the final products or intermediates of cellular activities and represent the overall response of organs or biological systems to various pathophysiological conditions (Wishart, 2019). Therefore, our results show the changes in pathways linked to DON toxicity. Metabolites from different tissues showed distinct profiles, indicating differences in metabolic profiles between the control and DON groups. These results are similar to those of our previous study that evaluated DON toxicity in weaned piglets (Jeong et al., 2024). Additionally, the correlation between the metabolites that contributed to the separation among treatment groups

and final body weight was analyzed to identify metabolic biomarkers associated with growth in pigs exposed to DON toxicity. In the present study, increases in phenylalanine and tyrosine contributed to weight loss in growing pigs, whereas increases in creatine were associated with weight gain in growing pigs. Phenylalanine is an essential amino acid that is a substrate for protein synthesis and other biochemical pathways (Xian et al., 2018). Phenylalanine suppresses food intake by inducing the secretion of satiety hormones (Alamshah et al., 2017). Tyrosine is the main metabolite of phenylalanine and is converted into other compounds, including dopamine, serotonin, and epinephrine. These are involved in biological processes such as stress response, appetite control, and behavioral regulation (Jeong et al., 2024). Creatine is endogenously synthesized from glycine, arginine, and methionine, primarily in the kidneys and pancreas (Wallimann et al., 2011; McBreairty et al., 2015). It is crucial in energy metabolism by providing the adenosine triphosphate required for cellular functions (Li et al., 2018). Additionally, creatine transporter mRNA expression is associated with the regulation of food intake, suggesting that creatine is closely related to feed intake and body weight gain (Li et al., 2018). Therefore, this suggests the possibility that creatine supplementation may improve growth performance in pigs impaired by DON toxicity. Indeed, several studies have shown that creatine monohydrate supplementation improves growth performance in pigs by stimulating muscle energy metabolism and increasing protein synthesis (Young et al., 2007; Li et al., 2018). However, few studies have directly linked DON toxicity to creatine supplementation. Therefore, further studies are needed to elucidate the effect of creatine supplementation on DON toxicity. Consequently, our findings suggest that DON toxicity affects the imbalance of various metabolic pathways in the body of growing pigs, which affected the weight loss of growing pigs in the high DON group. In this study, phenylalanine, tyrosine, and creatine synthesis were considered as potential biomarkers of DON toxicity affecting growth performance.

In conclusion, we demonstrated that there are no significant health effects at low DON levels for growing pigs, whereas high DON levels decreased growth performance and altered blood biochemical characteristics. Furthermore, our results showed that DON toxicity caused significant dose-related histological changes, including fibrosis and apoptosis, in specific organs of growing pigs. Additionally, DON toxicity induced metabolic changes in growing pigs, which were linked to their final body weights. Therefore, our findings suggest that DON levels above the maximum residue limits cause adverse health effects in growing pigs, with these effects intensifying as DON levels increase. However, because DON toxicity can manifest differently in chronic versus acute exposure, we will conduct future studies to clarify its effects throughout the lifespan of pigs (Pestka & Smolinski, 2005). In addition, a substantial proportion of feed is contaminated with multiple mycotoxins. DON toxicity may be exacerbated by interactions with other mycotoxins—such as zearalenone, fumonisin, and aflatoxin B1—that are frequently detected in animal feeds (Holanda & Kim, 2021; Lei et al., 2013; Weaver et al., 2013). In this context, several studies have reported that mitigation strategies, including inorganic compounds, adsorption, antioxidants, yeast, and bacteria, can help alleviate the toxic effects of these mycotoxins (Zhu et al., 2016; Holanda & Kim, 2021). In particular, biological detoxifiers such as probiotics and yeast are considered a promising approach to reduce toxic effects without compromising the nutritional value of feed, compared to physical and chemical methods (Recharla et al., 2022). Therefore, we will conduct further research to elucidate how DON interacts with other major mycotoxins commonly found in feed, while additional investigations are also needed to develop the most effective biological detoxifiers for application in conventional farming systems. Although further research is needed, this study can be used as a basis for toxicity studies and as a criterion for DON-contaminated diets for growing pigs.

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552 **Table 1.** Composition of the conventional diets in growing pigs

Ingredients	Percentage (%)
Corn	57.30
Soybean meal	25.00
Wheat bran	11.50
Molasses	1.40
Soybean oil	2.00
Limestone	1.00
L-Lysine	0.40
Salt	0.40
Sweet whey	0.50
Tricalcium phosphate	0.50
Total	**100**

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554

555 **Table 2.** Effects of increasing deoxynivalenol intake on growth performance in growing pigs for

556 4 weeks[1]

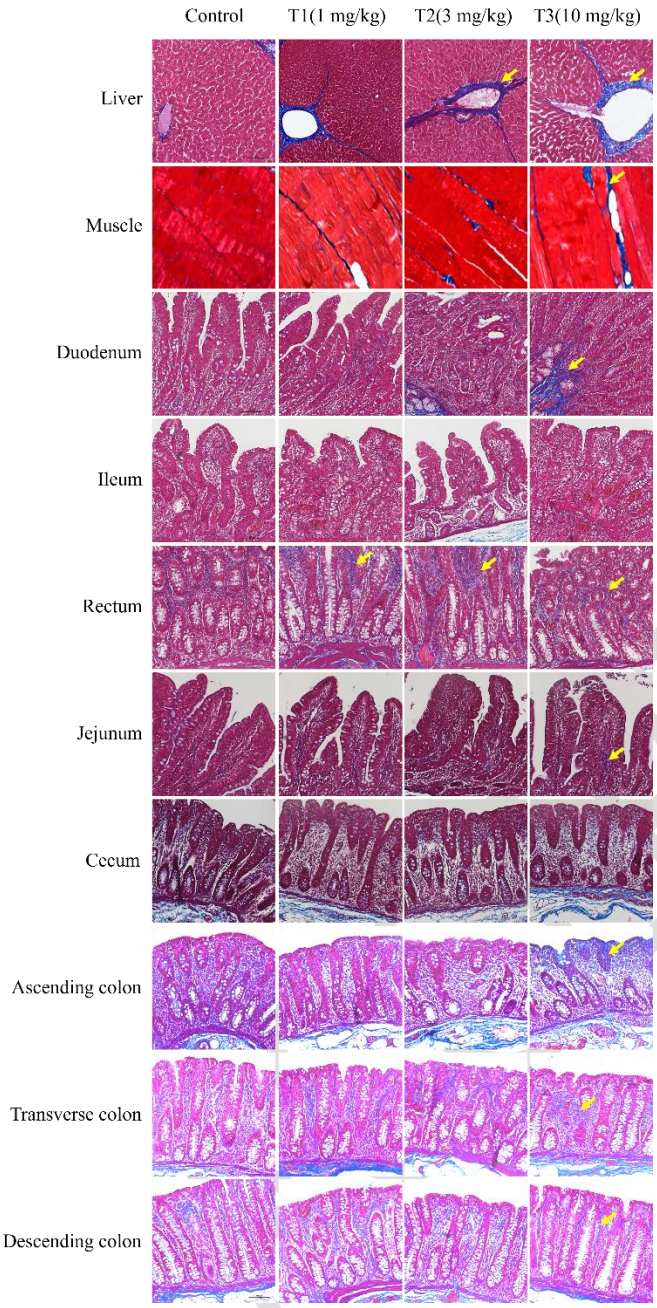
Parameters[2]	CTL (n=3)	T1 (n=3)	T2 (n=3)	T3 (n=3)	SEM	p-value
Initial BW, kg	34.8	34.5	34.4	34.4	0.53	0.995
Final BW, kg	52.5[a]	49.3[ab]	50.0[ab]	46.4[b]	0.84	0.045
ADFI, kg	1.30	1.30	1.33	1.27	0.04	0.974
ADG, kg	0.63	0.53	0.53	0.46	0.03	0.259
FCR	2.06	2.51	2.41	3.17	0.20	0.271

557 [1] CTL, control (basal diet); T1, basal diet + DON 1 mg/kg feed; T2, basal diet + DON 3 mg/kg
558 feed; T3, basal diet + DON 10 mg/kg feed
559 [2] BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed
560 conversion ratio
561 [a, b] Different superscript letters indicate that the variables within a row are significantly different
562 ($p < 0.05$).
563

564 **Table 3.** Biochemical effects of increasing deoxynivalenol intake in growing pigs for 4 weeks[1]

Parameters	CTL (n=3)	T1 (n=3)	T2 (n=3)	T3 (n=3)	SEM	p-value
Alkaline phosphatase, U/L	150.0[b]	201.3[a]	175.7[ab]	221.3[a]	7.94	0.003
Lipase, U/L	25.7[a]	25.2[a]	10.5[b]	11.3[b]	1.27	0.007

565 [1] CTL, control (basal diet); T1, basal diet + DON 1 mg/kg feed; T2, basal diet + DON 3 mg/kg
566 feed; T3, basal diet + DON 10 mg/kg feed
567 [a, b] Different superscript letters indicate that the variables within a row are significantly different
568 ($p < 0.05$).
569



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571
572 **Fig. 1.** Effects of increasing deoxynivalenol (DON) intake on histological analysis in growing
573 pigs. Images of the liver, muscle, duodenum, ileum, rectum, jejunum, cecum, and colon
574 (ascending, transverse, descending) of growing pigs treated with different concentrations of DON
575 were obtained after 28 d of the experiment using Masson's trichrome staining (blue). Signs of
576 fibrosis increased as DON concentrations increased in the liver, muscle, ileum, and duodenal

tissues. Control, basal diet; T1, basal diet + DON 1 mg/kg feed; T2, basal diet + DON 3 mg/kg feed; T3, basal diet + DON 10 mg/kg feed. The arrows indicate fibrosis. Observations were performed at 200 × magnification.

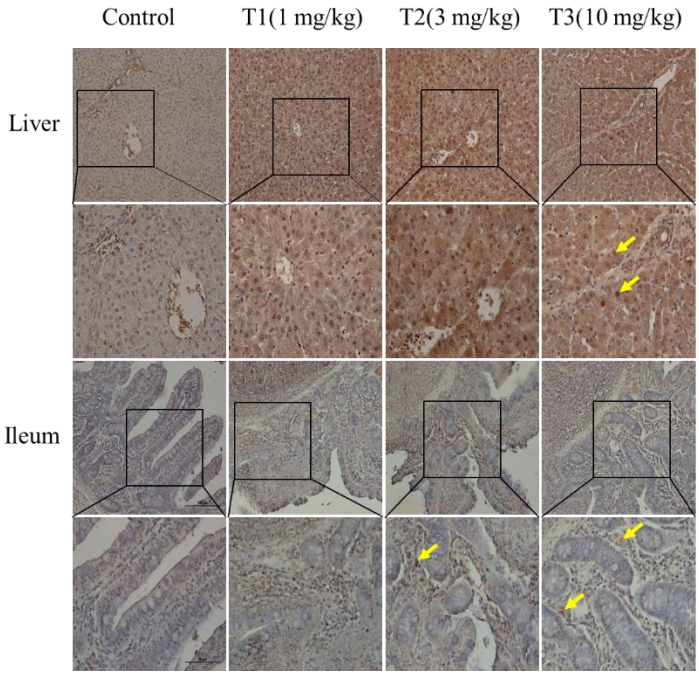
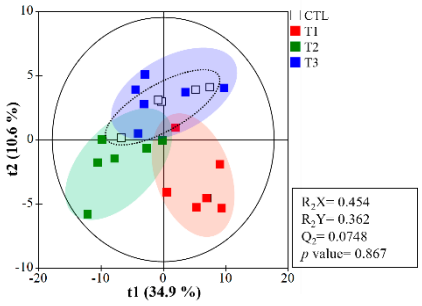
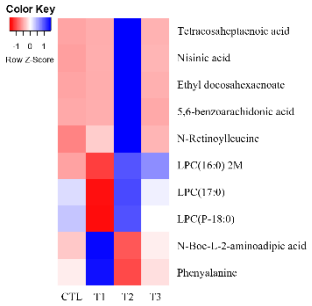


Fig. 2. Effects of increasing deoxynivalenol (DON) intake on apoptosis in growing pigs. Images at 200 × and 400 × magnification of the liver and ileum from growing pigs treated with increasing concentrations of DON were obtained after 28 d using terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining. In both organs, TUNEL-positive staining increased with elevated DON concentrations. Control, basal diet; T1, basal diet + DON 1 mg/kg feed; T2, basal diet + DON 3 mg/kg feed; T3, basal diet + DON 10 mg/kg feed. The arrows indicate apoptosis.

590 A





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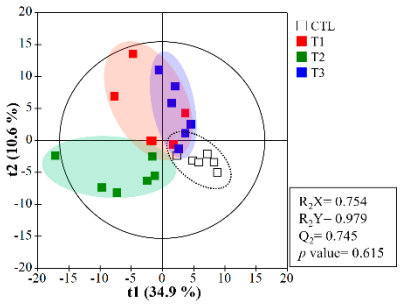

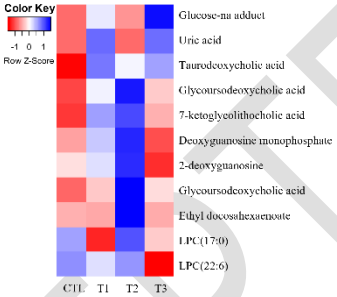

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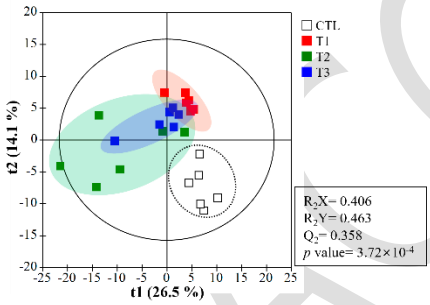

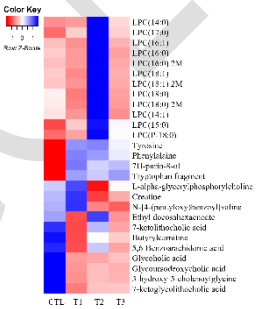

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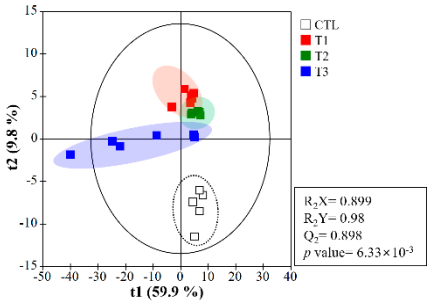

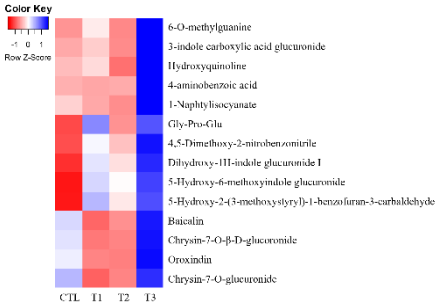

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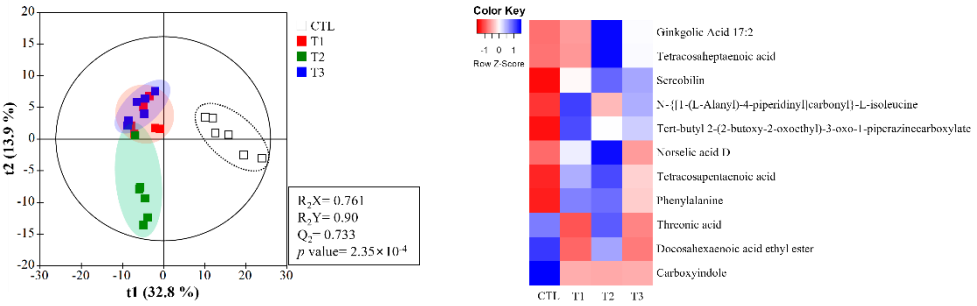

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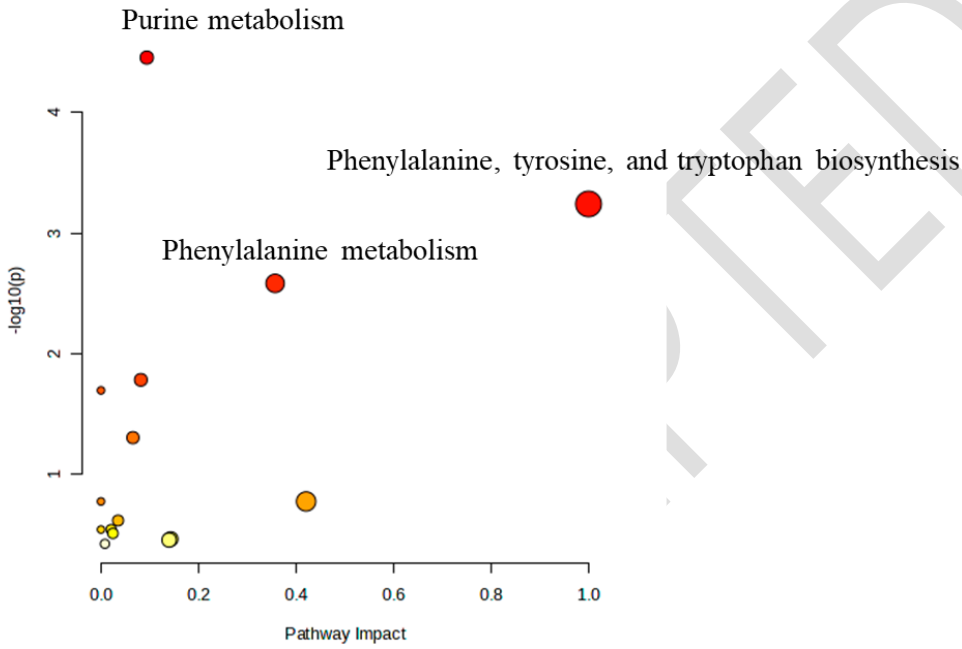

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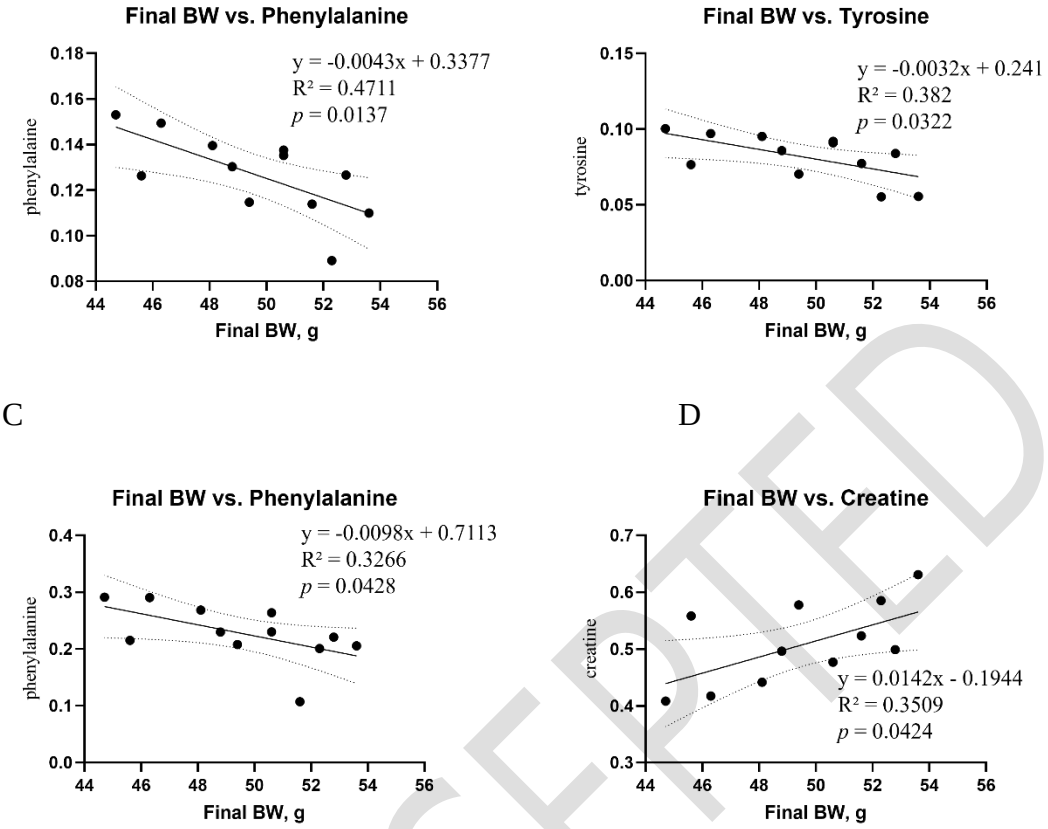
602 **Fig. 3.** Metabolite profiling of blood, liver, cecum, urine, and feces from deoxynivalenol (DON)-
603 contaminated growing pigs. Partial least squares discriminant analysis (PLS-DA) scores scatter
604 plot and heatmap of blood (A), liver (B), cecum (C), urine (D), and feces (E). (F) Metabolic
605 pathways from blood and four tissues metabolomics data were obtained using Holm–Bonferroni
606 and FDR correction. The most enriched pathways were identified, including purine metabolism.
607 “Pathway Impact Score” in the x-axis represents the impact of these enriched pathways computed
608 from topology analysis. “–log P” in the y-axis refers to the negative natural logarithmic value of
609 the original P-value from statistical analysis. Variations in score plots were defined using a 95%

confidence interval. Metabolites from the control and DON treatment groups showed distinct cluster separation in the cecum, urine, and feces but not in the blood and liver. The heatmap shows the significantly different data visualization of multiple parameters for the potential indicators of VIPs (VIPs > 1, $p < 0.05$). In the PLS-DA and biplots, the colored and white circles represent the metabolites identified in the DON-contaminated piglet tissue groups. CTL, control (basal diet); T1, basal diet + DON 1 mg/kg feed; T2, basal diet + DON 3 mg/kg feed; T3, basal diet + DON 10 mg/kg feed.

A B



C D

Fig. 4. Simple linear regression analysis showing the association between final body weight and metabolic parameters. Phenylalanine (A) and tyrosine (B) in cecum metabolites and phenylalanine (C) in feces metabolites were negatively correlated with final body weight. (D) Creatine in cecum metabolites was positively correlated with final body weight. The correlation coefficient and p-value were calculated using GraphPad Prism software. BW, body weight.