Deoxynivalenol damages the intestinal barrier and flora of the broiler chickens

shuangxiu wan
Shanxi Agricultural University
Na Sun
Shanxi Agricultural University
Hongquan Li
Shanxi Agricultural University
Ajab Khan
Shanxi Agricultural University
Xiaozhong Zheng
The University of Edinburgh
Yaogui Sun
Shanxi Agricultural University
Ruiwen Fan (ruiwenfan@163.com)
Shanxi Agricultural University

Research Article

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Abstract

Background

In livestock feed industry, feed and feed raw materials are extremely susceptible to mycotoxin contamination. Deoxynivalenol (DON) is one of the main risk factors of mycotoxin contamination in broiler feed and feedstuff, however, there are still few knowledges about tihs. Hence, the purpose of this study was to explore that the toxicity effect of DON on the intestinal barrier of broiler chickens and the microecological balance of the flora.

Results

In our present study, we compared the pathological scores of the small intestines of broilers on the 5th, 7th and 10th day, and chose the 7th day to analyze the small intestine histomorphology, tight junctions and cecal flora of the broilers. The results showed the damage to the small intestine worsened over time, the small intestinal villi of broilers were breakaged, the tight junctions of the small intestine were destroyed, the cecal flora was unbalanced, and the growth performance of broilers was reduced on the 7th day.

Conclusions

DON could damage the functional and structural completeness of intestinal tract, disorder the Intestinal flora, and finally lead to declined broiler's performance. Our study provided a basis for the prevention and treatment of DON in broiler production.

Background

In livestock feed industry, feed and feed raw materials are extremely susceptible to mycotoxin contamination. Deoxynivalenol (DON) in the trichothecene group B, produced by *Fusarium graminearum* is one of the main risk factors of mycotoxin contamination in broiler feed and feedstuff [1]. DON is also known as deoxynivalenol (3a, 7a, 15-trihydroxyfusarium-9-en-8-one), and its toxic effect could be maintained for more than one year under natural conditions, even for four years [2]. The main toxic effects of DON are cytotoxicity [3, 4], immunotoxicity [5, 6], neurotoxicity [7, 8] and synergistic effects with other biological toxins [9]. According to the tests conducted by Alltech Laboratories (China), deoxynivalenol and zearalenone were two of the main source of mycotoxin contamination in animal feed in 2018. The analysis of 44 types of mycotoxins from 411 animal feed samples in 24 provinces, autonomous regions and municipalities across the country revealed that the detection rate of fumonisins, trichothecenes B (deoxynivalenol), and zearalenone were all greater than 85% in 2019. Among the 149 feed and 34 litter samples in the first half of 2020, each sample was contaminated with 8.34 types of mycotoxins on average. The detection rates of fumonisins, trichothecenes B, and zearalenone were all greater than 92%. Therefore, deoxynivalenol contamination of feed was still a problem that seriously affects broiler production.

Intestinal mucosal biological, mechanical, chemical and immune barriers as well as the microecological balance of the intestinal flora are important for maintaining healthy growth and production of animals. However, in recent years, there have been few studies on DON's damage to the intestinal barrier of broiler chickens and the microecological balance of the flora [1, 10, 11]. The mechanism underlying the damages of intestinal barrier, the changes in the intestinal microbiota, and the impact on production performance of broilers by DON need to be elucidated. The purpose of this experiment was to study the toxic effects of DON on the growth performance, mechanical barrier of the small intestine, and the cecal flora of broilers which could provide a basis for the prevention and treatment of DON toxicity in broiler production.

Results

The effect of DON on the growth performance of broilers

Compared with the control group, the ADG, ADFI, and F/G of broilers in the DON group were decreased, among which ADG and ADFI were decreased significantly ($p < 0.05$). The results showed that the consumption of DON contaminated feed reduced the growth performance of broilers (Table 1).

Table 1
The effect of DON on the growth performance of broilers

Items	Control group	DON group	SEM	P-value
ADG(g)	16.78***	12.98	0.824	0.0001
ADFI(g)	24.71**	20.15	1.253	0.0097
F/G	1.82	1.44	0.258	0.171
n = 12 per treatment group. **$p < 0.01$, ***$p < 0.001$.				

Intestinal pathology score

As shown in Table 2, compared with the control group, no significant difference was observed for the intestinal pathology scores on the 5th day ($p > 0.05$). In contrast, the intestinal scores increased significantly ($p < 0.05$) on both 7th day and 10th day (Table 2).

Table 2
Results of pathological scoring of intestinal injury caused by DON in broilers

Item	5 d		7 d		10 d	
	Control group	DON group	Control group	DON group	Control group	DON group
Visual score	0	0.38 ± 0.48	0.25 ± 0.43	1.75 ± 1.56**	0.13 ± 0.33	4.25 ± 0.83***
Histological score	0.002	0.25 ± 0.43	0.38 ± 0.41	2.75 ± 1.50**	0.25 ± 0.43	5.25 ± 1.50***

Note: **$p < 0.01$, ***$p < 0.001$.

Morphological Changes Of Small Intestinal Villi

As shown in Fig. 1A, scanning electron microscopy analysis demonstrated that comparing with the control group, the duodenal mucosal surface of the DON group possessed less chyme and was swollen (blue arrow). Ulcers of different sizes (cyan arrow) were observed in the jejunal surface and ulcer foci of different sizes. Broken villi (yellow arrow) were also observed in the mucosal surface of ilium.

Comparing with the control group, H&E staining demonstrated that the edges of duodenal villi in the DON group were damaged. The outer cells were loosely arranged. The villus height and the villi/crypt ratio were significantly reduced ($p < 0.05$). The crypt depth was significantly increased ($p < 0.05$). There were shedding in the jejunum. The villus height was significantly reduced ($p < 0.05$). The crypt depth and the ratio between villi and crypt was reduced, although not significantly. In ileum the villi were irregular, and their heights were different (not significantly). The crypt depth and the villi/crypt ratio were significantly reduced ($p < 0.05$) (Fig. 1B and Table 3).

Table 3
Effect of DON on intestinal pathology

Parameter	Control group	DON group	SEM	P-value
Duodenum				
Villus height (μm)	787.99 ± 31.57***	657.39 ± 50.50	19.850	P < 0.0001
Crypt depth (μm)	81.20 ± 16.64	124.02 ± 19.55***	8.558	P < 0.0001
Villus/crypt ratio	10.03 ± 1.65***	5.43 ± 0.92	0.630	P < 0.0001
Jejunum				
Villus height (μm)	540.62 ± 17.40***	462.19 ± 9.67	6.634	P < 0.0001
Crypt depth (μm)	70.77 ± 10.42	63.12 ± 10.17	4.854	0.132
Villus/crypt ratio	7.85 ± 1.45	7.49 ± 1.04	0.595	0.547
Ileum				
Villus height (μm)	441.29 ± 25.55	428.62 ± 10.32	9.186	0.185
Crypt depth (μm)	55.64 ± 10.99	66.47 ± 6.70*	4.289	0.021
Villus/crypt ratio	8.30 ± 1.97*	6.52 ± 0.72	0.700	0.020
Note: ** $p < 0.01$, *** $p < 0.001$.				

Change in the digestive enzyme activities of small intestine in broilers

Compared with the control group, the amylase, trypsin and lipase activities in the DON treated group were reduced. The activity of amylase and trypsin in the duodenum were significantly reduced ($p < 0.05$), the lipase activity was non-significantly different ($p > 0.05$); the trypsin in the jejunum was significantly reduced ($p < 0.05$), but the activity of amylase and lipase showed a decreasing trend ($p > 0.05$); the amylase and trypsin activities in the ileum were significantly decreased ($p < 0.05$), while that of lipase were non-significantly changed ($p > 0.05$), as shown in Table 4.

Table 4
The digestive enzyme activities in each segment of the small intestine

Items	Control group	DON group	SEM	P-value
Duodenum				
Amylase U/mg prot	15.39*	14.97	0.201	0.0472
Trypsin U/mg prot	64.43***	63.57	0.179	$p < 0.0001$
Lipase U/g prot	3.01	2.96	0.0552	0.352
Jejunum				
Amylase U/mg prot	445.43	443.64	1.402	0.215
Trypsin U/mg prot	88.31**	87.37	0.300	0.0048
Lipase U/g prot	3.93	3.87	0.0433	0.225
Ileum				
Amylase U/mg prot	198.91*	195.58	1.267	0.0155
Trypsin U/mg prot	172.61**	171.88	0.207	0.0019
Lipase U/g prot	10.72	10.47	0.623	0.174
n = 12 per treatment group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.				

Expression analysis of the tight junction proteins in the small intestine

qRT-PCR was used to detect the mRNA expression of ZO-1, Occludin and Claudin-1 in each segment of the small intestine. The relative mRNA expression of ZO-1, Occludin and claudin-1 in the jejunum, ileum and duodenum of the DON group were significantly lower than the control group ($p < 0.05$, Fig. 2A). Western Blotting showed that the expression of Occludin and Claudin-1 proteins in the small intestine of DON group were decreased ($p > 0.05$) but not significantly compared with the control group. The expression of Claudin-1 and Occludin were significantly reduced in the jejunum ($p < 0.001$). Both Occludin and Claudin-1 expression in the ileum were significantly reduced ($p < 0.05$) (Fig. 2B). Immunohistochemistry showed that ZO-1, Occludin and Claudin-1 proteins were highly expressed in the small intestinal villi of control group and each protein was evenly expressed around the villi. The optical density results showed that compared with the control group, the expression of ZO-1, Occludin and Claudin-1 in the duodenum and jejunum of DON group were significantly decreased ($p < 0.05$), but the expression of ZO-1, Occludin and Claudin-1 in the ileum were not significantly decreased ($p > 0.05$) (Fig. 2C). Taken together, we demonstrated that the consumption of DON-contaminated feed inhibited the expression of ZO-1, Occludin and Claudin-1 in the small intestine.

The Changes In Microecology Of Caecal Flora In Broilers

The PCA method in 16S rDNA high-throughput sequencing was used for principal component analysis. The results showed that the bacterial community composition of control and the DON groups were significantly different. The dominant bacterial communities of the two groups were the same at the phylum level, but the relative abundance was different (Fig. 3A). At the phylum level, the dominant bacteria in the two groups were *Firmicutes*, *Proteobacteria*, *Tenericutes* and unknown bacteria. The proportions of these mentioned bacteria at the phylum levels were 88.01%, 10.66%, 1.13% and 0.20% for control group, and 96.16%, 1.52%, 2.16%, and 0.15% for DON group, respectively (Fig. 3B, C). The analysis of differences between the CON and DON groups showed that the *Firmicutes*, *Mounculus* and *Bacteroides* in the DON group were increased at the phylum level, while *Actinomycetes*, *Proteobacteria* and unknown bacteria were decreased, and *Proteobacteria* and unknown bacteria decreased significantly ($p < 0.05$), as shown in Fig. 3D.

Discussion

DON is the most common member of the trichothecene group of mycotoxins. In recent years, DON-contaminated feed has caused great economic loss to the livestock industry. It is well-documented that DON-contaminated feed leads to reduced performance, feed intake, body weight gain and feed conversion rate. High-dose acute DON exposure results in vomiting, diarrhea and neurological symptoms in humans and animals [10]. After oral administration, DON passes through the body's small intestinal barrier and is rapidly absorbed. It destroys the small intestinal mucosal layer, damages villi and causes epithelial shedding, which consequently affects the absorption of nutrients by mucosal epithelium [12, 13]. Therefore, intestines are the primary target of DON attack [14] and studying the molecular mechanism involved in the influence of DON on the intestinal tract of broilers is very crucial to successful broiler production.

The small intestine is the main site for transportation and absorption of nutrients in the body. The digestion, absorption and function execution of body are closely related to the small intestinal villi. The intact structure and good functions of the small intestinal villi are essential for better digestion and absorption of nutrients which leads to healthy growth of animal body [15]. The small intestinal villi increase the surface area of the inner wall of the small intestine as well as the capacity for the absorption of nutrients [16, 17]. The crypt between two villi is the site where the villi cells regenerate. The depth of the crypt reflects the rate of cell production and the shallower crypts indicate an increase in cell maturation rate and secretory function. The villus height to crypt depth ratio represents the secretory function of the small intestine [18]. This study found that the small intestinal villi of broilers fed with DON-contaminated feed were swollen, ulcerative, fractured, and the shorter villi reduced the absorption area of the intestinal tract, preventing the chyme from being in good contact with the intestinal tract and reducing the intestinal swing ability which resulted in overall reduction both in digestion and absorption of nutrients in the intestine. The increase of the crypt depth indicates a decrease of the intestinal epithelial mature cell numbers, which reduced the function of upper small intestinal cells. A decrease in the ratio of villus height to crypt depth indicated that the regeneration and metabolism of small intestinal epithelial cells slows down. Therefore, DON destroyed the mechanical barrier of small intestinal mucosa. The tight

junction proteins between adjacent epithelial cells are the basic structure of small intestinal mucosa. The expression of ZO-1, Occludin and Claudin-1 in the tight junction protein directly affects the intestinal barrier function [19]. The results of this study showed that DON reduced the expression of ZO-1, Occludin and Claudin-1 in the small intestine, especially in the duodenum and jejunum, which provided favourable condition for pancreatic juice and bile to damage duodenal and jejunal epithelial cells and resulted in reduced broiler performance.

The digestive enzymes in the small intestine play important roles in the digestion and absorption of nutrients. The level of digestive enzyme in the small intestine directly reflects the ability of animal nutrient utilization [20, 21]. Amylases catalyze the hydrolysis of glycogen, produce maltose and glucose and provide energy for the body [22]. Trypsin breaks down protein and provides amino acids for the body [23]. Under the combined action of bile salts, lipases break down fat into glycerol, fatty acids and monoglycerides, and thus provide energy for the body. Consumption of DON-contaminated feed reduced the amylase, trypsin, and lipase activities in the small intestine of broilers, impaired the digestion and absorption of nutrients in the small intestine and resulted in reduction of broilers performance.

In addition, the broilers intestinal microflora is of physiological importance in the host health and production performance in terms of host nutrient absorption, immune barrier, detoxification, immune system development and regulation [24]. Therefore, intestinal microbes are essential for stable production performance. As an active “organ” that interacts with the gastrointestinal environment, the flora provides nutrients and vitamins to the organism, transduce hormone information and ultimately affect the main metabolic pathways [25, 26]. Bacterial groups dominate the microbial community inhabiting broilers [27]. This study found that *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Tenericutes* were the main phyla in the broiler’s intestines, which was consistent intestine with the results of Oakley [28] and Qu [29]. Post consumption of the DON-contaminated feed, the *Firmicutes*, *Tenericutes* and *Bacteroides* phyla were increased. *Firmicutes* and *Bacteroides* are involved in energy regulation [30, 31, 7], fat metabolism [32, 33] and they work together to promote absorption or energy storage [34] in the body. So, these might be the important factors to reduce the body weight of broilers. The actinomycete flora was relatively less populated in the animal intestines, but it was of great importance. The *Bifidobacterium* under this phylum was believed to exert positive health benefits on their host. It can acidify the intestinal environment and inhibit the growth of pathogenic bacteria [35]. However, we found that DON consumption caused reduction of the *Actinomycetes* in the broiler’s intestines, indicating that the broiler’s intestinal injury was damaged. The phylum laminatum has been found to be increased in this research, which indicated that the growth environment of the flora had been destroyed. The *Proteobacteria* includes many pathogenic bacteria, such as *E. coli*, *Salmonella*, etc., their reduction in our study was probably the result of compensatory intestinal flora.

Conclusions

DON damaged the intestinal tract by destroying the morphology of the small intestinal villi, the tight junction protein of the small intestine, the enzyme activity of the intestine and the flora of the caecum,

leading to declined broiler's performance.

Materials And Methods

Animals

A total of 180 1-day-old AA broilers weighed 55.67 ± 1.43 g was purchased from Shanxi Elephant Agriculture and Animal Husbandry Group Co., Ltd, and randomly divided into two groups, control group and DON groups. Control group received basal diet and DON group received 10 mg/kg DON contaminated basal diet respectively, the ingredients and composition of the basal diets were as follows (Table 5). All broilers were free drinking, immunized with routine vaccines and had routinely feeding management.

Table 5
Ingredients and nutrient composition of the basal diets

Ingredients	Percentage (%)	Nutrient composition	Percentage (%)
Corn	61.17	Metabolism energy, (MJ/kg)	12.97
Soybean meal	29.50	Crude protein (%)	20.8
Fishmeal	6.50	Available P (%)	0.45
DL-Met	0.19	Ca (%)	1.02
L-Lys•HCl	0.05	Lys (%)	1.20
Bone Meal	1.22	Met + Cys (%)	0.86
Sodium chloride	0.37		
Microelement and Vitamin Compound Premix	1.00		
Total	100		

Premix can provide per kilogram of basal diet, Vitamin A 9500 IU, Vitamin E 30 IU, Vitamin D3 62.5 μg, Vitamin K3 2.65 mg, Vitamin B1 2 mg, Vitamin B2 6 mg, Vitamin B12 0.025 mg, Biological Element C 0.0325 mg, folic acid 1.25 mg, pantothenic acid 12 mg, niacin 50 mg, copper 8 mg, zinc 80 mg, manganese 80 mg, iodine 0.35 mg, selenium 0.3 mg.

Reagents And Antibodies

Amylase (C016-1-1), Trypsin (A080-2-2) and Lipase (A054-2-1) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). TRIzol reagent was obtained from Tiangen Biotech Co., Ltd.

(Beijing, China). Reverse transcription kit from TaKaRa (Japan), 2×SYBR Green qPCR Master Mix from Bimake (Houston, USA), RIPA cell lysate, protease inhibitor and phosphatase inhibitor were purchased from Solarbio (Beijing, China). β-actin Monoclonal Antibody was purchased from Immunoway Bitechnology company (Jiangsu, China). ZO-1(ab96587) was obtained from Abcam (Cambridge, MA, USA). Occludin (bs-10011R) was obtained from Bioss (Wuhan, China). Claudin-1 (13050-1-AP) was obtained from Proteintech (Wuhan, China) and DAB kit was purchased from CWBIO (Beijing, China). *Fusarium graminearum* ACCC 37687 was provided by Professor Yang Xiaojun, College of Animal Science and Technology, Northwest A&F University. AA broilers were purchased from Shanxi Elephant Farming and animal husbandry group (China).

Preparation Of Don Contaminated Feed

According to the methods described by Yu [10] and Yang [11], *Fusarium graminearum* ACCC 37687 was inoculated into potato dextrose agar medium and cultured at 27°C for 7 d to obtain a solid culture of *Fusarium graminearum*. Post inoculation, the medium was incubated at 27°C and 180 rpm for 7 d to obtain a liquid culture of *Fusarium graminearum*. 25 g of solid culture of *Fusarium graminearum* was inoculated into 100 mL of liquid culture and mixed with 200 g of rice, then further cultivated for 28 d to obtain DON-contaminated fragrant rice. The crushed DON contaminated rice was added to normal feed in a ratio of 10 mg/kg.

Intestinal Injury Score Of Broilers

According to the method of the previous studies [36, 37], the small intestine of broilers was scored by gross morphology and histomorphology.

Scanning Electron Microscopy

The tissue samples were fixed in glutaraldehyde by conventional method [38], and observed under scanning electron microscope (JEM-6490LV, JEOL, Japan).

Histopathological Observation Of Small Intestine

The duodenum, jejunum, and ileum were fixed in Bouin's solution for 24 hours, tissues were sectioned, stained with H&E, and mounted with neutral gum. The histological changes in intestinal tissue were observed with a microscope. Image J software (National Institutes of Health, USA) was used to measure the villus height (V) and crypt depth (C) and the villi/crypt ratio (V/C) were calculated.

Detection Of Small Intestine Tight Junction Proteins

The total RNA and proteins of the duodenum, jejunum and ileum samples were extracted, qRT-PCR and WB were used to detect the expression of ZO-1, Occludin and Claudin-1 in each intestinal segment, and the $2^{-\triangle\triangle ct}$ method and Image J software were used to calculate the relative expression of ZO-1, Occludin and Claudin-1 mRNA and protein level, respectively [39–41]. Immunohistochemical methods were used to detect the localization of ZO-1, Occludin and Claudin-1 in the duodenum, jejunum and ileum. The optical density was measured by Image Pro Plus software.

Determination of Lipase, Amylase and Trypsin of the small intestine

The collected duodenum, jejunum and ileum were ground, and then the lipase, amylase, and trypsin of mucosal homogenates in the duodenum, jejunum, and ileum were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

High-throughput Sequencing Of Cecal Intestinal Flora

The 16S rDNA high-throughput sequencing method was used to detect the bacterial flora in the cecum by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

Growth Performance

Broilers in both groups were fed for 7 d and weighed on fasting basis. The average daily gain (ADG, G), the average daily feed intake (ADFI, F) and F/G were calculated.

Statistical analysis

All data were expressed as mean standard error of the mean (Mean SEM). The differences among groups were analyzed by t-test using Graphpad Prism 5 (Graphpad Software, USA), $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ were considered statistically significant.

Abbreviations

DON
Deoxynivalenol

Declarations

Ethics approval and consent to participate

The experimental procedure involved animals in this study was approved by the Experimental Animal Ethics Committee of Shanxi Agricultural University (2017(050)), and the study is reported in accordance with ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Competing Interests

The authors declare no conflict of interest.

Founding

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Authors' Contributions

Conceptualization: S.W., H.L. and R.F.; methodology: N.S.; validation, S.W. and R.F.; formal analysis: A.K.; investigation: S.W.; resources: R.F.; data curation: R.F.; writing-original draft preparation: S.W.; writing-review and editing: R.F., H.L. and X.Z.; visualization: Y.S.; supervision: R.F.; project administration: R.F. All authors have read and agreed to the published version of the manuscript.

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Figures

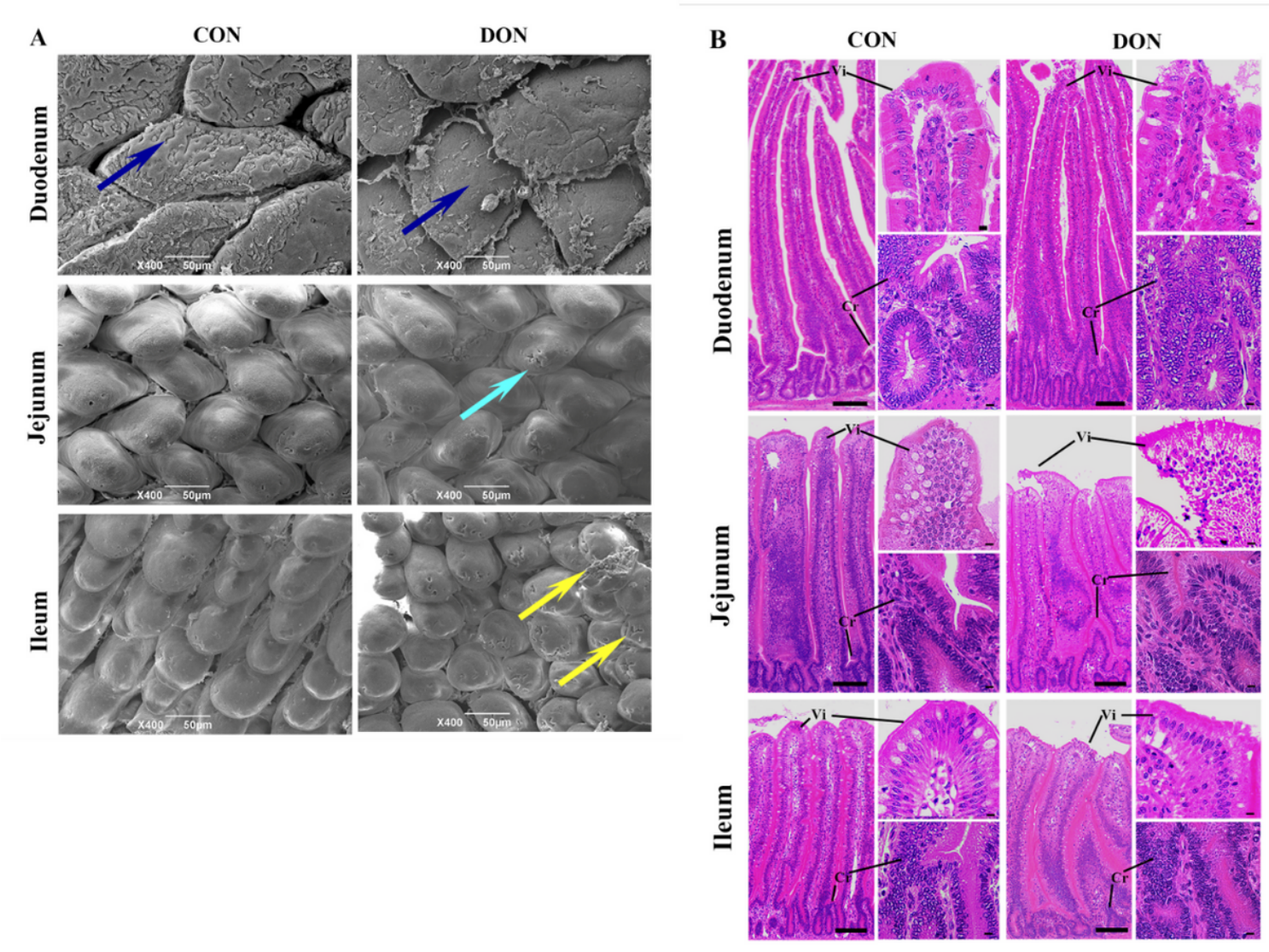

Figure 1

Morphology analysis of the small intestinal villi. (A) The scanning electron microscope images. (B) H&E staining.

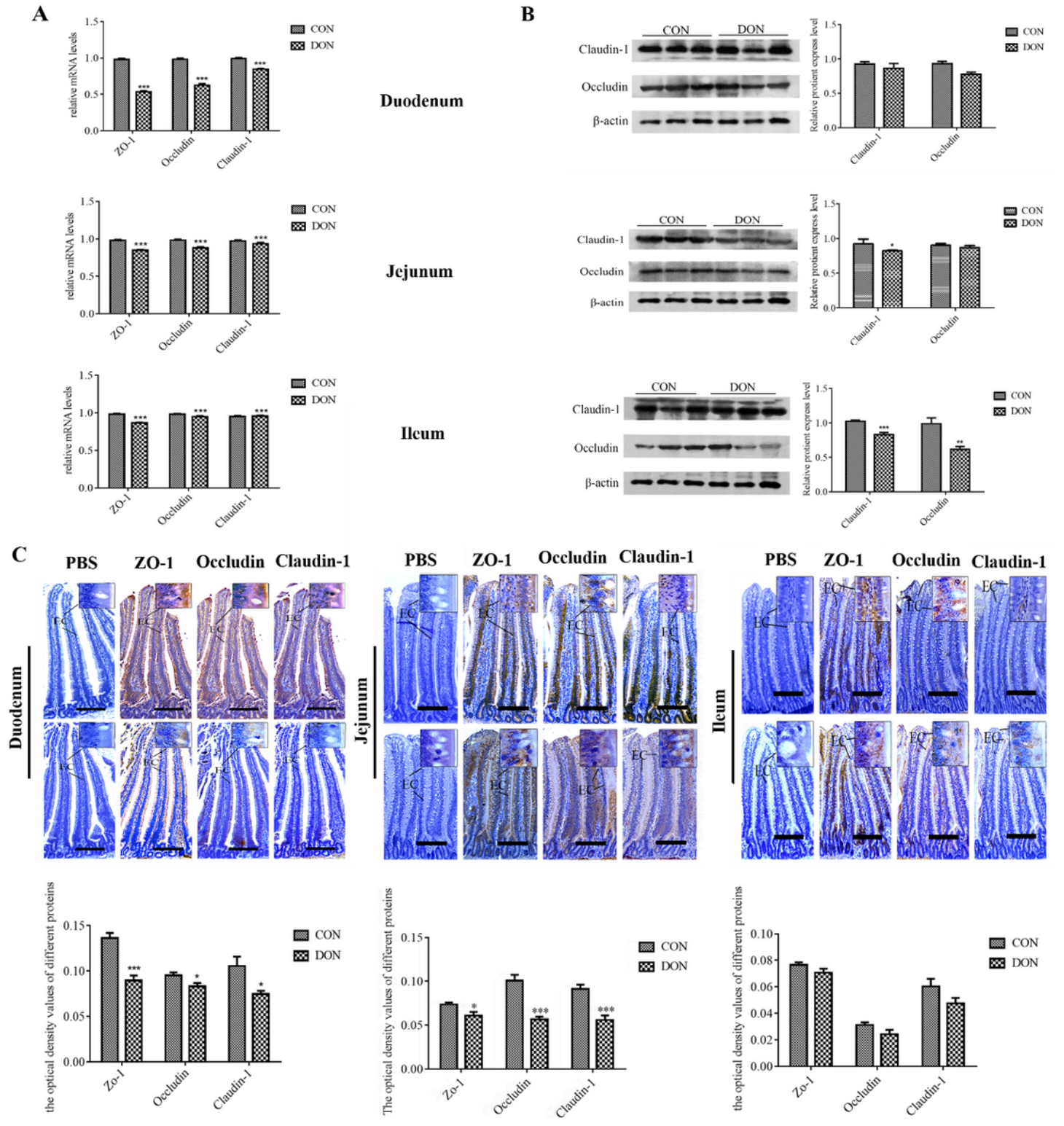

Figure 2

The expression of tight junction proteins in the small intestine of broilers. The mRNA levels (A) the representive western blotting images and the corresponding gray value analysis (B) and the immunohistochemical result and the corresponding optical density value analysis (C) of ZO-1, Occludin

and claudin-1 in each segment of the small intestine were shown. n=6 per treatment group. *p<0.05, **p<0.01, ***p<0.001.

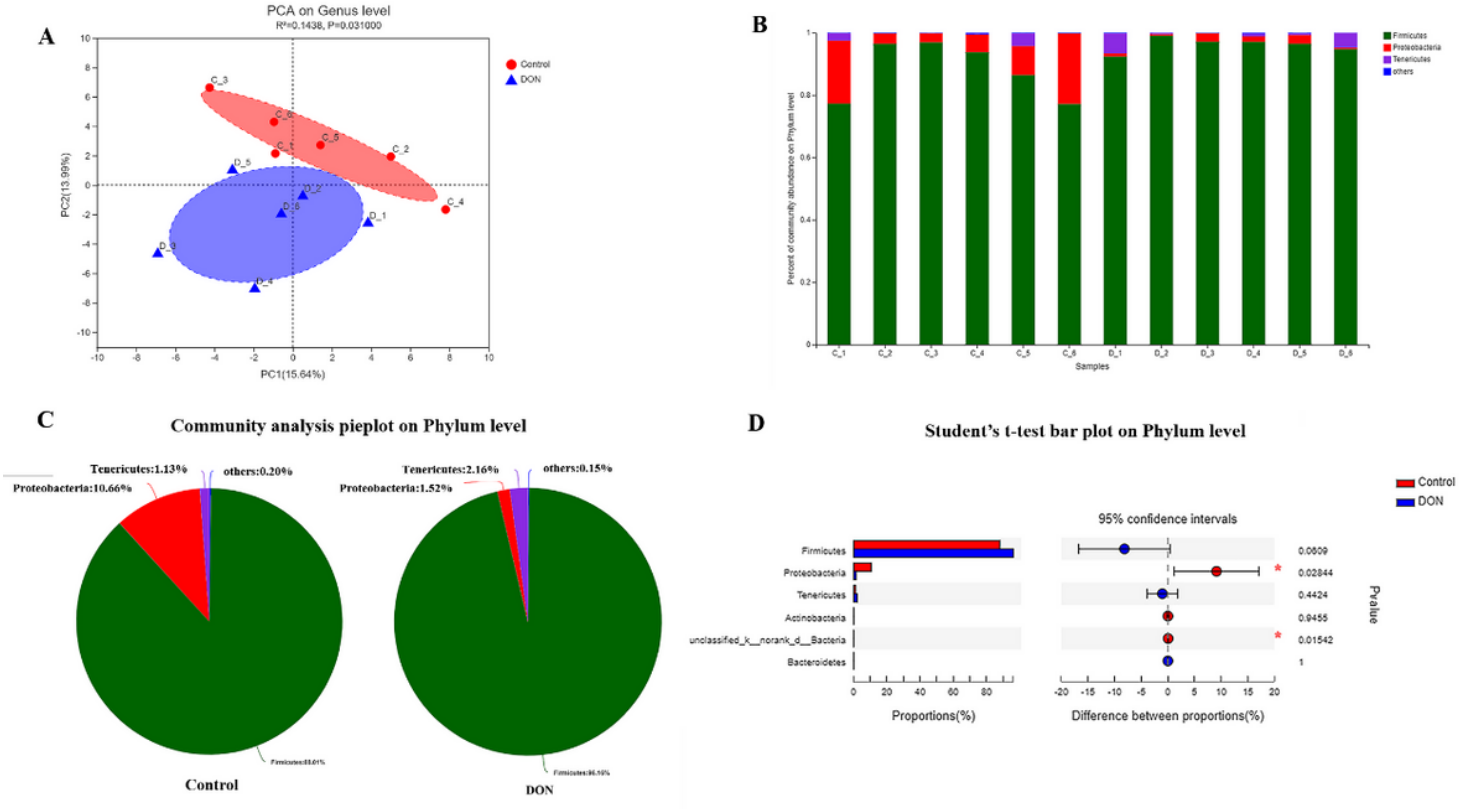

Figure 3

Microecological changes of caecal flora in broilers. The PCA principal component analysis diagram between microbial groups R2=0.144, P=0.031 (A), the microbial community composition structure diagrams (B, C), and the significant difference analysis diagram between microbial groups (D) were presented. n=6 per treatment group.