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Effects of Chinese gallnut tannic acid on growth performance, blood parameters, antioxidative status, intestinal histomorphology, and cecal microbial shedding in broilers challenged with aflatoxin B1

Zheng Fan Zhang,[1] Yu Xi,[1] Si Tian Wang,[1] Li Yun Zheng, Ya Qi, Shuang Shuang Guo, and Bin Ying Ding[2]

Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, Wuhan 430023, China

[1]These authors contributed equally to this research work.
[2]Corresponding author: dbying7471@126.com

Abstract

The objective of the present study was to investigate the effects of tannic acid (**TA**) on growth performance, blood parameters, antioxidant capacity, and intestinal health in broilers challenged with aflatoxin B1 (**AFB1**). A total of 480 broilers aged 1 d were randomly allotted into four treatments: 1) CON, control diet; 2) AF, CON + 60 μg/kg AFB1 of feed during days 1 to 21, CON + 120 μg/kg AFB1 of feed during days 22 to 42; 3) TA1, AF + 250 mg/kg TA; and 4) TA2, AF + 500 mg/kg TA. Average daily gain (**ADG**) and average daily feed intake (**ADFI**) were increased in the TA1 during days 1 to 21, days 22 to 42, and days 1 to 42 compared with CON and AF treatments ($P < 0.05$). Broilers fed the TA2 diet had greater ADG and ADFI than those fed the CON and AF diets during the finisher and the whole period ($P < 0.05$). Administration of TA decreased the relative weight of liver and kidney compared with broilers fed the AF diet on day 42 ($P < 0.05$). The blood activity of alanine transferase (**ALT**) and gamma-glutamyl transferase (**GGT**) was increased in the AF treatment compared with the CON ($P < 0.05$). Broilers fed the TA1 decreased the ALT content on day 21, and the level of ALT and GGT was decreased in the TA2 compared with the AF group on day 42 ($P < 0.05$). The activity of total superoxide dismutase (**T-SOD**) and glutathione peroxidase (**GSH-Px**) in plasma, and the hepatic glutathione S-transferase (**GST**) was decreased in the AF group compared with the CON group ($P < 0.05$). The TA decreased plasma malondialdehyde concentration, and increased plasma T-SOD, GSH-Px, total antioxidant capacity, and hepatic GST activity compared with the AF ($P < 0.05$). The crypt depth of the jejunum was decreased in the TA1 treatment on day 21, and the villus height of the ileum was increased in the TA2 group on day 42 compared with the AF treatment ($P < 0.05$). The cecal *Lactobacillus* counts on day 21 were tended to increase in the TA treatments compared with the AF ($P = 0.061$). In conclusion, dietary inclusion of 250 and 500 mg/kg TA could improve the growth, antioxidant capacity, and partially protected the intestinal health of broilers challenged with AFB1.

Lay Summary

Aflatoxin B1 (**AFB1**) is well known for its growth retardation, hepatotoxic, immunosuppressive, and other negative effects both in humans and poultry. Plant extracts such as tannic acid (**TA**) have been demonstrated as effective agents to control AFB1 contamination. The objective of this study was to evaluate the effects of Chinese gallnut TA in preventing aflatoxicosis in broilers. Broilers received one of four treatments: CON, control diet; AF, control diet with AFB1; TA1, AF + 250 mg/kg TA; TA2, AF + 500 mg/kg TA. Although AF did not decrease the growth performance of broilers, 250 and 500 mg/kg TA had greater average daily gain and average daily feed intake than those in the CON and AF. The relative weight of liver and kidney, blood alanine transferase, and gamma-glutamyl transferase activity were increased, and the antioxidant status was depressed in chicks fed the AF diet compared with the CON group. Dietary supplementation with 250 and 500 mg/kg TA ameliorated all the above-mentioned negative effects of AFB1. Moreover, the crypt depth of the jejunum was decreased, and the villus height of the ileum was increased in TA treatments compared with the AF. Conclusively, Chinese gallnut TA could be considered as a potential natural agent for the prevention of AFB1-induced oxidative and intestinal damage of broilers.

Key words: aflatoxin B1, antioxidant status, broiler, growth performance, intestinal health, tannic acid

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; AFB1, aflatoxin B1; ALB, albumin; ALT, alanine transferase; Ca, calcium; CD, crypt depth; CON, control; CREA, creatine; *E. coli*, *Escherichia coli*; FCR, feed conversion ratio; GGT, gamma-glutamyl transferase; GLU, glucose; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MDA, malondialdehyde; P, phosphorus; TA, tannic acid; T-AOC, total antioxidant capacity; TC, total cholesterol; TG, triglyceride; T-SOD, total superoxide dismutase; V/D, villus height and crypt depth ratio; VH, villus height

Introduction

Aflatoxin B1 (**AFB1**) is reported as the most wide spreading and toxic aflatoxin, which is well known for its growth retardation, hepatotoxic, immunosuppressive, and other negative effects both in humans and poultry (Yunus et al., 2011; Chen et al., 2013, 2014; Liu et al., 2018; Rajput et al., 2019). Physical, chemical, and biological methods to avoid AFB1 could cause decreased feed nutritional value, production of

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post toxic degradation products, and limited efficacy (Rasheed et al., 2021). Recently, the use of plant extracts such as polyphenols, alkaloids, and terpenes has been demonstrated as an effective strategy to control AFB1 contamination (El Khoury et al., 2017; Hernandez et al., 2021).

Hydrolysable tannic acid (**TA**) is a polyphenolic compound, which has positive effects on growth performance, intestinal morphology, and antioxidant activities in broilers under normal and stress situations (Schiavone et al., 2008; Xiong et al., 2016; Liu et al., 2017, 2020). Moreover, a previous study reported that tannins could inhibit the growth of bacteria and fungi, including *Aspergillus flavus*, which is the main producer of AFB1 (Hernandez et al., 2021). However, the effect of hydrolysable TA on broilers dietary exposed to AFB1 remains unclear.

Therefore, the objective of the present study was to determine the effects of Chinese gallnut TA on the growth performance, blood parameters, antioxidant capacity, intestinal morphology, and cecal bacterial population in broilers challenged with AFB1.

Materials and Methods

All animal procedures used in this study were approved by the Institutional Animal Care and Use Committee of Wuhan Polytechnic University (Number: 20161121).

AFB1 and TA

The AFB1 (purity ≥98%, HPLC) was produced from *A. flavus* provided by Qingdao Pribolab Biological Engineering Company Limited (Shandong, China), and the AFB1 concentration in the feed was designed to 60 μg/kg of feed during days 1 to 21, and 120 μg/kg of feed during days 22 to 42 in AFB1 treatments. Dietary AFB1 concentrations were confirmed by analysis (Gowda et al., 2009). Briefly, feed samples were extracted with acetonitrile:water (86:14), and an aliquot of the extract was passed through a puriTox TC-M160 cleanup column (Trilogy Analytical Laboratory Inc., Washington, MO, USA) and suitably diluted with water before analysis using HPLC with Kobra cell postcolumn derivatization with fluorescence detection at 365 nm excitation and 440 nm emission.

The hydrolysable TA was extracted from Chinese gallnut by the Wufeng Chicheng Biotechnology Company Limited (Yichang, China), which contained ≥80% tannin, crude fiber <2.00%, ash <2.50%, and moisture <8.00%.

Experimental design, animals, and diets

A total of 480 Arbor Acres broilers aged 1 d with an initial average body weight (**BW**) of 45 ± 1.6 g were randomly allotted into 4 treatments with 10 replicate pens per treatment and 12 broilers per pen for 42 d. Dietary treatments were: 1) CON, basal diet; 2) AF, CON + 60 μg/kg AFB1 of feed during days 1 to 21, CON + 120 μg/kg AFB1 of feed during days 22 to 42; 3) TA1, AF + 250 mg/kg TA; and 4) TA2, AF + 500 mg/kg TA.

The basal diet (phase 1 from days 1 to 21 and phase 2 from days 22 to 42) was formulated to provide nutrients to meet or exceed the nutrient requirements of Arbor Acres broilers (Table 1).

Table 1. Composition of experimental diets (as-fed basal)

Ingredients, %	Days 1 to 21	Days 22 to 42
Corn	51.45	51.49
Soybean meal	40.73	37.40
Soybean oil	3.36	7.18
Dicalcium phosphate	1.92	1.64
Limestone	1.16	1.06
Trace mineral premix[1]	0.20	0.20
Vitamin premix[2]	0.04	0.03
Sodium chloride	0.35	0.31
L-Lysine, 99%	0.28	0.22
DL-methionine, 98%	0.26	0.32
Choline chloride	0.25	0.25
Calculated composition		
ME, MJ/kg	12.55	13.18
Analyzed composition		
Crude protein, %	21.50	20.50
Lys, %	1.30	1.20
Met + Cys, %	0.90	0.70
Thr, %	0.82	0.74
Calcium, %	1.00	0.90
Available phosphorus, %	0.45	0.40

[1]Provided per kg of complete diet: 10 mg Mn ($MnSO_4$), 80 mg Zn ($ZnSO_4$), 5 mg Cu ($CuSO_4$), 0.5 mg I ($Ca(IO_3)_2$), and 0.3 mg Se (Na_2SeO_3).
[2]Provided per kg of complete diet: 10,000 IU vitamin A (transretinyl acetate), 3,000 IU vitamin D_3 (cholecalciferol), 30 IU vitamin E (all-rac-α-tocopherol acetate), 2.4 mg menadione, 6.0 mg riboflavin, 2.5 mg pyridoxine HCl, 13 mg calcium pantothenate, 23.5 mg niacin, and 0.04 mg biotin.

Animal management

All broiler chicks were reared in floor pens (1.4 m × 1.4 m) in an environmentally controlled room at the Animal Research Center of Wuhan Polytechnic University and given ad libitum access to diets and water throughout the study. The room temperature was maintained at 32 ± 2 °C for 1 to 5 d and then gradually decreased to 24 °C until the end of the experiment. Birds were maintained on a 24-h constant light schedule at day 1, and reduced to 23 h of light, 1 h of darkness during days 2 to 42.

Growth performance

Broilers were weighed on days 0, 21, and 42 of the experiment, and feed consumption per pen was recorded to calculate the average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**). The individual pen was considered as the experimental unit.

Sample collection

On days 21 and 42, blood samples were collected from wing vein of 2 broilers per pen (20 broilers per treatment) into K_3EDTA Vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA), one tube of blood was used for hematological evaluation and the other tube was centrifuged (3,000 × *g* for 15 min at 4 °C) for separating plasma, which was stored at –20 °C for the assay of antioxidative status.

After blood collection, the same broilers were weighed individually and slaughtered by cervical dislocation. The liver, spleen, bursa of Fabricius, thymus, and kidney were then removed by trained personnel and weighed. Organ weight was expressed as gram per kilogram of BW. The liver tissue (2 to 3 g) was collected, rinsed with ice-cold PBS, and quickly preserved in a centrifuge tube under ice-cold conditions for analysis of antioxidant status.

The small intestine was removed and gently flushed with ice-cold saline. Intestinal segments (~1 cm) taken from the mid-region of the duodenum (from gizzard outlet to the entry of the bile and pancreatic ducts), jejunum (from the end of the duodenum to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction) were immediately fixed in 4% paraformaldehyde for the examination of morphological parameters. Additionally, to determine the microbial counts, the digesta in the cecum was collected and frozen immediately at −80 °C until further processing. All samples were collected within 15 min after broilers were euthanized.

Blood parameters and antioxidative status

Plasma biochemical indicators were measured with corresponding kits using a Hitachi 7060 Automatic Biochemical Analyzer (Hitachi, Tokyo, Japan), and the activities of glutathione peroxidase (**GSH-Px**), total superoxide dismutase (**T-SOD**), glutathione S-transferase (**GST**), total antioxidant capacity (**T-AOC**) and the contents of malondialdehyde (**MDA**) in the plasma were determined by commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions (Oskoueian et al., 2014). Liver tissue was diluted with ice-cold PBS (pH 7·4) without heparin at a ratio of 1:9, homogenized in a homogenizer (Tekmar, SDT 1810, Cincinnati, OH, USA), and centrifuged (10,000 × *g*, 4 °C, 15 min). The clear supernatant was aspirated into vials and the antioxidant status was determined by the kits aforementioned. Total protein of the liver was determined using assay kits (Sigma Diagnostics, Sigma Chemical Co., St Louis, MO, USA). Each assay was performed in triplicate.

Intestinal histomorphology

Intestinal histomorphology was examined according to the method of Guo et al. (2018). Briefly, the fixed intestinal segments were embedded in paraffin. Consecutive sections (5 μm) were stained with hematoxylin and eosin and were observed for histomorphogical examination. The measurements were performed with an Olympus optical microscope using ProgRes CapturePro software (Jenoptik, Jena, Germany). The villus height and crypt depth were measured from 10 randomly selected villi and associated crypts on each section at 40 × magnification. Villus height was measured from the tip of the villus to the crypt opening and crypt depth was measured from the base of the crypt to the level of the crypt opening. The villus height to crypt depth (V:C) ratio was then calculated from these measurements.

Numeration of bacteria

The number of *Escherichia coli* and *Lactobacillus* in the cecal was performed via bacteria culture, as described by Zhang and Kim (2014). Briefly, approximately 1 g of each cecal digesta was diluted with 9 mL of 0.9% sterile saline solution. The suspension of each sample was serially diluted between 10^{-2} and 10^{-8} dilutions. Afterward, 100 μL of each dilution was plated onto MRS agar plates (HB0392, Qingdao Hopebio Co., Ltd., Shandong, China) and MacConkey agar plates (HB7001, Qingdao Hopebio Co., Ltd.) to enumerate the *Lactobacillus* and *E. coli*, respectively. The MRS agar plates were incubated anaerobically for 24 to 48 h at 37 °C. The MacConkey agar plates were incubated aerobically for 24 h at 37 °C. After the incubation period, the bacterial colonies were counted immediately after removing from the incubator in a colony counter, and the results were expressed as log colony-forming units per gram of sample.

Statistical analyses

All data were analyzed by one-way ANOVA using the SPSS 13.0 software. Data were expressed as means and SEMs. Differences between treatment means were determined by Duncan's multiple range test. A value of $P < 0.05$ was considered significant and $0.05 \leq P < 0.10$ as the trends.

Results

Dietary analyses

The CON diet used to prepare diets was negative for AFB1. Diets AF, TA1, and TA2 were analyzed and contained 63, 59, and 58 μg AFB1 per kg during days 1 to 21, and 125, 122, and 117 μg AFB1 per kg during days 22 to 42, respectively.

Growth performance

The effects of TA supplementation on the growth performance of broilers challenged by AFB1 are shown in Table 2. Results showed that no significant differences were observed in the growth performance of broilers challenged with AFB1 compared with the CON. Broilers fed the TA1 diet had greater ($P < 0.05$) ADG and ADFI than those fed the CON and AF diets throughout the experiment period. Relative to broilers receiving the CON diet, birds receiving the TA2 diet showed greater ($P < 0.05$) ADG and ADFI during days 1 to 21. The ADG and ADFI were also increased ($P < 0.05$) in the TA2 group compared with the CON and AF treatments during days 22 to 42, and overall period. However, no difference was observed in FCR of the four groups during the experiment.

Table 2. The effects of tannic acid on growth performance in broilers[1]

Items[2]	CON	AF	TA1	TA2	SEM	*P*-value
Days 1 to 21						
ADFI, g	47.01^{c}	47.96^{bc}	51.08^{a}	49.60^{ab}	1.603	0.002
ADG, g	36.76^{c}	37.52^{bc}	39.27^{a}	38.49^{ab}	1.245	0.017
FCR	1.28	1.28	1.30	1.29	0.018	0.107
Days 22 to 42						
ADFI, g	141.69^{b}	139.67^{b}	152.95^{a}	149.21^{a}	3.220	0.004
ADG, g	68.71^{b}	65.96^{b}	74.56^{a}	73.38^{a}	3.430	0.001
FCR	2.07	2.12	2.05	2.04	0.070	0.228
Days 1 to 42						
ADFI, g	94.35^{b}	93.62^{b}	102.01^{a}	99.41^{a}	3.495	0.001
ADG, g	58.10^{b}	56.94^{b}	62.63^{a}	61.13^{a}	2.053	<0.001
FCR	1.63	1.65	1.63	1.63	0.038	0.756

[1]Each mean represents 10 replications with 12 broilers per replication. CON, basal diet; AF, 60 μg/kg aflatoxin B1 of feed during days 1 to 21, 120 μg/kg aflatoxin B1 of feed during days 22 to 42; TA1, AF + 250 mg/kg tannic acid; TA2, AF + 500 mg/kg tannic acid.
[2]ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.
[a,b,c]Means in the same row with no common superscripts differ significantly ($P < 0.05$).

Relative organ weight

In Table 3, the relative weights of the liver, spleen, bursa of Fabricius, and kidney were unaffected by AFB1 and TA treatments on day 21. The relative weight of the liver and kidney was increased in the AF compared with the CON ($P < 0.05$) on day 42. The relative weight of the thymus on day 21 was tended to decrease ($P = 0.066$), and the relative weight of the spleen on day 42 was tended to increase ($P = 0.056$) in AF compared with CON, respectively. Dietary supplementation with TA could ameliorate all the above-mentioned effects compared with the AF.

Blood parameters

In Table 4, most blood characteristics (albumin, total cholesterol, triglyceride, glucose, calcium, phosphorus, creatine, high-density lipoprotein, and low-density lipoprotein) were not significantly affected by AFB1 and dietary supplementation of TA. However, the activity of alanine transferase (**ALT**) and gamma-glutamyl transferase (**GGT**) was increased in broilers fed the AF diet compared with the CON group on days 21 and 42, and on day 42, respectively. The TA1 decreased ($P < 0.05$) the ALT level on day 21, and the level of ALT and GGT in TA2 was decreased ($P < 0.05$) in TA2 compared with the AF.

Antioxidant capacity

The effects of TA on antioxidant capacity in broilers challenged with AFBI are presented in Table 5. Supplementation of the AFB1 diet with 250 and 500 mg/kg TA significantly improved antioxidant status in the plasma and liver in terms of T-SOD, GSH-Px, and GST on days 21 and 42 ($P < 0.05$). Furthermore, the level of MDA in the plasma was notably lower ($P < 0.05$) in the TA1 and TA2 groups compared with the AF group. No significant effect was observed on plasma GST level and liver MDA content among treatments.

Intestinal histomorphology and cecal bacterial population

The crypt depth was increased ($P < 0.05$) on day 21, and the ileum villus was decreased ($P < 0.05$) on day 42 in broilers fed the AF diet compared with the CON group (Table 6). At day 21, compared with the AF, dietary supplementation with 250 mg/kg of TA significantly decreased ($P < 0.05$) the crypt depth in the jejunum. The ileal villus height in the TA2 group was greater ($P < 0.05$) than that of the AF group at day 42, but was not significantly different from that in the CON.

The result showed that AFB1 and TA had no effect on the cecal population of *E. coli* and at 21 and 42 d of age in broilers (Table 7). The abundance of *Lactobacillus* in cecum on day 21 was tended to increase in the TA compared with the AF ($P = 0.061$).

Discussion

The current study investigated the protective effect of TA extracted from Chinese gallnut to control aflatoxicosis in broiler chickens. However, the growth performance was not affected by AFB1 challenge. In consistent with our result, Celik et al. (2005) and Kermanshahi et al. (2009) did not find any effect on BWG and FCR in broilers fed 0.5 to 1.0 mg/kg AF aged from 1 to 42 d. In addition, Slizewska et al. (2019) reported that 1 mg/kg AFB_1 of diet had a minor effect on the growth performance of broiler, but 5 mg/kg AFB1 reduced BWG by 20% to 50% compared with the control group. In contrast,

Table 3. The effects of tannic acid on relative organ weight in broilers[1]

Items, g/kg	CON	AF	TA1	TA2	SEM	*P*-value
Day 21						
Liver	21.06	19.44	20.28	20.24	2.273	0.425
Spleen	0.83	0.75	0.86	0.88	0.225	0.559
Bursa of Fabricius	2.46	2.52	2.18	2.07	0.513	0.121
Thymus	5.31	4.38	4.20	4.83	1.060	0.066
Kidney	7.63	7.73	7.73	7.95	0.708	0.731
Day 42						
Liver	17.06^{b}	21.59^{a}	18.21^{b}	16.97^{b}	3.118	0.034
Spleen	0.99	1.08	0.90	0.83	0.223	0.056
Bursa of Fabricius	1.95	1.84	1.96	1.73	0.450	0.621
Thymus	3.90	3.85	4.24	4.03	1.250	0.877
Kidney	5.43^{b}	6.95^{a}	5.63^{b}	5.54^{b}	0.883	0.011

[1]Each mean represents 10 replications with 2 broilers per replication. CON, basal diet; AF, 60 μg/kg aflatoxin B1 of feed during days 1 to 21, 120 μg/kg aflatoxin B1 of feed during days 22 to 42; TA1, AF + 250 mg/kg tannic acid; TA2, AF + 500 mg/kg tannic acid.
[a,b]Means in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 4. The effects of tannic acid on blood parameters in broilers[1]

Items[2]	CON	AF	TA1	TA2	SEM	*P*-value
Day 21						
ALB, g/dL	13.46	13.07	12.29	13.33	1.553	0.409
ALT, U/L	1.75^{b}	2.33^{a}	1.75^{b}	1.92^{ab}	0.520	0.049
TC, mg/dL	120.45	121.73	124.53	125.44	12.203	0.727
TG, mg/dL	31.92	29.00	32.34	33.50	10.000	0.770
GLU, mg/dL	215.52	217.03	218.3	213.84	9.275	0.684
Ca, mg/dL	11.00	10.65	11.43	10.94	1.115	0.034
P, mg/dL	6.59	6.63	7.51	6.90	1.720	0.830
CREA, mg/dL	1.75	1.17	1.67	2.08	1.490	0.546
HDL, mg/dL	104.18	108.61	102.45	108.34	14.990	0.727
LDL, mg/dL	24.19	24.57	25.12	28.52	7.275	0.456
GGT, U/L	15.75	16.46	16.75	16.67	2.988	0.329
Day 42						
ALB, g/dL	14.45	14.43	14.43	14.19	1.018	0.923
ALT, U/L	1.77^{b}	2.75^{a}	2.25^{ab}	1.83^{b}	0.288	0.024
TC, mg/dL	127.46	116.57	124.29	121.18	12.643	0.338
TG, mg/dL	35.47	31.66	33.00	35.10	10.890	0.814
GLU, mg/dL	217.76	215.54	211.57	213.59	11.188	0.589
Ca, mg/dL	10.57	10.83	10.75	11.03	0.600	0.328
P, mg/dL	6.93	6.71	6.88	7.23	0.630	0.301
CREA, mg/dL	3.33	2.83	2.75	3.67	1.618	0.514
HDL, mg/dL	108.55	101.97	105.82	102.49	13.258	0.671
LDL, mg/dL	30.98	27.01	29.16	28.43	7.173	0.627
GGT, U/L	15.42^{b}	18.50^{a}	16.92^{ab}	15.25^{b}	1.485	0.038

[1]Each mean represents 10 replications with 2 broilers per replication. CON, basal diet; AF, 60 μg/kg aflatoxin B1 of feed during days 1 to 21, 120 μg/kg aflatoxin B1 of feed during days 22 to 42; TA1, AF + 250 mg/kg tannic acid; TA2, AF + 500 mg/kg tannic acid.
[2]ALB, albumin; ALT, alanine transferase; Ca, calcium; CREA, creatine; GGT, gamma-glutamyl transferase; GLU, glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; P, phosphorus; TC, total cholesterol; TG, triglyceride.
[a,b]Means in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 5. The effects of tannic acid on antioxidant capacity in broilers[1]

Items[2]		CON	AF	TA1	TA2	SEM	*P*-values
Day 21	Plasma						
	T-SOD, U/mL	111.6[a]	109.1[b]	112.3[a]	113.2[a]	2.46	0.002
	GSH-Px, U/mL	1,748.6[b]	1,690.7[c]	1,742.5[b]	1,812.3[a]	47.30	<0.001
	GST, U/mL	27.8	27.6	28.2	25.5	3.19	0.204
	T-AOC, mM	0.41	0.36	0.42	0.44	0.095	0.307
	MDA, nmol/mL	7.75[a]	7.87[a]	5.87[b]	4.31[b]	1.373	<0.001
	Liver						
	T-SOD, U/mg	121.9	119.9	116.9	117.9	10.76	0.737
	GSH-Px, U/mg	49.5	44.8	51.9	52.6	13.07	0.473
	GST, U/mg	24.8[b]	16.7[a]	24.3[b]	23.4[b]	3.23	<0.001
	T-AOC, U/mg	0.07	0.07	0.07	0.06	0.010	0.245
	MDA, nmol/mg	1.66	1.68	1.54	1.54	0.338	0.634
Day 42	Plasma						
	T-SOD, U/mL	113.7	115.6	114.3	114.7	3.06	0.520
	GSH-Px, U/mL	1,775.5[b]	1,715.5[c]	1,834.8[a]	1,832.7[a]	49.57	<0.001
	GST, U/mL	31.6	30.1	32.6	27.7	4.42	0.085
	T-AOC, mM	0.39[b]	0.39[b]	0.48[a]	0.50[a]	0.085	0.005
	MDA, nmol/mL	6.51[ab]	7.04[a]	5.53[b]	5.63[b]	1.130	0.022
	Liver						
	T-SOD, U/mg	142.0	137.6	135.1	135.3	10.64	0.444
	GSH-Px, U/mg	60.2[b]	50.5[b]	88.0[a]	59.0[b]	19.07	0.001
	GST, U/mg	30.0[ab]	26.7[c]	28.0[bc]	32.2[a]	3.33	0.003
	T-AOC, U/mg	0.08	0.06	0.07	0.07	0.010	0.331
	MDA, nmol/mg	1.10	0.94	0.93	0.95	0.175	0.083

[1]Each mean represents 10 replications with 2 broilers per replication. CON, basal diet; AF, 60 μg/kg aflatoxin B1 of feed during days 1 to 21, 120 μg/kg aflatoxin B1 of feed during days 22 to 42; TA1, AF + 250 mg/kg tannic acid; TA2, AF + 500 mg/kg tannic acid.
[2]T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; T-AOC, total antioxidant capacity; MDA, malondialdehyde.
[a,b,c]Means in the same row with no common superscripts differ significantly ($P < 0.05$).

serious studies reported that dietary exposure to AFB1 has detrimental effects on the growth performance of broilers (Liu et al., 2018; Sarker et al., 2021a; Tavangar et al., 2021; Wan et al., 2021). We hypothesized that the toxic effects of AFB1 may be acute or chronic, affected by species, age, sex, dose, duration of exposure, health, and nutritional status of broilers.

Recent studies indicated that supplemented with 500 mg/kg to 5 g/kg TA in broiler diets could mitigate intestinal and footpad injury due to the antimicrobial, antioxidant, anti-inflammatory, and gut health-promoting effects (Tosi et al., 2013; Cengiz et al., 2017; Tonda et al., 2018). In the present study, both 250 and 500 mg/kg TA increased ADG and ADFI compared with the CON and AFB1 challenged groups. Based on the micrometric results of the intestine, it is possible to suggest that the growth-promoting effect in TA groups is related to the lower crypt depth and higher villus height when compared with the AF. The greater contact surface for nutrients may elevate the digestibility, which is reflected by greater ADG in the present study (Li et al., 2015).

Aflatoxicosis cause gross and pathological lesions in different organs, especially the liver, kidney, and bursa of Fabricius (Ortatatli and Oguz, 2001; Karaman et al., 2005; Cruz et al., 2019). Similarly, we observed AF exposure at a level of 60 and 120 μg/kg via feed increased relative weight of liver and kidney, as well as blood ALT and GGT, which is consistent with earlier studies (Denli et al., 2009; Zhao et al., 2010; Kumar et al., 2015). According to Subhani et al. (2018), the amelioration of clinical signs induced by AFB1 with plants extracts was similar to the present study in TA groups. Saleemi et al. (2020) demonstrated that a mycotoxin binder with 2% silymarin could ameliorate the gross and microscopic changes in the liver of boiler dietary challenged with AFB1. Several other studies have reported that plant extract can ameliorate the adverse effect of AFB1 in broilers (Gowda et al., 2009; Rashidi et al., 2020).

It has been demonstrated that AFB1 may induce liver and kidney damage through the overproduction of reactive oxygen species, leading to oxidative stress (Umaya et al., 2021). In the present study, the blood SOD and GSH-Px activity, as well as the hepatic GST were decreased by dietary AFB1 exposure. Tannins can remove oxygen free radicals in animals thus improving the antioxidant enzyme activity to reduce oxidative stress (Chamorro et al., 2013). In our study, we found that TA could alleviate the adverse effects of AFB1 on the antioxidant ability, which is similar to previous studies. For example, studies reported that plant tannins could increase the T-AOC, SOD, and GSH-Px activity in serum, muscle, liver, and intestine in broilers (Dong et al., 2015; Starčević et al., 2015; Liu et al., 2018, 2020). Moreover, the lower plasma MDA level in our trial was similar to the previous studies (Voljč et al., 2013; Faraha et al., 2016; Tong et al., 2022).

Table 6. The effects of tannic acid on intestinal morphology in broilers[1]

Items[2]		CON	AF	TA1	TA2	SEM	*P*-value
Day 21							
Duodenum	VH, μm	1,316.6	1,318.2	1,358.9	1,391.1	166.50	0.691
	CD, μm	159.5	137.4	139.2	156.4	31.72	0.303
	V/D, μm/μm	8.59	9.92	9.85	9.28	1.800	0.344
Jejunum	VH, μm	1,126.90	1,108.28	1,057.61	1,096.10	184.63	0.882
	CD, μm	114.5^{b}	149.4^{a}	115.2^{b}	125.6^{ab}	29.77	0.048
	V/D, μm/μm	10.07	7.50	9.48	8.03	2.113	0.051
Ileum	VH, μm	755.2	697.7	770.3	773.7	110.19	0.328
	CD, μm	111.8	120.2	120.3	124.0	23.84	0.822
	V/D, μm/μm	6.80	6.20	6.47	6.23	1.048	0.644
Day 42							
Duodenum	VH, μm	1,481.7	1,428.8	1,542.1	1,474.9	212.82	0.725
	CD, μm	171.2	180.6	149.0	163.7	31.24	0.243
	V/D, μm/μm	9.31	8.14	10.27	9.50	2.245	0.264
Jejunum	VH, μm	1,062.9	951.8	1,111.3	1,063.4	203.64	0.296
	CD, μm	141.3	121.4	124.7	133.1	23.94	0.224
	V/D, μm/μm	7.96	7.95	9.47	8.63	2.165	0.312
Ileum	VH, μm	673.7^{a}	503.3^{b}	606.6^{ab}	667.8^{a}	125.23	0.031
	CD, μm	91.2	83.4	87.1	94.2	16.59	0.448
	V/D, μm/μm	6.54	6.23	6.87	6.99	1.820	0.742

[1]Each mean represents 10 replications with 2 broilers per replication. CON, basal diet; AF, 60 μg/kg aflatoxin B1 of feed during days 1 to 21, 120 μg/kg aflatoxin B1 of feed during days 22 to 42; TA1, AF + 250 mg/kg tannic acid; TA2, AF + 500 mg/kg tannic acid.
[2]CD, crypt depth; V/D, villus height and crypt depth ratio; VH, villus height.
[a,b]Means in the same row with no common superscripts differ significantly ($P < 0.05$).

Table 7. The effects of tannic acid on cecal bacterial population in broilers[1]

Items, $\log_{10}$ cfu/g	CON	AF	TA1	TA2	SEM	*P*-value
Day 21						
Escherichia coli	7.08	7.76	6.48	6.82	0.953	0.566
Lactobacillus	8.08	7.24	8.01	8.11	1.135	0.061
Day 42						
Escherichia coli	7.27	7.66	6.73	6.77	1.178	0.507
Lactobacillus	8.02	7.52	7.84	7.62	1.663	0.888

[1]Each mean represents 10 replications with 2 broilers per replication. CON, basal diet; AF, 60 μg/kg aflatoxin B1 of feed during days 1 to 21, 120 μg/kg aflatoxin B1 of feed during days 22 to 42; TA1, AF + 250 mg/kg tannic acid; TA2, AF + 500 mg/kg tannic acid.

Collectively, the positive effect on antioxidant status indicated that TA could be used as a potential antioxidant additive in broiler diet containing AF.

In this study, we hypothesized that TA may have had a role in intestinal integrity in broilers challenged with AF. Zhang et al. (2014) reported that 0.3 mg/kg AFB1 decreased jejunum villus height and villus height to crypt depth ratio of broilers. Similarly, Sarker et al. (2021b) found that the villus height and villus height to crypt depth in the small intestine (duodenum, jejunum, and ileum) were decreased in 100 μg/kg AFB1-treated broilers. These results were in agreement with the present study, which could indicate that dietary AFB1 exposure decreased the broiler intestine function by depressing the intestinal histomorphological development. Liu et al. (2017) found that 1 and 2 g/kg chestnut tannins improved the villus height of the jejunum of broilers under heat stress, which indicates tannins can promote the proliferation of intestinal epithelial cells to promote intestinal development. Tong et al. (2022) also found that the addition of a low concentration of tannins (100 mg/kg) could promote the intestinal health of broilers. Data from the current study showed that villus height of ileum and crypt depth of jejunum were improved by dietary supplementation of different levels of Chinese gallnut tannins, thus alleviating AFB1-induced histological structure disruption of the small intestine.

Gastrointestinal microbial balance plays an important role in host defense mechanisms of broiler chickens, and lactic acid bacteria can synthesize bacteriocins to protect the host from pathogenic bacteria (Porto et al., 2017; Yang et al., 2022). According to the results of growth performance and intestinal morphology, the differences in cecal microbial shedding were further studied. It was found that the addition of TA in the diet tended to increase the count of *Lactobacillus*, which is one of the dominant bacteria in broilers. Redondo et al. (2022) reported no differences in gastrointestinal microbiota between birds under normal and tannin-based programs. Moreover, researchers demonstrated that *Lactobacillus*-based probiotics for detoxification of AFB1 (Chlebicz and Slizewska, 2020; Ragoubi et al., 2021; Zhu et al., 2021; Chen et al., 2022). Based on the results of growth performance, antioxidant capacity, and intestinal histomorphology, it is speculated that TA could promote the growth performance of broilers by improving antioxidant capacity, maintaining the integrity of the small intestine, and tending to increase the abundance of *Lactobacillus*.

Conclusions

The addition of 250 and 500 mg/kg TA to AFB1-contaminated diet had a positive effect on growth, antioxidant capacity, and partially protect the intestinal health of broilers. As a consequence, the TA may be used in the prevention of mycotoxicosis in chickens, contributing to the improvement of animal health.

Conflict of interest statement

There is no conflict of interest relevant to this publication.

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