Effects of tannic acid on growth performance, relative organ weight, antioxidative status, and intestinal histomorphology in broilers exposed to aflatoxin B₁

Yu Xi†, Jing Chen†, Shuanghuang Guo, Sitian Wang, Zhipeng Liu, Liyun Zheng, Ya Qi, Pengtao Xu, Lanlan Li, Zhengfan Zhang* and Binying Ding*

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

A total of 480 one-day-old AA broiler chicks were randomly allocated to one of four treatments in a 2 × 2 factorial to investigate the effects of tannic acid (TA) on growth performance, relative organ weight, antioxidative capacity, and intestinal health in broilers dietary exposed to aflatoxin B₁ (AFB₁). Treatments were as follows: (1) CON, control diet; (2) TA, CON + 250 mg/kg TA; (3) AFB₁, CON + 500 μg/kg AFB₁; and (4) TA+AFB₁, CON + 250 mg/kg TA + 500 μg/kg AFB₁. There were 10 replicate pens with 12 broilers per replicate. Dietary AFB₁ challenge increased the feed conversion ratio during days 1 to 21 (P < 0.05). The TA in the diet did not show significant effects on the growth performance of broilers during the whole experiment period (P > 0.05). The liver and kidney relative weight was increased in the AF challenge groups compared with the CON (P < 0.05). The addition of TA could alleviate the relative weight increase of liver and kidney caused by AFB₁ (P < 0.05). Broilers fed the AFB₁ diets had lower activity of glutathione peroxidase, catalase, total superoxide dismutase, S-transferase, and total antioxidant capacity in plasma, liver and jejunum, and greater malondialdehyde content (P < 0.05). Dietary supplemented with 250 mg/kg TA increased the activities of antioxidative enzymes, and decreased malondialdehyde content (P < 0.05). In addition, AFB₁ significantly reduced the villus height and crypt depth ratio in the ileum on day 42 (P < 0.05). In conclusion, supplementation with 250 mg/kg TA could partially protect the antioxidative capacity and prevent the enlargement of liver in broilers dietary challenged with 500 μg/kg AFB₁.

KEYWORDS
Aflatoxin B₁, antioxidant capacity, broiler, growth performance, intestinal health, tannic acid
Introduction

Aflatoxins are mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and widely exist in food and feed that are frequently caused health and economic problems in many countries (1). Among the 18 types of aflatoxin derivatives, aflatoxin B1 (AFB1) is the most common and toxic in the poultry feed industry (2). Poultry is extremely sensitive to AFB1, and long-term exposure to AFB1 may cause growth retardation, immunosuppression, hepatotoxic, and even death (3–5). Oxidative stress has been reported to play a significant role in the toxicity mechanism caused by AFB1 (6, 7). FDA (8) refines the maximum concentration of aflatoxin in poultry is 100 µg/kg of feed, whereas 500 µg/kg can be a practical testing concentration in feedstuff in the USA.

Chinese gallnut tannic acid (TA) belongs to the hydrolyzed tannin family, and is a polyphenolic compound of high molecular weight (500–3,000 Da), which can remove free radicals and prevent lipid oxidation (9). Because of the polyphenolic hydroxyl structure, the TA has various biological activities, such as antimicrobial, anti-inflammatory, anticancer, and immunomodulatory effects (10–12). Moreover, studies have shown that dietary supplementation with antioxidants, including plant extracts and tannins can protect broilers from AFB1-induced toxicity by enhancing the antioxidant capacity and immunity (6, 13–16). Nevertheless, it remains unclear whether dietary supplementation with TA could alleviate acute aflatoxicosis by improving the antioxidant capacity of broilers fed AFB1 contaminated diets.

Therefore, the aim of this study was to determine the effects of the TA on growth performance, antioxidative status, and intestinal histomorphology of broilers exposed to feed contaminated with 500 µg/kg AFB1.

Materials and methods

All animal procedures used in this study were performed in the experimental farm of Wuhan Polytechnic University, and 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 *Aspergillus flavus* provided by Qingdao Pribolab Biological Engineering Company Limited (Shandong, China), and the AFB1 concentration in the feed was designed to 500 µg/kg in AFB1 treatments. Dietary AFB1 concentrations were confirmed by analysis (17). 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%.

Dietary treatments and animal management

A 2 × 2 factorial complete randomized block design was employed and 480 one-day-old sex-mixed AA broilers were randomly assigned to 4 treatment groups, each with 10 replicates of 12 birds per pen. Experimental diets were as follows: (1) CON, basal diet; (2) TA, CON + 250 mg/kg TA; (3) AFB1, CON + 500 µg/kg TA; and (4) TA+AFB1, CON + 250 mg/kg TA + 500 µg/kg AFB1. The basal diet was formulated to meet or exceed the nutrient requirements of AA broilers. Diets were fed in 2 phases: phase 1 (from days 1 to 21) and phase 2 (from days 22 to 42). The composition and nutrient levels of the basal diets are presented in Table 1.

All broiler chicks were reared in stainless steel 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 33 ± 2°C for the first week and then gradually decreased to 24°C until the end of the experiment, and broilers were maintained on a 23 h constant light and 1 h darkness every day throughout the whole trial.

Growth performance

Broilers and feed were weighed on the beginning, days 21 and 42 of the trial, and calculated the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

Sample collection

On days 21 and 42, two broilers from each replicate (20 broilers per group) were randomly selected and blood samples were aseptically collected from the wing vein into vacuum blood vessels. Plasma was obtained by centrifuging (3,000 × g for 15 min at 4°C) the whole blood and stored at −20°C for the assay of antioxidative parameters.

Then, the same broilers were weighed individually and euthanized by cervical dislocation. The liver, spleen, bursa
TABLE 1 Composition of experimental diets (as-fed basal).

| Ingredients (%) | Days 1–21 | Days 22–42 |
|-----------------|-----------|------------|
| 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<sup>a</sup> | 0.20 | 0.20 |
| Vitamin premix<sup>b</sup> | 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       |
| ME (MJ/kg)      | 12.55     | 13.18      |

Calculated 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

<sup>a</sup> Provided per kg of complete diet: 10 mg Mn (MnSO<sub>4</sub>), 80 mg Zn (ZnSO<sub>4</sub>), 5 mg Cu (CuSO<sub>4</sub>), 0.5 mg I (Ca(IO<sub>3</sub>)<sub>2</sub>), and 0.3 mg Se (Na<sub>2</sub>SeO<sub>3</sub>).

<sup>b</sup> Provided per kg of complete diet: 10,000 IU vitamin A (transretinyl acetate), 3,000 IU vitamin D<sub>3</sub> (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.

Intestinal histomorphology

The intestinal histomorphology was measured as described by Guo et al. (19). Briefly, the fixed intestinal segments were embedded in paraffin. Consecutive sections (5 μm) were stained with hematoxylin and eosin and were observed for histomorphological 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 ratio (V/C) was then calculated from these measurements.

Statistical analyses

All experiment data were analyzed by a two-way ANOVA analysis using the GLM procedure of SPSS 26.0 software. In cases where the differences were significant, the means were compared by Duncan’s multiple range test. The results are shown as mean and the standard error of mean (SEM). Significance was considered at P < 0.05, and 0.05 ≤ P < 0.10 was considered to have a trend of difference.

Results

Dietary analyses of AFB<sub>1</sub>

Biochemical tests indicated that the CON and TA diets were negative for AFB<sub>1</sub> throughout the experiment. The analyzed concentration of AFB<sub>1</sub> in AFB<sub>1</sub> and AFB<sub>1</sub>+TA diets were 505.9 vs. 503.2 μg/kg during days 1 to 21, and 520.3 vs. 521.3 μg/kg during days 22–42, respectively.

Growth performance

As shown in Table 2, AFB<sub>1</sub> challenge increased the FCR during days 1–21 (P < 0.05). The addition of TA in the diet did not show significant effects on the ADG, ADFI, and FCR of broilers during the whole experiment period (P > 0.05). No interaction effect was observed between AFB<sub>1</sub> and TA on the growth performance (P > 0.05).
TABLE 2 Effects of tannic acid on growth performance of broilers challenged with AFB\textsubscript{1}.

| Items       | CON   | TA    | AFB\textsubscript{1} | TA + AFB\textsubscript{1} | SEM | P-value |
|-------------|-------|-------|-----------------------|---------------------------|-----|---------|
|             |       |       |                       |                           |     | AFB\textsubscript{1} | TA | AFB\textsubscript{1} × TA |
| Days 1–21   |       |       |                       |                           |     |         |     |                         |
| ADG (g)     | 32.92 | 32.76 | 33.41                 | 32.30                     | 0.23| 0.968   | 0.195 | 0.325                  |
| ADFI (g)    | 46.34 | 46.20 | 47.57                 | 46.55                     | 0.31| 0.227   | 0.371 | 0.497                  |
| FCR         | 1.41  | 1.41  | 1.42                  | 1.44                      | 0.01| 0.022   | 0.322 | 0.444                  |
| Days 22–42  |       |       |                       |                           |     |         |     |                         |
| ADG (g)     | 63.63 | 61.89 | 62.27                 | 61.19                     | 0.68| 0.474   | 0.328 | 0.817                  |
| ADFI (g)    | 114.11| 112.70| 114.67                | 112.12                    | 1.05| 0.997   | 0.387 | 0.802                  |
| FCR         | 1.80  | 1.82  | 1.84                  | 1.83                      | 0.02| 0.454   | 0.801 | 0.637                  |
| Days 1–42   |       |       |                       |                           |     |         |     |                         |
| ADG (g)     | 49.06 | 47.68 | 48.72                 | 48.72                     | 0.68| 0.774   | 0.153 | 0.923                  |
| ADFI (g)    | 81.33 | 79.59 | 81.30                 | 80.44                     | 0.60| 0.751   | 0.323 | 0.733                  |
| FCR         | 1.66  | 1.67  | 1.67                  | 1.69                      | 0.01| 0.475   | 0.460 | 0.757                  |

\* Each mean represents 10 replications with 12 broilers per replication. CON, control diet; AFB\textsubscript{1}, 500 µg/kg aflatoxin B\textsubscript{1} of feed; TA, 250 mg/kg tannic acid of feed; TA + AFB\textsubscript{1}, 250 mg/kg TA + 500 µg/kg AFB\textsubscript{1}.

TABLE 3 Effects of tannic acid on relative organ weight of broilers challenged with AFB\textsubscript{1}.

| Items (g/kg) | CON   | TA    | AFB\textsubscript{1} | TA + AFB\textsubscript{1} | SEM | P-value |
|-------------|-------|-------|-----------------------|---------------------------|-----|---------|
|             |       |       |                       |                           |     | AFB\textsubscript{1} | TA | AFB\textsubscript{1} × TA |
| Day 21      |       |       |                       |                           |     |         |     |                         |
| Liver       | 20.00\textsuperscript{b} | 20.54\textsuperscript{b} | 23.67\textsuperscript{a} | 20.90\textsuperscript{b}  | 0.28| <0.001  | 0.002 | <0.001                |
| Spleen      | 0.78  | 0.76  | 0.92                  | 0.84                      | 0.03| 0.049   | 0.356 | 0.608                  |
| Bursa of Fabricius | 2.89  | 2.93  | 3.10                  | 2.98                      | 0.07| 0.391   | 0.781 | 0.596                  |
| Thymus      | 3.51  | 3.70  | 3.38                  | 3.45                      | 0.11| 0.394   | 0.553 | 0.795                  |
| Kidney      | 7.75\textsuperscript{b} | 7.98\textsuperscript{b} | 9.27\textsuperscript{a} | 7.96\textsuperscript{b}  | 0.13| <0.001  | <0.001 | <0.001                |
| Day 42      |       |       |                       |                           |     |         |     |                         |
| Liver       | 18.20\textsuperscript{b} | 18.41\textsuperscript{b} | 22.18\textsuperscript{a} | 18.91\textsuperscript{a}  | 0.33| <0.001  | 0.002 | <0.001                |
| Spleen      | 0.93  | 1.18  | 1.22                  | 1.09                      | 0.06| 0.376   | 0.592 | 0.117                  |
| Bursa of Fabricius | 2.39  | 2.26  | 2.31                  | 2.47                      | 0.11| 0.781   | 0.955 | 0.529                  |
| Thymus      | 3.06  | 3.46  | 3.44                  | 3.27                      | 0.13| 0.716   | 0.670 | 0.302                  |
| Kidney      | 5.79\textsuperscript{b} | 5.53\textsuperscript{b} | 7.23\textsuperscript{a} | 5.65\textsuperscript{b}  | 0.13| <0.001  | <0.001 | <0.001                |

\* Each mean represents 10 replications with 2 broilers per replication. CON, control diet; AFB\textsubscript{1}, 500 µg/kg aflatoxin B\textsubscript{1} of feed; TA, 250 mg/kg tannic acid of feed; TA + AFB\textsubscript{1}, 250 mg/kg TA + 500 µg/kg AFB\textsubscript{1}.

\textsuperscript{a,b,c} Means in the same row with no common superscripts differ significantly (P < 0.05).

Relative organ weight

As shown in Table 3, on days 21 and 42, AFB\textsubscript{1} and TA exhibited significant interactive effects on the relative weight of the liver and kidney in broilers (P < 0.05). The liver and kidney relative weight was increased in the AFB\textsubscript{1} treatments compared with the CON (P < 0.05), while supplementation with TA into AFB\textsubscript{1} contaminated diet decreased liver and kidney relative weight (P < 0.05). The relative weights of the spleen, bursa of Fabricius, and thymus were unaffected by AFB\textsubscript{1} challenge and TA treatment on days 21 and 42 (P > 0.05).

Intestinal histomorphology

As presented in Table 4, on day 42, AFB\textsubscript{1} challenge reduced the villus height and crypt depth ratio in the ileum (P < 0.05). The ileal villus height tended to decrease (P = 0.079), and the crypt depth of the jejunum tended to increase (P = 0.082) in AFB\textsubscript{1} treatments compared with non-contaminated diets. The TA did not show significant effects on the intestinal histomorphology of broilers (P > 0.05). However, the villus height (P = 0.059) and villus height/crypt depth (P = 0.052) ratio were tended to increase in TA treatments. No interaction
TABLE 4 Effects of tannic acid on intestinal histomorphology of broilers challenged with AFB1.

| Items | CON | TA | AFB1 | TA + AFB1 | SEM | P-value |
|-------|-----|----|------|-----------|-----|---------|
|       |     |    |      |           |     | AFB1 × TA |
| **Day 21** |     |    |      |           |     |         |
| Duodenum | VH (µm) | 1144.03 | 1212.71 | 1014.64 | 1133.42 | 32.65 | 0.119 | 0.159 | 0.700 |
|          | CD (µm) | 89.57  | 92.50  | 86.41  | 86.29   | 2.94  | 0.464 | 0.825 | 0.811 |
|          | V/C (µm/µm) | 13.04 | 13.63  | 12.36  | 14.35   | 0.62  | 0.989 | 0.334 | 0.597 |
| Jejunum | VH (µm) | 814.13 | 883.12 | 715.60 | 861.82  | 28.00 | 0.281 | 0.059 | 0.485 |
|          | CD (µm) | 65.81  | 68.59  | 68.64  | 66.80   | 1.35  | 0.856 | 0.869 | 0.426 |
|          | V/C (µm/µm) | 12.33 | 12.87  | 10.44  | 12.83   | 0.38  | 0.195 | 0.052 | 0.209 |
| Ileum   | VH (µm) | 668.47 | 696.48 | 597.15 | 682.85  | 18.96 | 0.079 | 0.440 | 0.962 |
|          | CD (µm) | 80.93  | 74.12  | 76.11  | 85.60   | 2.89  | 0.583 | 0.825 | 0.184 |
|          | V/C (µm/µm) | 8.90  | 9.57   | 7.34   | 7.90    | 0.32  | 0.093 | 0.859 | 0.396 |
| **Day 42** |     |    |      |           |     |         |
| Duodenum | VH (µm) | 1417.00 | 1369.67 | 1261.61 | 1320.79 | 46.49 | 0.298 | 0.951 | 0.584 |
|          | CD (µm) | 139.15 | 113.19 | 108.09 | 114.06  | 7.08  | 0.298 | 0.489 | 0.272 |
|          | V/C (µm/µm) | 12.01 | 13.90  | 12.91  | 12.28   | 0.70  | 0.809 | 0.672 | 0.401 |
| Jejunum | VH (µm) | 962.97 | 915.73 | 896.66 | 940.08  | 27.25 | 0.715 | 0.973 | 0.431 |
|          | CD (µm) | 93.60  | 98.79  | 99.91  | 116.26  | 3.47  | 0.082 | 0.114 | 0.404 |
|          | V/C (µm/µm) | 9.45  | 9.87   | 9.72   | 8.73    | 0.31  | 0.501 | 0.654 | 0.277 |
| Ileum   | VH (µm) | 687.38 | 655.45 | 630.79 | 600.11  | 25.53 | 0.298 | 0.558 | 0.991 |
|          | CD (µm) | 80.06  | 81.49  | 87.07  | 90.84   | 2.50  | 0.116 | 0.612 | 0.818 |
|          | V/C (µm/µm) | 8.90  | 8.41   | 7.61   | 6.96    | 0.31  | 0.028 | 0.346 | 0.895 |

* Each mean represents 10 replications with 2 broilers per replication. CON, control diet; AFB1, 500 µg/kg aflatoxin B1 of feed; TA, 250 mg/kg tannic acid of feed; TA + AFB1, 250 mg/kg TA + 500 µg/kg AFB1.

b CD, crypt depth; V/C, villus height and crypt depth ratio; VH, villus height.

TABLE 5 Effects of tannic acid on plasma antioxidant capacity of broilers challenged with AFB1.

| Items | CON | TA | AFB1 | TA + AFB1 | SEM | P-value |
|-------|-----|----|------|-----------|-----|---------|
|       |     |    |      |           |     | AFB1 × TA |
| **Day 21** |     |    |      |           |     |         |
| T-AOC (mmol/L) | 0.52 | 0.48 | 0.42 | 0.49 | 0.01 | 0.103 | 0.723 | 0.034 |
| CAT (U/mL) | 3.35 | 3.60 | 2.64 | 2.85 | 0.12 | 0.002 | 0.304 | 0.936 |
| GST (U/mL) | 19.22 | 20.32 | 18.44 | 20.74 | 0.32 | 0.760 | 0.006 | 0.312 |
| GSH-Px (U/mL) | 1624.26 | 1698.25 | 1535.91 | 1550.99 | 20.09 | 0.002 | 0.222 | 0.417 |
| T-SOD (U/mL) | 105.51 | 105.02 | 99.98 | 103.11 | 1.25 | 0.147 | 0.603 | 0.475 |
| MDA (nmol/mL) | 4.22 | 4.05 | 4.52 | 4.27 | 0.10 | 0.198 | 0.293 | 0.865 |
| **Day 42** |     |    |      |           |     |         |
| T-AOC (mmol/L) | 0.43 | 0.40 | 0.43 | 0.46 | 0.01 | 0.368 | 0.831 | 0.351 |
| CAT (U/mL) | 3.82 | 3.89 | 3.69 | 3.99 | 0.04 | 0.826 | 0.014 | 0.118 |
| GST (U/mL) | 20.85 | 21.31 | 17.29 | 20.62 | 0.37 | 0.001 | 0.002 | 0.015 |
| GSH-Px (U/mL) | 1782.43 | 1888.16 | 1559.46 | 1894.93 | 31.21 | 0.026 | <0.001 | 0.019 |
| T-SOD (U/mL) | 108.19 | 107.23 | 105.91 | 110.43 | 1.60 | 0.889 | 0.591 | 0.409 |
| MDA (nmol/mL) | 3.34 | 3.21 | 3.92 | 3.16 | 0.07 | 0.030 | 0.001 | 0.011 |

* Each mean represents 10 replications with 2 broilers per replication. CON, control diet; AFB1, 500 µg/kg aflatoxin B1 of feed; TA, 250 mg/kg tannic acid of feed; TA + AFB1, 250 mg/kg TA + 500 µg/kg AFB1.

b T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; T-AOC, total antioxidant capacity; MDA, malondialdehyde.

Means in the same row with no common superscripts differ significantly (P < 0.05).
TABLE 6  Effects of tannic acid on liver antioxidant capacity of broilers challenged with AFB₁.

| Items          | CON      | TA     | AFB₁     | TA + AFB₁ | SEM      | AFB₁ | TA     | AFB₁ × TA |
|----------------|----------|--------|----------|-----------|----------|------|--------|-----------|
| **Day 21**     |          |        |          |           |          |      |        |           |
| T-AOC (nmol/mgprot) | 207.24   | 235.86 | 184.09   | 180.90    | 5.96     | <0.001 | 0.211 | 0.120     |
| CAT (U/mgprot)  | 15.28    | 18.58  | 15.81    | 18.66     | 0.66     | 0.813 | 0.021 | 0.861     |
| GST (U/mgprot)  | 23.35    | 26.88  | 16.69    | 20.32     | 0.84     | <0.001 | 0.006 | 0.969     |
| GSH-Px (U/mgprot) | 63.38    | 69.81  | 31.68    | 44.88     | 3.11     | <0.001 | 0.022 | 0.413     |
| T-SOD (U/mgprot) | 1692.04a | 1885.59a | 1443.62a | 1413.18a  | 38.77    | <0.001 | 0.103 | 0.027     |
| MDA (nmol/mgprot) | 1.71     | 1.65   | 1.73     | 1.58      | 0.06     | 0.864 | 0.404 | 0.730     |
| **Day 42**     |          |        |          |           |          |      |        |           |
| T-AOC (nmol/mgprot) | 143.34   | 147.58 | 125.06   | 126.11    | 3.78     | 0.008 | 0.713 | 0.824     |
| CAT (U/mgprot)  | 19.32    | 18.96  | 19.49    | 19.84     | 0.60     | 0.676 | 0.998 | 0.778     |
| GST (U/mgprot)  | 52.71    | 54.63  | 47.56    | 44.78     | 1.27     | 0.002 | 0.851 | 0.315     |
| GSH-Px (U/mgprot) | 58.87    | 56.95  | 51.96    | 60.32     | 1.51     | 0.552 | 0.282 | 0.090     |
| T-SOD (U/mgprot) | 1766.21  | 1723.15 | 1606.53  | 1802.90   | 37.69    | 0.595 | 0.310 | 0.117     |
| MDA (nmol/mgprot) | 2.01     | 1.86   | 2.00     | 1.70      | 0.09     | 0.642 | 0.202 | 0.674     |

| Items          | CON      | TA     | AFB₁     | TA + AFB₁ | SEM      | AFB₁ | TA     | AFB₁ × TA |
|----------------|----------|--------|----------|-----------|----------|------|--------|-----------|
| **Day 21**     |          |        |          |           |          |      |        |           |
| T-AOC (nmol/mgprot) | 143.34   | 147.58 | 125.06   | 126.11    | 3.78     | 0.008 | 0.713 | 0.824     |
| CAT (U/mgprot)  | 19.32    | 18.96  | 19.49    | 19.84     | 0.60     | 0.676 | 0.998 | 0.778     |
| GST (U/mgprot)  | 52.71    | 54.63  | 47.56    | 44.78     | 1.27     | 0.002 | 0.851 | 0.315     |
| GSH-Px (U/mgprot) | 58.87    | 56.95  | 51.96    | 60.32     | 1.51     | 0.552 | 0.282 | 0.090     |
| T-SOD (U/mgprot) | 1766.21  | 1723.15 | 1606.53  | 1802.90   | 37.69    | 0.595 | 0.310 | 0.117     |
| MDA (nmol/mgprot) | 2.01     | 1.86   | 2.00     | 1.70      | 0.09     | 0.642 | 0.202 | 0.674     |

a Each mean represents 10 replications with 2 broilers per replication. CON, control diet; AFB₁, 500 µg/kg aflatoxin B₁ of feed; TA, 250 mg/kg tannic acid of feed; TA + AFB₁, 250 mg/kg TA + 500 µg/kg AFB₁.

b T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; T-AOC, total antioxidant capacity; MDA, malondialdehyde.

cabc Means in the same row with no common superscripts differ significantly (P < 0.05).

TABLE 7  Effects of tannic acid on jejunum antioxidant capacity of broilers challenged with AFB₁.

| Items          | CON      | TA     | AFB₁     | TA + AFB₁ | SEM      | AFB₁ | TA     | AFB₁ × TA |
|----------------|----------|--------|----------|-----------|----------|------|--------|-----------|
| **Day 21**     |          |        |          |           |          |      |        |           |
| CAT (U/mgprot)  | 7.80     | 9.85   | 7.58     | 7.94      | 0.48     | 0.275 | 0.219 | 0.386     |
| GST (U/mgprot)  | 21.87    | 23.25  | 21.08    | 21.06     | 0.35     | 0.033 | 0.321 | 0.308     |
| GSH-Px (U/mgprot) | 20.72    | 21.19  | 18.31    | 19.07     | 0.44     | 0.009 | 0.460 | 0.860     |
| T-SOD (U/mgprot) | 275.90   | 277.80 | 266.84   | 277.97    | 3.07     | 0.479 | 0.301 | 0.462     |
| MDA (nmol/mgprot) | 5.02     | 3.90   | 4.94     | 4.47      | 0.16     | 0.407 | 0.010 | 0.264     |
| **Day 42**     |          |        |          |           |          |      |        |           |
| CAT (U/mgprot)  | 7.10     | 8.50   | 8.50     | 6.73      | 0.31     | 0.072 | 0.225 | 0.277     |
| GST (U/mgprot)  | 21.84    | 23.75  | 24.47    | 27.75     | 0.88     | 0.883 | 0.094 | 0.883     |
| GSH-Px (U/mgprot) | 19.95    | 21.09  | 17.89    | 19.37     | 0.58     | 0.112 | 0.261 | 0.892     |
| T-SOD (U/mgprot) | 300.37   | 330.85 | 295.25   | 329.45    | 5.49     | 0.747 | 0.003 | 0.854     |
| MDA (nmol/mgprot) | 4.37     | 3.99   | 4.51     | 4.21      | 0.12     | 0.451 | 0.166 | 0.876     |

a Each mean represents 10 replications with 2 broilers per replication. CON, control diet; AFB₁, 500 µg/kg aflatoxin B₁ of feed; TA, 250 mg/kg tannic acid of feed; TA + AFB₁, 250 mg/kg TA + 500 µg/kg AFB₁.

b T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; GST, glutathione S-transferase; T-AOC, total antioxidant capacity; MDA, malondialdehyde.

cabc Means in the same row with no common superscripts differ significantly (P < 0.05).

was found between AFB₁ and TA in intestinal histomorphology (P > 0.05).

Antioxidant capacity

The results of the antioxidant capacity in the plasma are shown in Table 5. AFB₁ challenge decreased plasma CAT and GSH-Px activities on day 21 (P < 0.05). Compared with the diet without TA, TA supplementation increased CAT activity in plasma on day 42 (P < 0.05). The AFB₁ and TA exhibited interactive effects on the T-AOC, GST, GSH-Px, and MDA (P < 0.05). Compared with the CON, dietary expose to AFB₁ decreased the T-AOC, GST, and MDA activities on days 21 and 42, and increased the MDA content on day 42, respectively (P < 0.05). The addition of TA to AFB₁ contaminated diet
significantly improved the CAT, GSH-Px, and, GST activities, and decreased the MDA content on day 42 (P < 0.05).

As presented in Table 6, AFB1 challenge decreased the GST and T-AOC in the liver on days 21 and 42, as well as GSH-Px and T-SOD activity on day 21 (P < 0.05). Broilers fed the TA diet had greater hepatic CAT, GST, and GSH-Px activities on day 21 (P < 0.05). Furthermore, on day 21, AFB1 and TA showed interactive effects on the T-SOD in the liver (P < 0.05).

In Table 7, AFB1 challenge decreased the GST and GSH-Px activities in the jejunum on day 21 (P < 0.05). Dietary supplemented with TA increased the T-SOD activity in jejunum on day 42. The MDA content of jejunum was also decreased in the TA treatments compared with other treatments (P < 0.05).

**Discussion**

Dietary exposure to AFB1 can cause tremendous economic losses by reducing growth performance, feed efficiency, and increasing mortality in the poultry industry (20–24). In our study, we found that the administration of 500 µg/kg AFB1 diets increased FCR during days 1–21 in broilers. These results are in alignment with several studies, which demonstrated the detriment of broiler health and performance by feeding diets contaminated with 0.1–1 mg/kg AFB1 (25, 26). These adverse effects can be explained as AFB1 could inhibit protein synthesis and lipogenesis, reduce the activity of digestive enzymes, and change the energy metabolism of the cell (15, 27). We hypothesized that a commercially relevant concentration of AFB1 (500 µg/kg) during 42 days could decrease the growth rate in broilers. Unfortunately, the ADG and ADFI were not affected by the AFB1 challenge in the present study. Slizewska et al. (28) also reported that fed 1 mg/kg AFB1 of diet did not affect the ADG and ADFI of broilers. Likewise, Chen et al. (29) and Mesgar et al. (30) noted that feed intake, body weight gain, and feed efficiency were not affected by the 500 and 1,000 µg/kg of AFB1. Therefore, the toxic effects of AFB1 may be acute or chronic, influenced by the age, dose, diet composition, and duration of exposure (31).

In a previous study, we found that 250 and 500 mg/kg TA increased growth performance of broilers (32). In the contrary, supplementation with 250 mg/kg TA had no beneficial effect on the growth performance of broilers. Similar to the current results, Jamroz et al. (33) found that 250–500 mg/kg sweet chestnut tannin had no effect on performance, whereas 1,000 mg/kg TA reduced the final body weight in broilers. In addition, Choi et al. (34) reported that dietary supplementation of 500–5,000 mg/kg TA linearly decreased body weight of broilers infected with *Eimeria Maxima*. On the contrary, Liu et al. (35) found that 1,000 mg/kg chestnut tannins did not affect the body weight gain and feed intake in broilers. Cengiz et al. (36) also indicated that supplemented with 2,000 mg/kg chestnut tannin in broiler diets did not affect the performance. The dosage effect of TA on the growth performance of broilers seems to be unclear. However, it is reported that high dose of TA has negative effects on the growth of broilers, and biological effects are strongly dose-dependent (37, 38). Redondo et al. (39) hypothesized that the addition of excessive TA to the diet may increase the astringency and bitterness of the feed, thereby reducing the feed intake. Based on the different results, the inconsistency might be attributed to the source of tannic acid, administration dosage, diet composition, and age of the bird (40).

Aflatoxin has been known to mainly accumulated and metabolized in the liver and kidney after absorption, causing impairment of the liver and kidney (41, 42). In the present study, we observed that 500 µg/kg AFB1 caused a significant increase in the relative weight of liver and kidney, which is consistent with other studies (43–45). The enlargement of organ weight is attributed to disorders of lipid metabolism, and the inhibition of lipid transportation, leading to lipid deposition, which results in hepatic enlargement (15, 46). Many studies describe the role of plant extract could ameliorate the adverse effect of AFB1 in broilers (47–49). In our previous study, we also found that the increase in liver and kidney relative weight in the AFB1 group was ameliorated by the supplementation of 250 and 500 mg/kg TA (32). Therefore, these results confirmed that TA has a protective effect on the liver and kidney damage caused by AFB1.

Intestinal villus height, crypt depth, and villus height/crypt depth ratio are important indexes to evaluate intestinal nutrient digestion and absorption capacity of poultry (50). These parameters especially the villus height/crypt depth ratio was positively related to the absorptive efficiency of the intestine (51). In the present study, intestinal histomorphology result revealed that dietary AFB1 exposure decreased the villus height and crypt depth ratio in the ileum of 42-day-old broilers. Similar to the results by Tavangar et al. (22), who reported that 1 mg/kg AFB1 decreased small intestine villus height and villus height to crypt depth ratio of broilers. These results showed that AFB1 could decrease the capacity of intestinal mucosa to digest and absorb nutrients by depressing intestinal development. Brus et al. (32) found that tannin extract could promote the proliferation of intestinal epithelial cells to promote intestinal development in vitro. Therefore, further studies need to be conducted to confirm the positive effect of TA on intestinal morphology in broilers.

It has been demonstrated that AFB1 could induce the production of reactive oxygen species (ROS) and oxidative stress, thereby inducing cell and DNA damage (53). The antioxidant system of organism can eliminate the adverse effects of ROS, and the GST, T-SOD, CAT, and GSH-Px are important endogenous antioxidant enzymes, which play a key role in scavenging free radicals and maintaining the intracellular redox equilibrium (15). In the present study, AFB1 significantly increased the concentrations of MDA.
and decreased the antioxidant enzyme activities of T-SOD, GSH-Px, GST, and CAT in the liver, jejunum, and the plasma of broilers when compared with the CON. These results are in consistent with previous studies, which demonstrated that different dosage of AFB₁ decreased the activity of antioxidant enzymes, increased the lipid peroxidation, and inhibited the antioxidant capacity of broilers (14, 15, 54, 55). Recently, researchers have been interested in the usage of antioxidants to counter the toxic effects of aflatoxins (56). Our present results confirmed that 250 mg/kg TA could enhance antioxidative capacity, and alleviate the adverse effects of AFB₁ on oxidative stress in the liver, jejunum, and plasma, which is similar to previous studies (57–59). Consequently, these results indicated that TA could play an important role in preventing the AFB₁-induced oxidative damage in broilers.

Conclusion

In conclusion, supplementation with 250 mg/kg TA could alleviate the oxidative damage, and prevent the enlargement of liver in broilers dietary challenge with 500 µg/kg AFB₁. Therefore, Chinese gallnut TA may be used as a feed additive in the prevention of aflatoxicosis and improve the health of poultry.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of Wuhan Polytechnic University (Number: 20161121).

Author contributions

ZZ and BD conceived and designed the experiment. YX, JC, SG, SW, ZL, and LL performed the experiment. YX, ZL, and YQ analyzed the data. YX and ZZ wrote the manuscript. All authors read and approved the final manuscript.

Funding

This research was financially supported by the Key Projects of Hubei Province (No. 2022BBA0014) and the Research and Innovation Initiatives of Wuhan Polytechnic University (No. 2022RZ068).

Acknowledgments

We highly appreciate all other members of the Animal Nutrition and Intestinal Health Research Group of Wuhan Polytechnic University for providing assistance during the animal experiment.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Negash D, A. review of aflatoxin: occurrence, prevention, and gaps in both food and feed safety. J Appl Microbiol Res. (2018) 1:35–43. doi: 10.15406/jnmfe.2018.08.00268
2. Mishra HN, Das C. A review on biological control and metabolism of aflatoxin. CRC Crit Rev Food Technol. (2003) 43:245–64. doi: 10.1080/10408690330026518
3. Hinton DM, Myers MJ, Raybourne RA, Francke-Carroll S, Sotomayor RE, Shaddock J, et al. Immunotoxicity of aflatoxin B₁ in rats: effects on lymphocytes and the inflammatory response in a chronic intermittent dosing study. Toxicol Sci. (2003) 73:362–77. doi: 10.1093/toxsci/kfg074
4. Liu WC, Yang YY, Pushparaj K, Balasubramanian B. Evaluation of hepatic detoxification effects of Enteromorpha prolifera polysaccharides against aflatoxin B1 in broiler chickens. Antioxidants (Basel). (2022) 11:1757. doi: 10.3390/antiox11091757
5. Rawal S, Kim JE, Coulombe R. Aflatoxin B₁ in poultry: Toxicology, metabolism and prevention. Res Vet Sci. (2010) 89:325–31. doi: 10.1016/j.rvsc.2010.04.011
6. Rajput SA, Sun L, Zhang NY, Khalil MM, Ling Z, Chong L, et al. Grape seed proanthocyanidin extract alleviates aflatoxinB₁-induced immunotoxicity and oxidative stress via modulation of NF-κB and Nrf2 signaling pathways in broilers. Toxins. (2019) 11:23. doi: 10.3390/toxins11010023
7. Ren Y, Jin J, Zheng M, Yang Q, Xing F. Ethanol inhibits aflatoxin B₁ biosynthesis in Aspergillus flavus by up-regulating oxidative stress-related genes. Front Microbiol. (2020) 10:2946. doi: 10.3389/fmicb.2019.02946
8. Food and Drug Administration. Sec. 683, 100. Action Levels for Aflatoxins in Animal Food. (2019). Available online at: https://www.fda.gov/regulatory-
mg/mL. (2015) 74:470–5. doi: 10.1016/j.fct.2015.07.009

35. Liu HS, Mahfuz SU, Wu D, Shang QH, Piao XS. Effect of chestnut wood on performance, meat quality, antioxidant status, immune function, and aflatoxin residues in broilers exposed to aflatoxin B1. Poult Sci. (2019) 98:3298–303. doi: 10.3382/ps/pez178

36. Dohmal V, Wu Q, Kuca K. Metabolism of aflatoxin: key enzymes and interindividual as well as interspecies differences. Arch Toxicol. (2014) 88:1635–44. doi: 10.1007/s00204-014-1312-9

37. Zhang ZF, Xi Y, Wang ST, Zheng YG, Q Y, Q Guo SS, et al. Effects of Chinese gallnut tannin acid on growth performance, blood parameters, antioxidative status, intestinal histomorphology, and cecal microbial shedding in broilers challenged with aflatoxin B1. J Anim Sci. (2022) 100:1–8. doi: 10.1093/jas/skaa999

38. Choi J, Tompkins YH, Yeng PY, Goyal JR, Kim WK. Effects of tannic acid supplementation on growth performance, oocyst shedding, and gut health of broilers infected with Eimeria Maxima. Animals. (2022) 12:1378. doi: 10.3390/animals12111378

39. Liu HS, Mahfuz SU, Wu D, Shang QH, Piao XS. Effect of chestnut wood on performance, meat quality, antioxidant status, immune function, and aflatoxin residues in broilers exposed to aflatoxin B1. Poult Sci. (2019) 98:3298–303. doi: 10.3382/ps/pez178

40. Choi J, Yadav S, Wang L, Loretz BJ, Lorence JM, Callaway TR, et al. Effects of dietary tannic acid supplementation in corn-or barley-based diets on growth performance, intestinal viscosity, litter quality, and incidence and severity of footpad dermatitis in broiler chickens. Livest Sci. (2017) 202:52–7. doi: 10.1016/j.livsci.2016.07.036

41. Aar P, Molit S, Ekes J. The central role of intestinal health on the effect of feed additives on feed intake in swine and poultry. Anim Feed Sci Technol. (2017) 233:64–75. doi: 10.1016/j.anifeeds.2016.07.019

42. Choi J, Yader S, Wang L, Lorentz BJ, Lorence JM, Callaway TR, et al. Effects of supplemental tannic acid on growth performance, gut health, microbiota, and fat accumulation and optimal dosages of a tannin acid in broilers. Front Physiol. (2022) 13:91927. doi: 10.3389/fphys.2022.91927

43. Redondo LM, Chacana PA, Domínguez IE, Fernández ME. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Front Microbiol. (2014) 5:118. doi: 10.3389/fmicb.2014.00118

44. Choi J, Marshall B, Ko H, Shi H, Singh AK, Thippareddi H, et al. Antimicrobial and immunomodulatory effects of tannic acid supplementation in broilers infected with Salmonella Typhimurium. Poult Sci. (2022). 101:20111. doi: 10.3382/ps.2022-010211

45. Huff WE, Doer JR. Synergism between aflatoxin and ochratoxin A in broiler chickens. Poult Sci. (1983) 62:550–5. doi: 10.3382/ps.0620550

46. Li S, Muhammad I, Yu H, Sun X, Zhang X. Detection of aflatoxin adds as potential markers and the role of curcumin in alleviating AFB1-induced liver damage in chickens. Ecotoxic Environ Saf. (2019) 176:137–45. doi: 10.1016/j.ecosafe.2018.03.089

47. Shi YH, Xu ZR, Feng J, Wang CZ. Efficacy of modified montmorillonite nanocomposite to reduce the toxicity of aflatoxin in broiler chicks. Anim Feed Sci Technol. (2006) 129:138–48. doi: 10.1016/j.anifeedsci.2005.12.006

48. Gowda NK, Ledoux DR, Rottinghaus GE, Bermudez AJ, Chen YC. Protective effect of date pits on growth performance, meat quality, antioxidant status, and aflatoxin residues in broilers exposed to aflatoxin B1. Poult Sci. (2011) 100:101441. doi: 10.3382/poultry.2011.101441

49. Deni M, Blandon JC, Guynot ME, Salado S, Perez JF. Effects of dietary DAFADetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B1. Poult Sci. (2009) 88:1444–51. doi: 10.3382/ps.2008-00341

50. Chen X, Horn N, Applegate TJ. Efficiency of hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of graded levels of aflatoxin B1 in broiler chicks. Poult Sci. (2004) 83:2037–47. doi: 10.3382/poultry.2004-03984

51. Rajput SA, Sun L, Zhang N, Khalil MM, Gao X, Ling Z, et al. Ameliorative effects of grape seed proanthocyanidin extract on growth performance, immune function, antioxidant capacity, biochemical constituents, liver histopathology, and aflatoxin residues in broilers exposed to aflatoxin B1. Poult Sci. (2017) 96:371. doi: 10.3390/toxins9110371

52. Zhang NY, Qi M, Zhao L, Zhu MK, Guo J, Liu J, et al. Effect of combined probiotics with aflatoxin B1-degrading enzyme on aflatoxin detoxification, broiler production performance and hepatic enzyme gene expression. Food Chem Toxicol. (2015) 79:470–5. doi: 10.1016/j.fct.2015.06.044

53. Bajgheraehde KF, Karimi TMA, Allameh A, Sharatiadami F. A novel aflatoxin-binding Bacillus probiotic. Performance, serum biochemistry, and immunological parameters in Japanese quail. Poult Sci. (2012) 91:1846–53. doi: 10.3382/ps.2011-01830

54. Slizewska K, Cukrowksa B, Smialokowska S, Cielecka-Kuszyk J. The effect of probiotic supplementation on performance and the histopathological changes in liver and kidney broiler chickens fed diets with aflatoxin B1. Toxins. (2019) 11:112. doi: 10.3390/toxins11020112

55. Mesgar A, Aghdam SH, Bailey CA, Ebrahimzadeh Y, Mohan A. Effect of dietary L-Threonine and threonin binder on performance, blood parameters, and immune response of broilers exposed to aflatoxin B1. Toxins. (2022) 14:10192. doi: 10.3390/toxins14010192
47. Yin HB, Chen CH, Kollanoor-Johny A, Darre MJ, Venkitanarayanan K. Controlling Aspergillus flavus and Aspergillus parasiticus growth and aflatoxin production in poultry feed using carvacrol and trans-cinnamaldehyde. Poul Sci. (2015) 94:2183–90. doi: 10.3382/ps/pev207

48. Makki OF, Omidi A, Ansari Nik H, Hasheminejad SA, Senjedak HSM. Antiaflatoxin B1 effects of shirazi thyme (Zataria multiflora) in broilers, evaluation of performance and liver histopathology. Vet Sci Dev. (2016) 6:36–40. doi: 10.4081/vsd.2016.6090

49. Nazarizadeh H, Hosseini MS, Pourreza J. Effect of plant extracts derived from thyme and chamomile on the growth performance, gut morphology and immune system of broilers fed aflatoxin B1 and ochratoxin A contaminated diets. Ital J Anim Sci. (2019) 18:1073–81. doi: 10.1080/1828051X.2019.1615851

50. Jiang M, Fang J, Peng X, Cui H, Yu Z. Effect of aflatoxin B1 on IgA+ cell number and immunoglobulin mRNA expression in the intestine of broilers. Immunopharmacol Immunotoxicol. (2015) 37:450–7. doi: 10.3109/08923973.2015.1081933

51. Liu HW, Li K, Zhao JS, Deng W. Effects of chestnut tannins on intestinal morphology, barrier function, pro-inflammatory cytokine expression, microflora and antioxidant capacity in heat-stressed broilers. J Anim Physiol Anim Nutr. (2018) 102:717–26. doi: 10.1111/jpn.12839

52. Brus M, Gradnik L, Trapecar M, Škorjanc D, Frangež R. Beneficial effects of water-soluble chestnut (Castanea sativa Mill.) tannin extract on chicken small intestinal epithelial cell culture. Poult Sci. (2018) 97:1271-82. doi: 10.3382/ps/pxe424

53. Choi KC, Chung WT, Kwon JK, Yu JY, Jang YS, Park SM, et al. Inhibitory effects of quercetin on aflatoxin B1-induced hepatic damage in mice. Food Chem Toxicol. (2010) 48:2747–53. doi: 10.1016/j.fct.2010.07.001

54. Cheng P, Ishaq M, Yu H, Yang Y, Li S, Li X, et al. Curcumin ameliorates doxodonal toxicity of AFB1 in chicken through inducing P-glycoprotein and downregulating cytochrome P450 enzymes. Poul Sci. (2020) 99:7035–45. doi: 10.1016/j.psj.2020.09.055

55. Sarker MT, Wan X, Yang H, Wang Z. Dietary lycopene supplementation could alleviate aflatoxin B1 induced intestinal damage through improving immune function and anti-oxidant capacity in broilers. Animals. (2021) 11:3165. doi: 10.3390/ani11113165

56. Umaya SR, Vijayalakshmi YC, Sejian V. Exploration of plant products and phytochemicals against aflatoxin toxicity in broiler chicken production: present status. Toxicon. (2021) 200:55–68. doi: 10.1016/j.toxicon.2021.06.017

57. Dong S, Li H, Casco L, Xiong Y, Guo KJ, Zoccarato I, et al. Antioxidative activity of the polyphenols from the involucres of Castanea mollissima blume and their mitigating effects on heat stress. Poult Sci. (2015) 94:1096–104. doi: 10.3382/ps/pev101

58. Farahat MH, Abdallah FM, Ali HA, Hernandez-Santana A. Effect of dietary supplementation of grape seed extract on the growth performance, lipid profile, antioxidant status and immune response of broiler chickens. Animal. (2017) 11:771–7. doi: 10.1017/S1751731116002251

59. Tong Z, Lei F, Liu L, Wang J, Guo A. Effects of Plotytarya strohilacea Sieb. et Zuce tannin on the growth performance, oxidation resistance, intestinal morphology and cecal microbial composition of broilers. Front Vet Sci. (2022) 8:806105. doi: 10.3389/fvets.2021.806105