Effects of Aflatoxin B1 on Growth Performance, Liver Tissue and Intestinal Health in Weaned Piglets: Postprint

Authors: Bi Xiaojuan, Chen Daiwen, Yu Bing, He Jun, Mao Xiangbing, Zheng Ping, Huang Zhiqing, Luo Junqiu, Luo Yuheng, Jie Yu

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of aflatoxin B1 (AFB1) on growth performance, liver tissue, and intestinal health of weaned piglets. Thirty-two 21-day-old "Duroc × Landrace × Yorkshire" weaned piglets of similar parity were selected and randomly allocated into 2 groups (16 replicates per group, 1 pig per replicate) based on similar body weight, and fed a basal diet (control group) or a diet containing 0.3 mg/kg AFB1 (AFB1 group). Throughout the experiment, pigs had ad libitum access to feed and water under conventional management. The trial consisted of a 3-day adaptation period followed by a 21-day formal feeding period. At the conclusion of the experiment, all pigs were fasted and weighed, and 6 pigs with body weight approximating the group mean were selected from each group for sample collection to evaluate growth performance, liver health, intestinal mucosal morphology, and intestinal microbial counts. The results demonstrated that: compared with the control group, dietary AFB1 significantly reduced average daily gain (ADG) ($P<0.05$), significantly increased feed-to-gain ratio (F/G) ($P<0.05$), and tended to decrease average daily feed intake (ADFI) ($P=0.09$) in weaned piglets; dietary AFB1 significantly increased liver index ($P<0.05$) and resulted in indistinct hepatic lobule structure, moderate hydropic degeneration of hepatocytes, moderate to severe focal necrosis in some regions, and marked hepatic fibrous tissue proliferation; dietary AFB1 exerted no significant effects on mRNA expression levels of hepatic lipid metabolism-related genes including acetyl-CoA carboxylase-1, fatty acid synthase, carnitine palmitoyltransferase-1, lipoprotein lipase, and peroxisome proliferator-activated receptor ($P>0.05$); dietary AFB1 significantly increased duodenal villus height and crypt depth as well as jejunal crypt depth ($P<0.05$); dietary AFB1 significantly decreased cecal Bifidobacterium count ($P<0.05$), but had no significant effects on counts of total bacteria, Escherichia coli, Lactobacillus, and Bacillus ($P>0.05$). These results indicate that feeding a diet containing

0.3 mg/kg AFB1 leads to reduced growth performance and mild impairment of liver tissue and intestinal health in weaned piglets.

Full Text

Effects of Aflatoxin B1 on Growth Performance, Liver Tissue, and Intestinal Health of Weaned Piglets

BI Xiaojuan, CHEN Daiwen, YU Bing, HE Jun, MAO Xiangbing, ZHENG Ping, HUANG Zhiqing, LUO Junqiu, LUO Yuheng, YU Jie*

(Key Laboratory for Animal Disease-Resistance Nutrition of Ministry of Education, Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu 611130, China)

Abstract

This experiment was conducted to investigate the effects of aflatoxin B1 (AFB1) on growth performance, liver tissue, and intestinal health of weaned piglets. Thirty-two 21-day-old "Duroc × Large White × Landrace" weaned piglets of similar parity were randomly allocated into two groups (16 replicates per group, 1 pig per replicate) based on similar body weight principles. The groups were fed either a basal diet (control group) or a diet containing 0.3 mg/kg AFB1 (AFB1 group). Pigs had free access to feed and water throughout the experiment, which consisted of a 3-day adaptation period followed by a 21-day formal trial period. At the conclusion of the experiment, all pigs were weighed after fasting, and six pigs from each group with body weights close to the group average were selected for sample collection to evaluate growth performance, liver health, intestinal mucosal morphology, and intestinal microbial populations. The results demonstrated that compared with the control group, dietary AFB1 significantly reduced average daily gain ($P < 0.05$), significantly increased feed-to-gain ratio ($P < 0.05$), and tended to decrease average daily feed intake ($P = 0.09$) in weaned piglets. Dietary AFB1 significantly increased liver index ($P < 0.05$) and caused indistinct hepatic lobule structure, moderate hepatocellular hydropic degeneration, partial medium to severe focal necrosis, and obvious hepatic fibrosis proliferation. Dietary AFB1 had no significant effects on hepatic mRNA expression of lipid metabolism-related genes including acetyl-CoA carboxylase-1, fatty acid synthase, carnitine palmitoyltransferase-1, lipoprotein lipase, and peroxisome proliferator-activated receptor ($P > 0.05$). Dietary AFB1 significantly increased duodenal villus height and crypt depth as well as jejunal crypt depth ($P < 0.05$). Dietary AFB1 significantly reduced cecal Bifidobacterium populations ($P < 0.05$) but had no significant effects on total bacteria, Escherichia coli, Lactobacillus, or Bacillus populations ($P > 0.05$). These findings indicate that feeding a diet containing 0.3 mg/kg AFB1 reduces growth performance and causes mild impairment of liver tissue and intestinal health in weaned piglets.

Keywords: aflatoxin B1; weaned piglets; growth performance; liver damage; intestinal health

---

Aflatoxin B1 (AFB1) is an extremely toxic mycotoxin that causes substantial economic losses to the livestock industry through feed 原料 waste and reduced animal performance. Following ingestion of AFB1-contaminated diets, approximately 80% of aflatoxins are rapidly absorbed through passive transport in the anterior gastrointestinal tract and subsequently transferred to the liver for metabolism, resulting in hepatic damage, reduced appetite, and decreased growth performance. As the primary site of AFB1 absorption, intestinal toxin concentrations are substantially higher than in other tissues, yet research on AFB1' s gastrointestinal effects remains limited. Intestinal health represents a critical factor for both human and animal health, and whether AFB1 reduces animal productivity through intestinal damage requires further investigation. Most current AFB1 research utilizes naturally moldy corn containing multiple interacting toxins, which complicates understanding of AFB1' s specific toxic effects and mechanisms. Studies using purified AFB1 are limited and yield inconsistent results. Therefore, this experiment added AFB1 produced from cultured *Aspergillus flavus* to weaned piglet diets to investigate the effects of 0.3 mg/kg dietary AFB1 on growth performance, liver tissue, and intestinal health, providing reference data for further mechanistic studies of AFB1 toxicity.

1.1 Aflatoxin Cultivation and Detection

Aflatoxin cultivation consisted of four processes: strain activation, inoculation, fermentation, and toxin collection. (1) Activation: The aflatoxin-producing strain ATCC28539, purchased from the China Center of Industrial Culture Collection, was activated on potato dextrose agar (PDA) medium and cultured in an incubator at 28°C for 5–8 days until green spores formed. (2) Inoculation: Ten milliliters of 0.05% sterilized Tween-20 solution was used to wash the *Aspergillus flavus* spores from the PDA medium into a spore suspension; 4 mL of this spore suspension was then inoculated into sterilized rice medium. (3) Fermentation: After thorough mixing, the culture was incubated statically at 25–28°C for 8–12 days until green spores emerged, with stirring every 8–12 hours during the first 3 days. (4) Toxin collection: Following aflatoxin cultivation, chloroform was added to the Erlenmeyer flask to submerge and moisten the *Aspergillus*-covered medium. The mixture was stored at 4°C in darkness for 24 hours or overnight, after which the chloroform was evaporated at 65°C in a fume hood. The medium was finally dried, ground, bagged, and stored in darkness (this process requires wearing a mask or respirator).

1.2 Preparation and Detection of Aflatoxin-Contaminated Diets

The cultured aflatoxin was quantified according to national standard methods using an enzyme-linked immunosorbent assay (ELISA) kit (purchased from

Jiangsu Suvival Bio-Research Co., Ltd.) following the manufacturer' s instructions. The AFB1 concentration in the fermentation product was 400 mg/kg.

The cultured AFB1 was then added to the basal diet at a calculated rate of 0.3 mg/kg AFB1, requiring 0.75 kg of fermented aflatoxin product per ton of diet to replace 部分玉米 and mix thoroughly through stepwise addition. Dietary toxin content was measured using high-performance liquid chromatography (HPLC): AFB1 content was determined by GB/T 30955−2014 (immunoaffinity column purification-HPLC); deoxynivalenol content by GB/T 30956−2014 (immunoaffinity chromatography purification-HPLC); zearalenone content by GB/T 28716−2012 (immunoaffinity chromatography purification-HPLC); ochratoxin A content by GB/T 30957−2014 (immunoaffinity chromatography purification-HPLC); fumonisin content by GB/T 25228−2010 (immunoaffinity chromatography purification-HPLC); and T-2 toxin content by GB/T 23501−2009 (immunoaffinity chromatography purification-HPLC). Analysis revealed dietary AFB1 content of 363.3 μg/kg, AFB2 content of 34.6 μg/kg, and undetectable levels of AFG1 and AFG2, while other common mycotoxins remained within standard limits. Detailed results are presented in Table 1 .

1.2 Experimental Animals and Design

Thirty-two 21-day-old "Duroc × Large White × Landrace" weaned piglets of similar parity were randomly divided into two groups (16 replicates per group, 1 pig per replicate) based on similar body weight. The groups were fed either a basal diet (control group) or a diet containing 0.3 mg/kg AFB1 (AFB1 group). The experiment included a 3-day adaptation period followed by a 21-day formal trial period.

1.3 Basal Diet

The basal diet was formulated according to NRC (2012) nutrient requirements for piglets combined with practical considerations. Composition and nutrient levels are presented in Table 2 .

1.4 Animal Management

The experiment was conducted at the piglet facility of the Institute of Animal Nutrition, Sichuan Agricultural University. Animal husbandry followed standard practices for weaned piglets. After a 3-day adaptation period, pigs were fed four times daily during the formal trial (08:00, 12:00, 16:00, and 20:00). Feed allowance was adjusted to ensure slight excess remained after satiation, following principles of free water access, ad libitum feeding, and frequent small meals. All pigs were weighed on the morning of day 1 after fasting, and re-weighed on the morning of day 22. Six pigs from each group with body weights closest to the group average were selected for blood collection and slaughter sampling.

1.5 Sample Collection

Diet samples: Diet samples were collected using the quartering method, with approximately 300 g per group placed in sample bags, labeled, and sent to Beijing Zhongjian Weikang Company for analysis of aflatoxin, zearalenone, deoxynivalenol, and six other common mycotoxins.

Liver samples: Following slaughter, livers were rapidly excised and weighed. One liver sample was immediately snap-frozen in liquid nitrogen for gene expression analysis and subsequently stored at -80°C. Another sample was fixed in 4% paraformaldehyde for histological examination.

Intestinal samples: The intestinal mesentery was completely dissected from all experimental piglets and immediately placed on ice. The segment from the pylorus to the Treitz ligament was designated as duodenum, the terminal 10 cm of the small intestine near the ileocecal junction as ileum, and the remaining portion as jejunum. Digesta from duodenum, jejunum, ileum, and cecum were collected in sterile cryovials, snap-frozen in liquid nitrogen, and stored for analysis. Middle segments (4 cm each) of duodenum, jejunum, and ileum were fixed in 4% paraformaldehyde.

1.6.2 Growth Performance

All experimental pigs were weighed after fasting at 08:00 on days 1 and 22. Daily feed allocation, residual feed, and wasted feed were accurately recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.6.3 Liver Index

Liver index (g/kg) = liver weight (g) / live body weight (kg).

1.6.4 Histomorphological Observation

Liver, duodenal, jejunal, and ileal tissues fixed in 4% paraformaldehyde were processed through dehydration, paraffin embedding, and sectioning, followed by hematoxylin-eosin (HE) staining. Results were photographed using a Leica DM1000 microscopic imaging system for histopathological evaluation. Intestinal villus height and crypt depth were measured in 10 representative fields using ImageProPlus 6.0, and villus height-to-crypt depth (V/C) ratios were calculated. Samples were analyzed at Chengdu Lilai Biotechnology Company.

1.6.5 Determination of Hepatic Lipid Metabolism-Related Gene mRNA Expression

Total RNA was extracted from liver samples following the TaKaRa RNAiso Plus protocol. Reverse transcription was performed using the TaKaRa PrimeScript™

RT reagent Kit. Real-time quantitative PCR was conducted using SYBR Premix Ex Taq II in a 10 µL system containing 5.0 µL SYBR Premix Ex Taq II (Tli RNaseH Plus) (2×), 0.2 µL ROX Reference Dye II (50×), 1.0 µL DNA template, 0.4 µL each of forward and reverse primers, and 3.0 µL ddH O. PCR conditions were: 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 30 s. Melting curve analysis was performed at 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. -actin served as the internal reference gene, and relative gene expression was calculated using the 2^(-ΔΔCt) method.

Primer sequences were designed by searching target gene fragments in NCBI and using Primer 3 software, then synthesized by Sangon Biotech (Shanghai) Co., Ltd. Specific primer sequences are listed in Table 3 .

1.6.6 Intestinal Digesta Microbial Populations

Reverse transcription PCR (RT-PCR) was used to quantify microbial populations in ileal and cecal digesta, with results expressed as log (CFU/g) based on Ct values and standard curves. Digesta DNA was extracted using the E.Z.N.A Stool DNA Kit (Omega, USA). PCR conditions were: 95°C for 10 s; 40 cycles of 95°C for 5 s and optimal annealing temperature for 25 s; followed by 95°C for 10 s. Melting curve analysis was performed from 65°C to 95°C with 0.5°C/s increments.

Lactobacillus, Escherichia coli, and Bacillus were detected using a 20 µL reaction system containing 1 µL Probe Enhance Solution, 8 µL Real Master Mix, 1 µL each of forward and reverse primers, 1 µL DNA template, 0.3 µL probe, and 7.7 µL ddH O. Bifidobacterium was detected using a 20 µL system containing 1 µL Probe Enhance Solution, 8 µL Real Master Mix, 1 µL DNA template, 1 µL each of forward and reverse primers, 0.8 µL probe, and 7.2 µL ddH O. Total bacteria were detected using a 25 µL system: 12.5 µL SYBR Premix Ex Taq (2×), 1 µL each of forward and reverse primers, 1 µL DNA template, and 9.5 µL ddH O. Probe Enhance Solution and Real Master Mix were obtained from a probe mix kit from Beijing Tiangen Biotech Co., Ltd. Primer sequences and annealing temperatures are listed in Table 4 .

1.7 Data Processing

Experimental data were organized using Excel 2007 and analyzed by t-test using SPSS 19.0 software. Results are expressed as means ± standard error, with $P < 0.05$ considered statistically significant.

2.1 Effects of AFB1 on Growth Performance of Weaned Piglets

As shown in Table 5 , compared with the control group, the AFB1 group exhibited a 23.81% reduction in ADG ($P < 0.05$), a 15.66% decrease in ADFI ($P = 0.09$), and a significant increase in F/G ($P < 0.05$).

2.2.1 Effects of AFB1 on Liver Index of Weaned Piglets

As shown in Figure 1 [Figure 1: see original paper], dietary AFB1 significantly increased liver index in weaned piglets compared with the control group ($P < 0.05$).

2.2.2 Effects of AFB1 on Liver Morphology of Weaned Piglets

As shown in Figure 2 [Figure 2: see original paper], compared with the control group, AFB1 treatment resulted in indistinct hepatic lobule structure, moderate hepatocellular hydropic degeneration, partial medium to severe focal necrosis, and obvious hepatic fibrosis proliferation.

2.2.3 Effects of AFB1 on Hepatic Lipid Metabolism-Related Gene mRNA Expression

As shown in Table 6 , dietary AFB1 had no significant effects on hepatic mRNA expression of acetyl-CoA carboxylase-1 (ACC-1), fatty acid synthase (FAS), carnitine palmitoyltransferase-1 (CPT-1), lipoprotein lipase (LPL), or peroxisome proliferator-activated receptor (PPAR) ($P > 0.05$).

2.3.1 Effects of AFB1 on Intestinal Morphology of Weaned Piglets

As shown in Table 7 , dietary AFB1 significantly increased duodenal villus height and crypt depth ($P < 0.05$) and significantly decreased jejunal crypt depth ($P < 0.05$), while ileal villus height, crypt depth, and V/C ratio showed no significant differences ($P > 0.05$).

2.3.2 Effects of AFB1 on Intestinal Microbial Populations of Weaned Piglets

As shown in Table 8 , dietary AFB1 significantly reduced cecal Bifidobacterium populations ($P < 0.05$) but had no significant effects on cecal total bacteria, Escherichia coli, Lactobacillus, or Bacillus populations ($P > 0.05$). Ileal microbial populations also showed no significant differences ($P > 0.05$).

3 Discussion

Meta-analysis by Grenier et al. indicates that commonly used AFB1 doses in current research range from 0.3–2.0 mg/kg, with 0.3 mg/kg representing the critical threshold between normal conditions and high-risk scenarios. Therefore, this experiment selected 0.3 mg/kg AFB1 as the test dose. Furthermore, AFB1 was produced using modified fermentation conditions based on Shotwell et al.' s method, with toxin concentration determined by the internationally accepted immunoaffinity column purification-HPLC method. Results showed that 91% of the aflatoxin produced was AFB1, with no other mycotoxins exceeding standard limits, indicating high purity of the fermented product and its suitability for single AFB1 toxicity studies.

As a class-1 carcinogen, AFB1 primarily impairs swine growth and nutrient absorption, causing subclinical disease that wastes feed and delays market time, resulting in economic losses. Research demonstrates that AFB1 reduces ADFI and ADG while increasing feed conversion ratio in pigs, with greater damage observed in younger animals, at higher doses, and with longer exposure. This study showed that 0.3 mg/kg AFB1 significantly reduced ADG, tended to decrease ADFI, and significantly increased F/G, indicating growth inhibition in piglets. These results differ from Rustemeyer et al., who found that 0.25 mg/kg AFB1 significantly reduced ADFI only after 5 weeks without affecting ADG in growing barrows. The earlier manifestation of toxicity in our study (within 2 weeks) may be attributed to younger animals having lower AFB1 tolerance.

The liver is the primary metabolic organ and main target of AFB1 toxicity. Studies show AFB1 increases liver weight and affects broiler performance. This experiment demonstrated significantly increased liver index with histopathological changes including indistinct lobular structure, moderate hydropic degeneration, focal necrosis, and fibrosis proliferation, confirming hepatic damage. Previous research indicated AFB1 reduces PPAR expression, potentially disrupting lipid metabolism and increasing hepatic fat accumulation. However, this study found no significant differences in lipid metabolism-related gene expression. Combined with HE staining results (Figure 2), the increased relative liver weight may reflect histological changes resulting from damaged mitochondrial oxidative enzyme systems. When hepatic injury occurs, disruption of mitochondrial oxidative enzymes reduces ATP production and impairs cell membrane sodium pump function, leading to increased intracellular sodium and water influx, causing cellular swelling and hydropic degeneration.

Intestinal villus height, crypt depth, and V/C ratio are important indicators of small intestinal digestive and absorptive capacity. Crypt depth reflects crypt cell proliferation and maturation, while villus height indirectly indicates the proportion of absorptive and secretory cells and intestinal nutrient absorption capacity. This study showed that AFB1 significantly increased duodenal villus height and crypt depth while decreasing jejunal crypt depth, indicating altered intestinal morphology and impaired normal development and function, consistent with Feng' s findings. This may occur because 80% of AFB1 is absorbed through passive transport in the anterior gastrointestinal tract, with 50% absorbed in the duodenum. To reduce the AFB1 concentration gradient across cells, duodenal villus height increases. However, AFB1 reduces cellular synthesis, decreasing crypt cell generation and secretion rates and increasing duodenal crypt depth. The reduced jejunal crypt depth may represent a compensatory response to AFB1-induced metabolic disruption.

Intestinal microbiota significantly impacts animal health, and disruption of ecological balance may compromise host health. Studies show that 250 μg/kg BW AFB1 significantly reduces beneficial bacteria including Lactobacillus, Bifidobacterium, and total anaerobes in male Kunming mouse cecal digesta, causing dysbiosis. Our results align with Ezz El-Arab et al., who found AFB1 signifi-

cantly reduced hindgut Bifidobacterium populations, suggesting AFB1' s impact on intestinal microbial homeostasis primarily involves reducing beneficial bacteria. Additionally, liver function status is closely related to intestinal microecological balance, as the liver metabolizes harmful substances from the intestine and secretes free bile acids to regulate hindgut pH and maintain microbial balance. Therefore, alterations in intestinal microbiota may also be associated with AFB1-induced liver damage.

4 Conclusion

Feeding a diet containing 0.3 mg/kg AFB1 reduces growth performance and causes mild impairment of liver tissue and intestinal health in weaned piglets.

References

[1] YUNUS A W, RAZZAZI-FAZELI E, BOHM J. Aflatoxin B1 in affecting broiler' s performance, immunity, and gastrointestinal tract: a review of history and contemporary issues[J]. Toxins, 2011, 3(6): 566-590.

[2] ATHERSTONE C, GRACE D, LINDAHL J F, et al. Assessing the impact of aflatoxin consumption on animal health and productivity[J]. African Journal of Food, Agriculture, Nutrition and Development, 2016, 16(3): 10949-10966.

[3] DIAO Hui. Effects of benzoic acid and thymol on growth performance and intestinal health of weaned piglets[D]. Master' s thesis. Ya' an: Sichuan Agricultural University, 2013.

[4] GRENIER B, APPLEGATE T J. Modulation of intestinal functions following mycotoxin ingestion: meta-analysis of published experiments in animals[J]. Toxins, 2013, 5(2): 396-430.

[5] SHOTWELL O L, HESSELTINE C W, STUBBLEFIELD R D, et al. Production of aflatoxin on rice[J]. Applied and Environmental Microbiology, 1966, 14(3): 425-428.

[6] LIU Lifang. Progress in detection and degradation methods of aflatoxin[J]. China Brewing, 2014, 33(1): 23-26.

[7] YI Zhonghua, WU Xingli. Common mycotoxin poisoning and hazards in feed[J]. Hunan Feed, 2008(4): 14-17.

[8] RUSTEMEYER S M, LAMBERSON W R, LEDOUX D R, et al. Effects of dietary aflatoxin on health and performance of growing barrows[J]. Journal of Animal Science, 2010, 88(11): 3624-3630.

[9] HERZALLAH S M, AL-AMEIRI N, AL DMOOR H, et al. Meat and organs quality of broiler chickens fed diet contaminated with aflatoxin B1[J]. Global Veterinaria, 2014, 12(3): 376-380.

[10] ALLAMEH A, SAFAMEHR A, MIRHADI S A, et al. Evaluation of biochemical and production parameters of broiler chicks fed ammonia treated afla-

toxin contaminated maize grains[J]. Animal Feed Science and Technology, 2005, 122(3/4): 289-301.

[11] LIU Yanli, WANG Mingshu, CHENG Anchun, et al. Dynamic pathological changes in ducklings artificially infected with aflatoxin[J]. Chinese Veterinary Science, 2006, 36(5): 396-400.

[12] ZENG Dong, TANG Yurui, NI Xueqin, et al. Effects of *Lactobacillus plantarum* F22 on liver and intestinal microflora[J]. Acta Nutrimenta Sinica, 2010, 32(4): 370-374.

[13] FENG Guangde. Study on the effects and mechanisms of naturally moldy corn on production performance and digestive physiology of meat ducks[D]. PhD dissertation. Ya' an: Sichuan Agricultural University, 2011.

[14] EZZ EL-ARAB A M, GIRGIS S M, HEGAZY E M, et al. Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice[J]. BMC Complementary and Alternative Medicine, 2006, 6(1): 6.

[15] HU Wenhao, DONG Liyang, YANG Yunjun, et al. Study on the effect of adjusting intestinal flora on liver function and endotoxin after interventional therapy for liver cancer[J]. Chinese Journal of Microecology, 2008, 20(3): 251-252, 354.

[16] ZHU Ningchuan, CHEN Yan. Intestinal flora dysbiosis and gut-derived endotoxemia in liver disease[J]. Chinese Journal of Microecology, 2004, 16(1): 61-62.

Corresponding author: Associate Professor YU Jie, E-mail: yujie@sicau.edu.cn

Note: Figure translations are in progress. See original paper for figures.