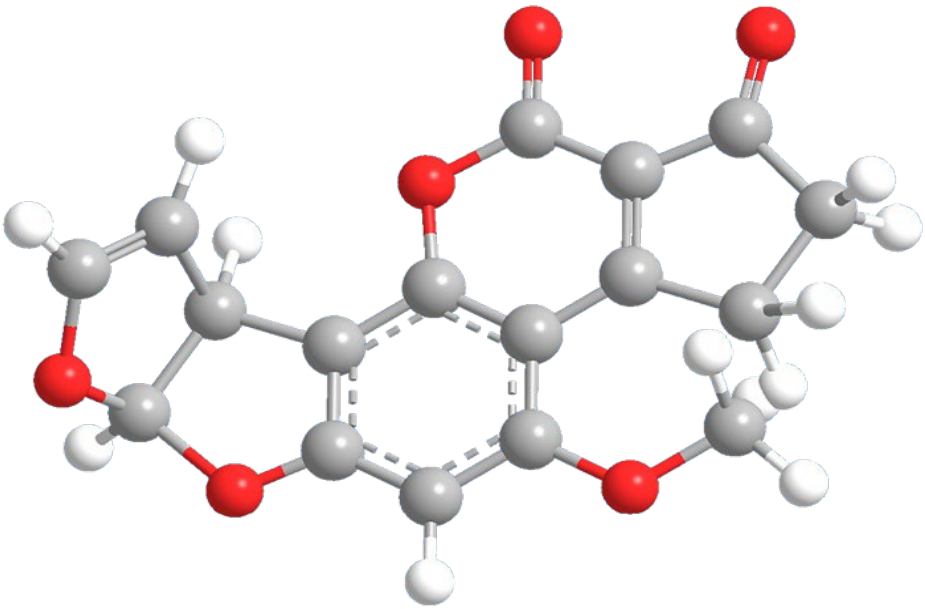
BIBLIOGRAFI AFLATOXIN B1



PERPUSTAKAAN BALAI BESAR PENGUJIAN STANDAR INSTRUMEN VETERINER

BADAN STANDARISASI INSTRUMEN PERTANIAN

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KATA PENGANTAR

Bibliografi ini disusun sebagai referensi bagi para peneliti, akademisi, serta praktisi di bidang kesehatan hewan dan keamanan pangan yang ingin mendalami informasi terkait Aflatoxin B1. Aflatoxin B1 merupakan salah satu mikotoksin berbahaya yang dihasilkan oleh jamur Aspergillus spp. dan memiliki dampak signifikan terhadap kesehatan manusia dan hewan.

Dokumen ini berisi daftar artikel ilmiah yang membahas berbagai aspek terkait Aflatoxin B1, termasuk mekanisme toksisitas, dampaknya terhadap kesehatan, strategi mitigasi, serta metode deteksi dan analisis. Dengan adanya bibliografi ini, diharapkan dapat membantu dalam penelitian dan pengambilan keputusan terkait pengendalian risiko aflatoksin dalam produk pangan dan pakan.

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Semoga bibliografi ini bermanfaat dan dapat mendukung upaya peningkatan keamanan pangan dan kesehatan hewan di Indonesia.

Perpustakaan BBPSI Veteriner

DAFTAR ISI

1. **Acute aflatoxin B1-induced hepatic and cardiac oxidative damage in rats: Ameliorative effects of morin,** Ahmed E. Altyar, Osama A. Kensara, Amany A. Sayed, Lotfi Aleya, Mikhlid H. Almutairi, Mohamed Sayed Zaazouee, Alaa Ahmed Elshanbary, Fatma M. El-Demerdash, Mohamed M. Abdel-Daim,Heliyon,Volume 9, Issue 11,2023,e21837,https://doi.org/10.1016/j.heliyon.2023.e21837.

Abstract:

Aflatoxins (AFs) are secondary metabolites produced by the fungus Aspergillus flavus, of which Aflatoxin-B1 (AFB1) appears to be the most cancerogenic and of the highest toxicity. AFB1 causes serious effects on several organs including the liver. Morin is a flavonol that exists in many fruits and plants and has diverse biological properties including anticancer, anti-atherosclerotic, antioxidant, anti-inflammatory, immunomodulatory, and multi-organ protective activities. The present study aims to evaluate the potential protective effects of morin against acute AFB1-induced hepatic and cardiac toxicity in rats. Forty rats were divided into five groups (n = 8) as follows: control received the vehicle, morin was orally administered 30/mg/kg body weight (MRN30), the AFB1 was administered orally at a dose of 2.5 mg/kg, twice on days 12 and 14 of the experiment for the 3rd, 4th, and 5th groups., AFB1-MRN15 was orally given morin at a dose of 15 mg/kg body weight, and AFB1-MRN30 orally received morin at 30 mg/kg body weight. The results indicated a significant decrease in serum AST, ALP, LDH, GGT, CK, CK-MB, 8-OHdG, IL-1β, IL-6, TNF-a levels in MRN30 compared to AFB1, and AFB1-MRN15 groups. However, the results indicated non-significant differences in the serum levels between MRN30, control, and AFB1-MRN30 groups. Meanwhile, regarding the hepatic and cardiac parameters, there were significant differences in the levels of MDA, NO, GSH, GSH-Px, SOD, and CAT in MRN30 compared to AFB1, and AFB1-MRN15 groups, overall implying the protective effects of morin. To conclude, morin at a dose of 30 mg/kg b. wt. showed significant enhancements in acute AFB1-induced hepatic and cardiac toxicity in rats, which could play a role in limiting the public health hazards of AFs.

Keywords: Mycotoxin; Flavonoids; Morin; Oxidative stress; Inflammation; Liver; Heart

2. **Dual effects of zearalenone on aflatoxin B1–induced liver and mammary gland toxicity in pregnant and lactating rats,** Kuntan Wu, Sifan Jia, Dongfang Xue, Shahid Ali Rajput, Minjie Liu, Desheng Qi, Shuai Wang,Ecotoxicology and Environmental Safety,Volume 245,2022,114115,https://doi.org/10.1016/j.ecoenv.2022.114115.

Abstract:

Food and feed are frequently co-contaminated with aflatoxin B1 (AFB1) and zearalenone (ZEN). This study investigated the effects of ZEN on the AFB1–induced liver and mammary gland toxicity in pregnant and lactating rats. AFB1 and ZEN co-exposure inhibited the growth of rats and caused oxidative stress and inflammatory responses in the liver and mammary gland. Compared with the AFB1-only group, damage was aggravated in the AFB1 + 10 mg/kg ZEN group, and the AFB1 + 1 mg/kg ZEN group showed a reduction in some metrics. The metabolomic results of the mammary gland showed that metabolite

changes were mainly in lipid, amino acid, and glucose metabolism. Compared with the AFB1 + 0 mg/kg ZEN group, the AFB1 + 1 mg/kg ZEN group had the most metabolite changes. Moreover, AFB1 and ZEN co-exposure reduced the levels of sex hormones and RNA m6A methylation in the mammary gland. We speculate that ZEN affects the toxicity of AFB1 to the liver and mammary gland by interfering with the function of sex hormones, regulating cell proliferation and metabolic processes.

Keywords: Aflatoxin B1; Zearalenone; Rat; Liver; Mammary gland

3. **The protective effects of Lactobacillus SNK-6 on growth, organ health, and intestinal function in geese exposed to low concentration Aflatoxin B1,**Guangquan Li, Huiying Wang, Junhua Yang, Zhi Qiu, Yi Liu, Xianze Wang, Huaxiang Yan, Daqian He,Poultry Science,Volume 103, Issue 8,2024,103904,https://doi.org/10.1016/j.psj.2024.103904.

Abstract:

Aflatoxin B1 (AFB1) is a prevalent mycotoxin present in feed ingredients. In this study, we investigated the effects of Lactobacillus salivarius (L. salivarius) on the Landes geese exposed to AFB1. The 300 one-day-old Landes geese were randomly divided into five groups: The control group received a basic diet, while the other groups were fed a basic diet supplemented with 10 μg/kg AFB1, 10 μg/kg AFB1+ 4*108 cfu/g L. salivarius, 50 μg/kg AFB1, and 50 μg/kg AFB1 + 4*108 cfu/g L. salivarius for 63 d. Results showed that high level AFB1 exposure significantly decreased final BW and ADG, increased feed/gain ratio (F/G) and liver index ($P < 0.05$). L. salivarius improved levels of IL-1, IL-6, and IL-12 under low level of AFB1 exposure ($P < 0.05$), along with similar trends observed in serum IgA, IgG, IgM, T3, T4, TNF-α, and EDT ($P < 0.05$). AFB1 exposure reduced jejunum villus high and villus high/crypt depth ratio, and suppressed expression of ZO-1, Occludin, and Claudin-1 mRNA, and significant improved with L. salivarius supplementation under low level AFB1 exposure ($P < 0.05$). AFB1 significantly increased expression levels of TLR3 and NF-kB1, with supplementation of L. salivarius showing significant improvement under low AFB1 exposure ($P < 0.05$). Cecal microbiota sequencing revealed that under low level AFB1 exposure, supplementation with L. salivarius increased the abundance of Bacteroidetes and Lactococcus. In summary, supplementation with 4*108 cfu/g L. salivarius under 10 μg/kg AFB1 exposure improved growth performance and immune capacity, enhanced jejunum morphology, reduced liver inflammation, altered the cecal microbial structure, and positively affected the growth and development of geese.

Keywords: goose; growth performance; liver; intestinal; cecum microbiota

4. **Investigating the individual and mixture cytotoxicity of co-occurring aflatoxin B1, enniatin B, and sterigmatocystin on gastric, intestinal, hepatic, and renal cellular models,**Soraia V.M. de Sá, Miguel A. Faria, José O. Fernandes, Sara C. Cunha,Food and Chemical Toxicology,Volume 188,2024,114640,https://doi.org/10.1016/j.fct.2024.114640.

Abstract:

This study investigates the individual and combined effects of the mycotoxins, Aflatoxin B1 (AFB1), Enniatin B (ENNB) and Sterigmatocystin (STG), on the cellular viability of gastric (NCI–N87), intestinal (Caco-2), hepatic (Hep-G2) and renal (Hek-293) cells, shedding light on synergistic or antagonistic effects using a constant ratio combination design proposed by Chou-Talalay. These toxins are prevalent in cereal-based foods, frequently consumed by children which raises concerns about their exposure to these mycotoxins. This population is particularly vulnerable to the effects of these toxins due to their underdeveloped organs and incompletely structured physiological processes. Results showed that ENB was the most toxic of the three mycotoxins across all cell lines, while STG and AFB1 showed lower toxicity. The combination of ENNB + STG was found to be the most potent in terms of binary mixtures. In regard to ternary combinations, Caco-2 cells are more sensitive to the tested mycotoxins, whereas NCI–N87 cells show lower levels of cell damage. Worrying dose reduction values (>10-fold) were found for ENNB in binary and ternary combinations at low exposure levels. These findings are significant for establishing initial reference values, which play a pivotal role in estimating reference doses that are subsequently incorporated into the broader risk assessment process.

Keywords: Citotoxicity; Children; Human cells; Cell viability; Co-occurrence; Inhibitory concentration

5. **Hesperetin alleviates aflatoxin B1 induced liver toxicity in mice: Modulating lipid peroxidation and ferritin autophagy,**Chao Song, Zixu Wang, Jing Cao, Yulan Dong, Yaoxing Chen,Ecotoxicology and Environmental Safety,Volume 284,2024,116854,https://doi.org/10.1016/j.ecoenv.2024.116854.

Abstract:

One of the ways Aflatoxin B1 damages the liver is through ferroptosis. Ferroptosis is characterized by the build-up of lipid peroxides and reactive oxygen species (ROS) due to an excess of iron. Dietary supplements have emerged as a promising strategy for treating ferroptosis in the liver. The flavonoid component hesperetin, which is mostly present in citrus fruits, has a number of pharmacological actions, such as those against liver fibrosis, cancer, and hyperglycemia. However, hesperetin's effects and mechanisms against hepatic ferroptosis are still unknown. In this study, 24 male C57BL/6 J mice were randomly assigned to CON, AFB1 (0.45 mg/kg/day), and AFB1+ hesperetin treatment groups (40 mg/kg/day). The results showed that hesperetin improved the structural damage of the mouse liver, down-regulated inflammatory factors (Cxcl1, Cxcl2, CD80, and F4/80), and alleviated liver fibrosis induced by aflatoxin B1. Hesperetin reduced hepatic lipid peroxidation induced by iron accumulation by up-regulating the levels of antioxidant enzymes (GPX4, GSH-Px, CAT, and T-AOC). It is worth noting that hesperetin not only

improved lipid peroxidation but also maintained the dynamic balance of iron ions by reducing ferritin autophagy. Mechanistically, hesperetin's ability to regulate ferritin autophagy mostly depends on the PI3K/AKT/mTOR/ULK1 pathway. In AFB1-induced HepG2 cells, the addition of PI3K inhibitor (LY294002) and AKT inhibitor (Miransertib) confirmed that hesperetin regulated the PI3K/AKT/mTOR/ULK1 pathway to inhibit ferritin autophagy and reduced the degradation of ferritin in lysosomes. In summary, our results suggest that hesperetin not only regulates the antioxidant system but also inhibits AFB1-induced ferritin hyperautophagy, thereby reducing the accumulation of iron ions to mitigate lipid peroxidation. This work provides a fresh perspective on the mechanism behind hesperetin and AFB1-induced liver damage in mice.

Keywords: Hesperetin; Aflatoxin B1; liver; ferroptosis; ferritin autophagy

6. **Bovine Lactoferrin alleviates aflatoxin B1 induced hepatic and renal injury in broilers by mediating Nrf2 signaling pathway,** Hong Chen, Jameel Ahmed Buzdar, Roshan Riaz, Dalia Fouad, Nisar Ahmed, Qurban Ali Shah, Shulin Chen,Poultry Science,2024,104316,https://doi.org/10.1016/j.psj.2024.104316.

Abstract:

Aflatoxin B1 (AFB1) a mycotoxin found in chicken feed that possess a global hazard to poultry health. However different potent compounds like bovine lactoferrin (bLF) may prove to be protective effects against AFB1. This study aims to explore the protective effect of bLF against AFB1-induced injury in the liver and kidney in broiler. For this purpose, 600 broilers chicks were randomly alienated into five groups (n=120 each): negative control; positive control (3mg/kg AFB1), and bLF high, medium, and low dosage groups (600 mg/kg, 300 mg/kg, and 150 mg/kg, respectively). The results highlight that AFB1 toxicity in birds exhibited low feed intake, reduction in weight gain, and a decrease in FCR while, bLF regulated these adverse effects. Meanwhile, AFB1 group showed higher levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) and lower levels of superoxide dismutase (SOD) and glutathione (GSHpx) in liver, while urea and creatinine were decline in kidney. Supplementation with bLF effectively controlled these biomarkers and control the negative effects of toxicity. Furthermore, hematoxylin and eosin (H&E) staining exhibited normal morphological structures within liver and kidney in the bLF treated groups, while degenerative changes were observed in AFB1 group. Similarly, bLF, decreased oxidative stress and thus prevented apoptosis in the liver and kidney cells of the birds. Whereas, mRNA level of mitochondrial apoptosis related gene including Bcl-2 (Bak and Bax), caspase-3 and caspase-9 was upregulated, while bcl2 gene were downregulated in AFB1 group. Dietary supplementation of bLF effectively normalizes the expression of these genes. AFB1 exposed birds shown to decrease gene expression level of the crucial component of Nrf2 pathway, responsible to regulate antioxidant defense. Interestingly, bLF reverse these detrimental effects of and restore the normal expression levels of Nrf2 pathway. Conclusively, our findings demonstrate that bLF mitigates the detrimental effects of AFB1, besides regulation of the apoptosis-related genes via mitochondrial pathways. These findings validate that the bLF (600 mg/kg) could be used as protective agent against AFB1-induced liver and kidney damage.

Keywords: Bovine Lactoferrin; aflatoxin B1; hepatic and renal injury; Nrf2 signaling pathway; broilers

7. **Hesperetin protects hippocampal neurons from the neurotoxicity of Aflatoxin B1 in mice,**Chao Song, Zixu Wang, Jing Cao, Yulan Dong, Yaoxing Chen,Ecotoxicology and Environmental Safety,Volume 269,2024,115782,https://doi.org/10.1016/j.ecoenv.2023.115782.

Abstract:

Aflatoxin B1 (AFB1) is a major food and feed pollutant that endangers public health. Previous studies have shown that exposure to AFB1 causes neurotoxicity in the body. However, the mechanism of neurotoxicity caused by AFB1 is not well understood, and finding a workable and practical method to safeguard animals from AFB1 toxicity is essential. This study confirmed that AFB1 caused endoplasmic reticulum stress (ER stress) and apoptosis in hippocampal neurons using C57BL/6 J mice and HT22 cells as models. In vitro experiments showed that the aryl hydrocarbon receptor (AHR) plays a significant role in the cytotoxicity of AFB1. Finally, we assessed how hesperetin protecting against the neurotoxicity caused by AFB1. Our findings demonstrated that AFB1 increased the levels of BAX and Cleaved-Caspase3 proteins, while decreasing the levels of BCL2 protein in the CA1 and CA3 regions of the hippocampus. The AFB1 increased the expression of AHR and activated nuclear translocation. It also elevated the expression levels of Chop, GRP78, p-IRE1/ Xbp1s, and p-PERK/p-EIF2a. Importantly, we also discovered for the first time that blocking AHR in HT22 cells dramatically reduced the level of ER stress and apoptosis caused by AFB1. In vivo and in vitro studies, supplementation of hesperetin effectively reversed AFB1-induced cytotoxicity. We have demonstrated that hesperetin effectively restored the imbalance in the GSH/GST system in HT22 cells treated with AFB1. Furthermore, we observed that elevated GSH levels facilitated the formation of AFB1-GSH complexes, which enhanced the excretion of AFB1. Therefore, hesperetin improves ER stress-induced apoptosis by reducing AFB1 activation of AHR.

Keywords: Hesperetin; Aflatoxin B1; Aromatic hydrocarbon receptors; Apoptosis; ER stress

8. **Simultaneous determination of aflatoxins B1, B2, G1 and G2 in commercial rices using immunoaffinity column clean-up and HPLC-MS/MS,**Iván Romero-Sánchez, Lorena Ramírez-García, Emma Gracia-Lor, Yolanda Madrid-Albarrán,Food Chemistry,Volume 395,2022,133611,https://doi.org/10.1016/j.foodchem.2022.133611.

Abstract:

Rice is frequently contaminated with aflatoxins, that are highly toxic fungal substances and strongly involved on hepatic cancer. In this work, different extraction and clean-up methods were evaluated for the simultaneous extraction and clean-up of aflatoxins B1, B2, G1 and G2 from rice. Favourable results were obtained by using methanol – water (80:20, v/v) extraction followed by immunoaffinity columns for clean-up, with recoveries of 86–92%, standard deviations between 5 and 11%, LOD ranged between 0.09 and 0.32 μg/kg, and LOQ between 0.31 and 1.06 μg/kg. Method validation and sample analysis were performed by using HPLC-MS/MS. Nine rice samples from different origin, varieties and specific

characteristics, acquired in Spanish supermarkets were analysed. In two basmati samples from the same batch aflatoxin B1 was detected at (1.62 ± 0.08) µg/kg and (0.77 ± 0.03) µg/kg, both lower than the levels established by European Regulation for aflatoxin B1 in cereals.

Keywords: Aflatoxins; Cereals; Rice; Immunoaffinity columns; HPLC-MS/MS; Bioanalytical Chemistry

9. **Hepatoprotective effects of Radix Bupleuri extract on aflatoxin B1-induced liver injury in ducks,**Tianyi Feng, Siyu Li, Pengpeng Wang, Di Zhu, Zhixiang Xu, Lidan Wang, Aoyun Li, Md. F. Kulyar, Yaoqin Shen,Ecotoxicology and Environmental Safety,Volume 283,2024,116781,https://doi.org/10.1016/j.ecoenv.2024.116781.

Abstract:

Aflatoxin B1 (AFB1) is recognized as the most toxic mycotoxin, widely present in nature and known to specifically target the liver, leading to severe consequences to animal and human health. The mechanisms underlying AFB1-induced hepatotoxicity involve oxidative stress and apoptosis. Radix Bupleuri (RB) and its extracts (RBE), traditional Chinese herbs with a rich history spanning over 2000 years, have been reported to possess hepatoprotective properties. Nevertheless, the impact of RBE on AFB1-induced liver injury remains to be fully elucidated. The current study utilized Pekin ducks as experimental models to explore the effects of RBE on AFB1-induced liver injury both in vitro and in vivo. In vitro findings indicated that RBE mitigated AFB1-induced cytotoxicity, improved primary duck hepatocytes (PDHs) morphology, and reduced intracellular reactive oxygen species (ROS) levels. In vivo experiments demonstrated that: I) RBE alleviated the growth inhibitory caused by AFB1, as evidenced by improved final body weight and weight gain. II) AFB1 led to significant alterations in serum biochemical parameters (AST, ALT, TP, and ALB) and liver lesions attenuated by RBE supplementation at 2.5 g/kg. III) RBE significantly mitigated oxidative stress induced by AFB1. IV) AFB1-induced changes in mRNA and protein levels associated with oxidative stress and apoptosis were counteracted by RBE. In conclusion, our results suggest that RBE offers protection against AFB1-induced liver injury in ducks, primarily through its antioxidative and anti-apoptotic properties. These findings indicate the potential of RBE in preventing and treating AFB1 poisoning.

Keywords: Aflatoxin B1; Radix Bupleuri extract; Duck; Oxidative stress; Apoptosis

10. **The relationship between aflatoxin B1 with the induction of extrinsic/intrinsic pathways of apoptosis and the protective role of taraxasterol in TM3 leydig cell line,** Cyrus Jalili, Ardeshir Abbasi, Nasim Rahmani-Kukia, Salar Andarzi, Seyran Kakebaraie, Touraj Zamir Nasta,Ecotoxicology and Environmental Safety,Volume 276,2024,116316,https://doi.org/10.1016/j.ecoenv.2024.116316.

Abstract:

Aflatoxins B1 (AFB1) a dangerous type of aflatoxin, poses a serious threat to human health. Meanwhile, Taraxasterol, a bioactive compound in dandelion, exhibits strong anti-inflammatory and antioxidant activity. Therefore, the aim of this study was to investigate the impact of AFB1 on the intrinsic and extrinsic pathways of apoptosis, as well as evaluate the protective role of taraxasterol in the TM3 Leydig cell line. Cell viability was evaluated using an MTT assay, measuring the effects of 3.6 µM AFB1 and varying concentrations of taraxasterol. Expression levels of Caspase 3,8, and 9 were analyzed with RT-qPCR, and flow cytometry was used to assess cell cycle progression and apoptotic alterations. The findings of this study demonstrated that exposure to 3.6 µM of AFB1 resulted in an upregulation of Caspase 3 and Caspase 9 expression, indicating an activation of apoptotic pathways in TM3 cells. Additionally, the analysis of apoptosis revealed a significant increase in cellular apoptosis at this AFB1 concentration. However, when TM3 cells were exposed to 5 µM of taraxasterol, a downregulation of Caspase 3 and Caspase 9 expression was observed, suggesting a protective effect against apoptosis. Moreover, the apoptotic rate in TM3 cells was reduced in the presence of 5 µM of taraxasterol. Consequently, this study highlights the potential of taraxasterol as a protective agent against AFB1-induced apoptosis and suggest its potential application in regulating cell survival and apoptosis-related processes. Further investigations are necessary to elucidate the underlying mechanisms and evaluate the clinical implications of taraxasterol in the context of fertility disorders and other conditions associated with AFB1 exposure.

Keywords: Taraxasterol; Aflatoxin B1; TM3 Leydig cells; Apoptosis; Caspase 3; Caspase 9; Caspase 8

11. **Effects of aflatoxin B1 on subacute exposure of hybrid groupers (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂): Growth, liver histology, and integrated liver transcriptome and metabolome analysis,** Hao Liu, Shuqing Liang, Weibin Huang, Yuanzhi Yang, Menglong Zhou, Baiquan Lu, Biao Li, Wenshan Cai, Hengyang Song, Beiping Tan, Xiaohui Dong,Animal Nutrition,2024,https://doi.org/10.1016/j.aninu.2024.08.002.

Abstract:

With the increasing incorporation of plant-based ingredients into the grouper diet, the issue of aflatoxin B1 (AFB1) contamination in the diet has become a significant concern. In this study, the negative effects of AFB1 on the growth and liver health of hybrid grouper (Epinephelus fuscoguttatus ♀ × Epinephelus lanceolatus ♂) were investigated in the context of growth, liver histology, serum biochemical indices, and integrated transcriptomic and metabolomic data. A total of 540 healthy hybrid groupers, initially weighing 11.59 ± 0.03 g, were randomly divided into six groups (three replicates of 30 fish each): the

Control group was fed a basal diet, and the experimental groups were supplemented with 7 (AF7), 30 (AF30), 111 (AF111), 445 (AF445) and 2230 μg/kg AFB1 (AF2230) in the basal diet respectively, for 56 days. Groups Control, AF445, and AF2230 were selected for subsequent histological, muscle fatty acid, and transcriptomic and metabolomic analyses based on the results of hybrid grouper growth and serum biochemical indices. Compared to the Control group, both whole-body crude lipid and muscle crude lipid contents showed significant decreases in the AF2230 group ($P < 0.05$), while only muscle crude lipid content showed a significant decrease in the AF445 group ($P = 0.001$). Liver damage was seen in the histology of the liver of AF445 and AF2230 groups. Muscle fatty acid results showed that the addition of 445 and 2230 μg/kg AFB1 to the ration increased saturated fatty acids and monounsaturated fatty acids and decreased polyunsaturated fatty acids and highly unsaturated fatty acids in muscle ($P < 0.05$). Transcriptome analyses revealed multiple metabolic pathways associated with AFB1 metabolism, and metabolomics analyses further confirmed changes in the activity of these pathways. The results of the combined transcriptomic and metabolomic analyses indicated that AFB1 causes liver injury mainly by affecting liver retinol metabolism, metabolism of xenobiotics by cytochromes P450, drug metabolism-cytochromes P450 and biosynthesis of unsaturated fatty acids. In conclusion, dietary AFB1 levels above 445 μg/kg resulted in growth inhibition, liver injury, liver AFB1 accumulation, and reduced muscle polyunsaturated fatty acid content in grouper, thereby affecting muscle quality. This study provides novel insights into the detrimental effects of AFB1 on aquatic species and contributes to the scientific basis for the health and sustainability of aquaculture practices.

Keywords: Aflatoxin B1; Hybrid grouper; Transcriptomic; Metabolomic; Liver

12. **Study on the mechanism of aflatoxin B1 degradation by Tetragenococcus halophilus,**Wei Li, Wenjun Li, Chao Zhang, Ning Xu, Caixia Fu, Chao Wang, Deyuan Li, Qian Wu LWT,Volume 180,2023,114662,https://doi.org/10.1016/j.lwt.2023.114662.

Abstract:

The degradation conditions of aflatoxin B1 (AFB1) by T. halophilus in MRS liquid culture were studied. The inoculum amount of T. halophilus, fermentation time, and fermentation temperature were recorded as experimental factors, and the degradation rate of AFB1 was measured as the experimental index. AFB1 degradation increased with the increase of temperature, with a maximum value of 75.9 ± 4.92%. When the temperature exceeded 32 °C, the degradation rate showed a downward trend. Experimental results showed that the degradation of AFB1 by T. halophilus was related to the starting concentration of the strain. The molecular weight of the extracellular enzymes that degraded AFB1 ranged from 55 KD to 70 KD,Zn2+ can promote the activity of AFB1 degradation by this enzyme, while Al3+, Ba2+, Ca2+ and Mn2+ can inhibit the activity of AFB1 degradation by this enzyme. Especially, the inhibitory effect of Ca2+ is the strongest, up to 48.54%, and the optimal temperature is 32 °C. The degradation products of AFB1 were identified as M/Z327.08 ($C_{17}H_{10}O_7$) and M/Z285.08 ($C_{16}H_{12}O_5$). Taken together, these results demonstrated that T. halophilus has application potential for AFB1 degradation.

Keywords: Tetragenococcus halophilus (T.halophilus); Aflatoxin B1; Degradation rate; Extracellular enzyme; Degradation pathway

13. **Impact of Kluyveromyces marxianus VM004 culture conditions on the cell wall structure and its influence on aflatoxin B1 binding,**Carina Pereyra, María del Pilar Monge, Silvestre Bongiovanni, Andrea Cristofolini, Sergio Campos, Lilia Cavaglieri,Revista Argentina de Microbiología,2024,https://doi.org/10.1016/j.ram.2024.07.004.

Abstract:

The aim of this study was to determine the impact of Kluyveromyces marxianus VM004 culture conditions on the cell wall (CW) structure and its influence on aflatoxin B1 binding. The yeast was inoculated into two types of culture media: yeast extract-peptone-dextrose (YPD) broth and dried distiller's grains with solubles (DDG). The CW was extracted from the biomass produced in these media. AFB1 (150ng/ml) adsorption tests using the biomass (1×107cells/ml) and the CW (0.001g) were performed at pH 2 and pH 8. Transmission electron microscopy (TEM) evaluated the CW thickness, and infrared spectroscopy (IR) determined the CW composition. Biomass production in YPD was higher than that in DDG. Cell diameter (μm) and CW thickness (μm) increased in the DDG medium. The CW percentage obtained in DDG was higher than that in YPD. The absorbance of carbohydrates by IR was higher in YPD. pH influenced AFB1 adsorption, which was lower at pH 8. The proportion of β-glucan and chitin present in CW was higher in the YPD medium. The IR method allowed to study the CW carbohydrate variation under the influence of these carbon sources. In conclusion, the culture media composition influenced the β-glucan and chitin composition and consequently, AFB1 adsorption.

Resumen

El objetivo de este estudio fue determinar el impacto de las condiciones de cultivo de Kluyveromyces marxianus VM004 en la estructura de la pared celular (PC) y su influencia en la adsorción de la aflatoxina B1 (AFB1). La levadura fue inoculada en dos medios de cultivo, extracto de levadura-peptona-dextrosa (YPD) y granos de destilería secos con solubles (DDG). De la biomasa obtenida en estos medios se extrajo la PC. Los ensayos de adsorción de la AFB1 (150ngml−1) utilizando la biomasa (1×107celml−1) y la PC (0.001g) se realizaron con pH 2 y pH 8. El espesor y la composición de la PC se determinaron por microscopía electrónica de transmisión (TEM) y espectroscopía infrarroja (IR), respectivamente. La producción de biomasa en YPD fue superior a la lograda en DDG. El diámetro de las células (μm) y el espesor de la PC (μm) aumentaron en el medio DDG. El porcentaje de PC obtenido en DDG fue superior al obtenido en YPD. La absorbancia de carbohidratos por IR fue mayor en YPD. El pH influyó en la adsorción de la AFB1, que fue menor al pH 8. La relación de β-glucano/quitina presente en la PC fue mayor en medio YPD. El método de espectroscopía IR permitió estudiar la variación de carbohidratos en la PC bajo la influencia de estas fuentes de carbono. En conclusión, la composición de los medios de cultivo incidió en la relación de β-glucano y quitina y, en consecuencia, en la adsorción de la AFB1.

Keywords: Aflatoxin B1; β-Glucans; Cell wall; Chitin; Kluyveromyces marxianus; Aflatoxina B1; β-Glucanos; Pared celular; Quitina; Kluyveromyces marxianus

14. **Pre-harvest host-resistance to Aspergillus infection and aflatoxin B1 contaminations in groundnut (Arachis hypogaea L.) genotypes,**Zeyede Akale, Abdi Mohammed, Amare Kebede, Seltene Abady,Heliyon,Volume 9, Issue 12,2023,e23034,https://doi.org/10.1016/j.heliyon.2023.e23034.

Abstract:

Groundnut (Arachis hypogaea L.) is an important oil crop in the tropical and sub-tropical countries. Pod and seed coat crack-inducing factors favour Aspergillus species infections and aflatoxin B1 (AFB1) contamination of groundnut. Aflatoxin B1 (AFB1), a toxic secondary metabolite of Aspergillus species, remains a global concern due to its human and animal health, and economic impacts. Thus, the study was conducted at Babile in 2018 with the objective to identify groundnut genotypes resistant to pre-harvest fungal infections, aflatoxin contaminations and associated effects in crop physiology. Seventeen advanced groundnut breeding lines including one commercial cultivar (Werer-961), were evaluated using randomized complete block design and completely randomized design under field and with four replications for laboratory experiments, respectively. Aflatoxin B1 analysis was carried out using Enzyme-Linked Immunosorbent Assay (ELISA) kits. Appropriate statistical procedures, including regression, were employed for data analyses. Highly significant ($p<0.01$) variation existed among the genotypes for A. flavus and A. niger infections, and the AFB1 contamination ranged from 13.98 (G14) to 1990.86 ppb (G12). The more A. flavus infection, the more reduction in harvest yield and seedling vigour. Fortunately, 53 % of the test materials were found to be resistant to AFB1 production, and frighteningly, none of the AFB1 contaminated genotypes were within the acceptable limit of the lenient standard (10 ppb). All in all, the groundnut genotype (G4) was identified as a good source of pre-harvest resistance to A. flavus infection, AFB1 contamination and seedling vigour so that its inclusion in breeding programs is worthwhile utmost, specifically, in the test environment as pathogen-crop-environment interaction is natural. Since the experiment was employed at one location and for only one year, it is suggested to repeat the experiment across multiple locations and over seasons for reliable recommendation.

Keywords: Aflatoxin B1 (AFB1); Aspergillus flavus; A. niger; Enzyme-linked immunosorbent assay (ELISA); Genotypes

15. **Effect of Lactiplantibacillus plantarum on the growth, hemato-biochemical, inflammation, apoptosis, oxidative stress markers, involved gens and histopathological alterations in growing rabbits challenged with aflatoxin B1,**Sultan A.M. Saghir, Amir M. Al Hroob, Ayat H. Al-Tarawni, Mahfoudh A.M. Abdulghani, Yasser Tabana, Ahmed K. Aldhalmi, Ramzi A. Mothana, Hanan M. Al-Yousef,Poultry Science,Volume 103, Issue 9,2024,104002,https://doi.org/10.1016/j.psj.2024.104002.

Abstract:

Aflatoxin B1 (AFB1) is a significant pollutant found in food and feed, posing a threat to public health. The objective of this study was to assess the effect of Lactiplantibacillus plantarum (LACP) against AFB1 in growing rabbits by investigating growth, serum metabolites, immunity, antioxidant capacity, and inflammatory response. A total of 60 growing male rabbits (721.5 ± 2.68g) were allocated to 4 experimental groups. The control group receiving only a basal diet, the AFB1 group (0.3 mg AFB1/kg diet), the LACP group (1 × 109 cfu/g /kg diet), and the combination group (1 × 109 cfu/g + 0.3 mg AFB1/kg diet; AFB1+ LACP) for 8 wk. The administration of AFB1 alone significantly decreased the final body weight, body gain, and feed intake, while significantly increasing the feed conversion ratio ($P < 0.05$). A significant decline in total proteins and globulins, along with elevated levels of hepatic enzymes (AST, ALP, ALT, and GGT) and renal function markers (creatinine and uric acid), were observed in the AFB1-contaminated group ($P < 0.05$). Immunoglobulins (IgG and IgM) were significantly decreased, alongside a significant elevation of triglycerides, direct bilirubin, and indirect bilirubin in growing rabbits fed diets with AFB1 ($P < 0.05$). Supplementing the AFB1 diet with LACP restored the growth reduction, improved liver (AST, ALP, ALT, and GGT) and kidney (creatinine and uric acid) functions, and enhanced immune markers in rabbit serum ($P < 0.05$). Antioxidant indices (SOD, GSH, and CAT) were significantly decreased in the AFB1 group ($P < 0.05$). However, the addition of LACP to the AFB1-contaminated diets improved antioxidant capacity and malondialdehyde (MDA) and protein carbonylation (PC) in hepatic tissues of rabbits ($P < 0.05$). Serum interlukin-4 (IL-4) and interferon gamma (IFN-γ) levels were significantly increased in the AFB1 group ($P < 0.05$), but the addition of LACP significantly reversed this elevation. AFB1 downregulated the expression of immune-inflammatory genes such Nrf2, IL-10, and BCL-2 genes, while up-regulating the caspase-3 (CASP3) gene ($P < 0.05$). Supplementing AFB1 diet with LACP significantly decreased the expression of immune-inflammatory genes (Nrf2, IL-10, and BCL-2) and reduced the expression of the apoptotic-related gene CASP3. This study highlights the potential of L. plantarum (1 × 109 cfu/g /kg diet) as a protective agent against AFB1 in growing rabbits by enhancing antioxidant and immune function and reducing apoptosis and inflammation pathways.

Keywords: aflatoxin B1; rabbit; oxidative stress and immunity; probiotic; apoptosis; inflammation

16. **A comparative study for evaluating the binding affinity of MARAS-selected aptamer and a patented aptamer towards aflatoxin B1 by electrochemical impedimetric aptasensing**,Kuang-Yi Chang, Chin-Yih Hong, Kai-Chien Yang, Bo-Chuan Hsieh,International Journal of Electrochemical Science,Volume 18, Issue 10,2023,100307,https://doi.org/10.1016/j.ijoes.2023.100307.

Abstract:

Magnetic-assisted rapid aptamer selection (MARAS) is a technique that uses magnetic nanoparticles to efficiently separate aptamer-target complexes under an alternating magnetic field, thereby reducing the time and number of selection rounds required. This study investigated the applicability of MARAS in selecting aptamers against a small molecule, aflatoxin B1 (AFB1). Among the six MARAS-selected aptamers, DNA 1 demonstrated the highest binding affinity. Its Kd value, further estimated from aptasensing data, was 43.7 nM, which is comparable to the Kd value of the patented aptamer (55.6 nM). The DNA 1-immobilized aptasensor exhibited good repeatability (RSD < 7 %, N = 3), linearity (R2 = 0.9613 from 1 to 100 nM), and a calculated LOD of 0.42 nM. Specificity testing indicated that interferences from ochratoxin A, aflatoxin B2, aflatoxin G1, and aflatoxin G2 were all below 7 %. The developed aptasensor was also applied to detect AFB1 content in the peanut sample matrix, achieving a good recovery rate ranging from 94.3 % to 112.2 %. Overall, this study confirms the potential of MARAS as a promising technique for identifying aptamers with strong binding affinities towards low molecular weight compounds such as AFB1. Additionally, the DNA 1-immobilized aptasensor provided a simple, accurate, and cost-effective alternative for AFB1 monitoring.

Keywords: Electrochemical impedance spectroscopy; Aflatoxin B1; Ochratoxin A; Aptasensor; Peanut; Magnetic nanoparticle

17. **Contamination of the traditional medicine Radix Dipsaci with aflatoxin B1 impairs hippocampal neurogenesis and cognitive function in a mouse model of osteoporosis**,Chengyan Yang, Weike Jiang, Dapeng Su, Changgui Yang, Qingsong Yuan, Chuanzhi Kang, Chenghong Xiao, Lulu Wang, Cheng Peng, Tao Zhou, Jinqiang Zhang,Ecotoxicology and Environmental Safety,Volume 283,2024,116831,https://doi.org/10.1016/j.ecoenv.2024.116831.

Abstract:

Aflatoxin B1, which can penetrate the blood-brain barrier and kill neural cells, can contaminate traditional herbal medicines, posing a significant risk to human health. The present study examined cellular, cognitive and behavioral consequences of aflatoxin B1 contamination of the anti-osteoporotic medicine Radix Dipsaci.

Methods

A mouse model of osteoporosis was created by treating the animals with all-trans-retinoic acid. Then the animals were treated intragastically with water decoctions of Radix Dipsaci that contained detectable aflatoxin B1 or not. The animals were compared in terms of mineral density and mineral salt content of

bone, production of pro-inflammatory factors, neurogenesis and microglial activation in hippocampus, as well as behavior and cognitive function.

Results

Contamination of Radix Dipsaci with aflatoxin B1 significantly reduced the medicine's content of bioactive saponins. It destroyed the ability of the herbal decoction to improve mineral density and mineral salt content in the bones of diseased mice, and it induced the production of the oxidative stress marker malondialdehyde as well as the pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α. Aflatoxin B1 contamination inhibited formation of new neurons and increased the proportion of activated microglia in the hippocampus. These neurological changes were associated with anhedonia, behavioral despair, and deficits in short-term memory and social memory.

Conclusion

Contamination of Radix Dipsaci with aflatoxin B1 not only eliminates the herbal decoction's anti-osteoporotic effects, but it also induces neurotoxicity that can lead to cognitive decline and behavioral abnormalities. Such contamination should be avoided through tightly regulated production and quality control of medicinal herbs.

Keywords: Radix Dipsaci; aflatoxin B1; neurotoxicity; neurogenesis; cognitive function

18. **Low transfer of cadmium, lead and aflatoxin B1 to eggs and meat of laying hens receiving diets with black soldier fly larvae reared on contaminated substrates,**M. Heuel, M. Kreuzer, I.D.M. Gangnat, E. Frossard, C. Zurbrügg, J. Egger, B. Dortmans, M. Gold, A. Mathys, J. Jaster-Keller, S. Weigel, C. Sandrock, M. Terranova,Animal Feed Science and Technology,Volume 304,2023,115733,https://doi.org/10.1016/j.anifeedsci.2023.115733.

Abstract:

Replacing soybeans with insects in egg and poultry meat production could improve environmental sustainability. Black soldier fly larvae (BSFL) have a favorable nutrient composition and can be reared on low-grade waste, but this is associated with the risk of feed and food contamination. The aim of this study was to assess the transfer of selected contaminants from larval substrates to poultry-derived food. Two different control substrates were used. Substrate CCH (produced in Switzerland) was based on side streams approved for insect rearing in the European Union (EU), while substrate CIND (produced in Indonesia) included non-EU approved waste. In addition, substrate CIND was spiked with either heavy metals (HM; 1.9 mg cadmium and 18.8 mg lead/kg dry matter (DM)) or 1.5 mg aflatoxin B1/kg DM (AF)). The larvae fed HM contained 7 mg cadmium and 16 mg lead/kg DM. These values were about 30 times the concentrations of cadmium and 30–60 times the concentrations of lead found on average in the BSFL reared with the two non-spiked substrates. Although substrate AF contained 842 μg aflatoxin B1/kg DM as analysed, the AF larvae contained only 4 μg aflatoxin B1/kg DM. Larval meals were integrated at 200 g/kg in two control diets (diets CCH and CIND) and two diets based on contaminated BSFL (diets HM

and AF) designed for late-laying hens (n = 9/treatment). After feeding these diets for 4 weeks, the hens were slaughtered. Diet HM and AF did not affect laying performance or egg quality compared with the control diets. In the body tissue, the cadmium concentrations (per kg DM) were nearly doubled by diet HM in the breast meat (13.3 µg), kidneys (12.3 mg) and liver (1.86 mg) compared to diet CIND. The same diet increased lead in kidneys from below 0.1 to 0.5 mg/kg DM. No lead was detected in the meat and eggs, and no cadmium was found in the eggs. In conclusion, despite cadmium and lead also occurring in BSFL meals of CCH and CIND, the levels in all corresponding hen-based feed and food materials were below the maximum content, except for the kidneys. The aflatoxin B1 level of diet AF (1 µg/kg DM) suggests that the risk might also be small when BSFL are reared on moldy substrate containing aflatoxin-producing fungi. In conclusion, postconsumer waste apparently poses a lower risk than expected in poultry food chains for these contaminants when used as larval substrate.

Keywords: Poultry; Hermetia illucens; Insect feeding; Heavy metal; Mycotoxin; Food waste

19. **Rhodococcus turbidus PD630 enables efficient biodegradation of aflatoxin B1,** Haocheng Liu, Yuqian Tang, Weili Si, Jiaru Yin, Yujuan Xu, Jiguo Yang,LWT,Volume 186,2023,115225,https://doi.org/10.1016/j.lwt.2023.115225.

Abstract:

Microbial degradation is a promising method for eliminating aflatoxin B1 (AFB1). In this study, we used a co-culture method to screen for microorganisms capable of efficiently degrading AFB1 and identified the strain Rhodococcus turbidus PD630 as a highly effective degrader. Our results demonstrated that PD630 had a high degradation efficiency, and the degradation efficiency of PD630 strain could be further enhanced by AFB1 induction or change of carbon and nitrogen sources. The extracellular fluid of PD630 exhibited the highest AFB1 degradation activity (81.67%), mainly due to the action of extracellular enzymes, with the extracellular enzyme precipitated by 70% saturated ammonium sulfate showing the highest degradation activity (55.01%). This extracellular enzyme maintained high degradation activity over a pH range (7–10) and broad temperature range (10–80 °C). Li+ and Cu2+ were identified as the primary activators of the enzyme, and the AFB1 lactone ring was cleaved and transformed into a substance completely different from the parent compound. In AFB1 contaminated corn, wheat, and peanut substrates, fermentation time, liquid-to-solid ratio, and inoculation amount all significantly impacted the degradation efficiency, with wheat showing the highest degradation efficiency. Additionally, PD630 showed a degradation rate of over 40% for the co-contaminant zearalenone.

Keywords: Mycotoxins; AFB1; Rhodococcus turbidus PD630; Biodegradation

20. **Factors influencing aflatoxin B1 levels in the groundnut (Arachis hypogaea L.) germplasm of Ethiopia**,Yonas Syraji, Jeyaramraja PR, Teklu Wegayehu,Heliyon,Volume 10, Issue 15,2024,e35023,https://doi.org/10.1016/j.heliyon.2024.e35023.

Abstract:

As there was no maximum permissible limit prescription for aflatoxin B1 (AFB1) in Ethiopia, this study has been conducted to generate data on AFB1 levels in Ethiopian groundnut accessions/landraces. Besides, an attempt was made to find out if there is any relationship between AFB1 and other parameters such as altitude of cultivation, individual seed weight, kernel colonization by Aspergillus flavus, total carbohydrates, protein and total free amino acids. Out of the 28 accessions studied, merely six accessions registered ≤2 ppb AFB1 and thus, they comply with maximum permissible limit set by European Union. Altitude of cultivation had no relationship with AFB1 levels. Interestingly, total carbohydrates in the seeds as well as kernel colonization by A. flavus showed statistically significant ($p < 0.01$) positive relationships with AFB1 levels. It is suggested to use kernel colonization measurement as an alternative to the expensive ELISA based AFB1 measurement. Besides, suitable pre- and post-harvest aflatoxin management strategies should be developed to alleviate the AFB1 levels in Ethiopian groundnut.

Keywords: Aflatoxin B1; Arachis hypogaea; Aspergillus flavus; Groundnut; Kernel colonization

21. **Aflatoxin B1 production: A time–water activity–temperature model**,Sonia Marín, Laila Aldars-García, Francisco Molino, Antonio J. Ramos, Vicente Sanchis,Fungal Biology,2024,https://doi.org/10.1016/j.funbio.2024.03.003.

Abstract:

Aspergillus flavus occurs as a contaminant of various foods and animal feeds and can produce the mycotoxin aflatoxin B1 that is a danger to human and animal health. Here, we develop models to predict the behaviour of A. flavus in maize extract agar and maize grains. Growth and aflatoxin B1 production were recorded on maize extract agar at 20–35 °C and water activities from 0.84 to 0.90. We then obtained probability models—using temperature, water activity, and time as explanatory variables—based on data of growth and aflatoxin B1 production. Additional data were generated under two dynamically changing temperature regimes. Initial water activity, and relative humidity during incubation, were recorded. Predicted probability of growth under dynamic conditions based on models built under static conditions depended on the temperature regime and substrate, concordance ranging from 66 to 100%, with lower concordances obtained for aflatoxin B1 production prediction. Interestingly, aflatoxin B1 production was higher on maize grains than on maize extract agar. Moreover, this work suggests that the safe water activity for a cereal may depend on the previous water activity and temperatures which may have allowed fungal growth and so trigger later toxin production under water stress.

Keywords: Aspergillus flavus; Aflatoxin; Predictive mycology; Probability; Temperature; Water activity

22. **Isolation of Bacillus licheniformis and its protective effect on liver oxidative stress and apoptosis induced by aflatoxin B1,**Wenwen Dong, Mingchao Liu, Bei Liu, Yaqing Xiao, Xia Liu, Menghao Yang, Xiaoyuan Yuan, Yuxia Zhang, Guiming Li, Kai Meng,Poultry Science,Volume 103, Issue 10,2024,104079,https://doi.org/10.1016/j.psj.2024.104079.

Abstract:

Aflatoxin B1 (AFB1) is one of the most toxic mycotoxins. The use of probiotics is an effective approach to reduce aflatoxins content in foods. To find efficient bacterial species that can eliminate or detoxify AFB1, a bacterial strain S51 capable of degrading AFB1 was isolated from chicken intestine and soil samples by using a culture medium containing coumarin as the sole carbon source. Based on the results of 16S rRNA gene sequence analysis, this isolate (strain S51) was identified as Bacillus licheniformis strain QT338. Further characterization of strain S51 showed that it could degrade AFB1 by 61.3% after incubation at 30°C for 72 h. Additional studies demonstrated that S51 promoted good growth performance of the treated chickens, showed no hemolytic activity, carried few drug resistance genes, and exhibited a certain level of tolerance to acid and bile salts. Furthermore, to verify whether strain S51 exerts a protective effect on AFB1-induced liver injury in chickens and to elucidate the underlying mechanism, a chicken toxicity model was induced with AFB1 (100 μg/kg BW) and treated with S51(1×109CFU/mL) for 12 d. The results showed that S51 decreased the level of alanine transaminase, aspartate transaminase, and total bilirubin ($P < 0.05$); increased glutathione activity and total antioxidant capacityin the liver induced by AFB1, and decreased malondialdehyde production ($P < 0.05$). S51 also up-regulated the mRNA expression level of the antioxidant proteins HO-1 and Nrf2 and down-regulated the expression of the oxidation-related factor Keap1 in the Nrf2/Keap1 signaling pathway ($P <0.05$). S51 inhibited hepatocyte apoptosis induced by AFB1 and decreased the mRNA expression levels of the apoptosis-related genes Bax, caspase-3, caspase-9, and Cyt-C ($P < 0.05$). These results indicate that S51 regulates apoptosis and alleviates AFB1-induced oxidative stress in chicken liver by controlling the Nrf2/Keap1 signaling pathway.

Keywords: aflatoxin B1; Bacillus licheniformis; liver oxidative stress; apoptosis

23. **Baicalin protects against hepatocyte injury caused by aflatoxin B1 via the TP53-related ferroptosis Pathway,**Han-Jing Zhang, Jian-Zhu Luo, Chen-lu Lan, Xiong Teng, Bin Ge, Jun-Qi Liu, Hai-Xiang Xie, Ke-Jian Yang, Chong-Jiu Qin, Xin Zhou, Tao Peng,Ecotoxicology and Environmental Safety,Volume 281,2024,116661,https://doi.org/10.1016/j.ecoenv.2024.116661.

Abstract:

Objective

Baicalin has antioxidative, antiviral, and anti-inflammatory properties. However, its ability to alleviate oxidative stress (OS) and DNA damage in liver cells exposed to aflatoxin B1 (AFB1), a highly hepatotoxic compound, remains uncertain. In this study, the protective effects of baicalin on AFB1-induced hepatocyte injury and the mechanisms underlying those effects were investigated.

Methods

Stable cell lines expressing CYP3A4 were established using lentiviral vectors to assess oxidative stress levels by conducting assays to determine the content of reactive oxygen species (ROS), malondialdehyde (MDA), and superoxide dismutase (SOD). Additionally, DNA damage was evaluated by 8-hydroxy-2-deoxyguanosine (8-OHdG) and comet assays. Transcriptome sequencing, molecular docking, and in vitro experiments were conducted to determine the mechanisms underlying the effects of baicalin on AFB1-induced hepatocyte injury. In vivo, a rat model of hepatocyte injury induced by AFB1 was used to evaluate the effects of baicalin.

Results

In vitro, baicalin significantly attenuated AFB1-induced injury caused due to OS, as determined by a decrease in ROS, MDA, and SOD levels. Baicalin also considerably decreased AFB1-induced DNA damage in hepatocytes. This protective effect of baicalin was found to be closely associated with the TP53-mediated ferroptosis pathway. To elaborate, baicalin physically interacts with P53, leading to the suppression of the expression of GPX4 and SLC7A11, which in turn inhibits ferroptosis. In vivo findings showed that baicalin decreased DNA damage and ferroptosis in AFB1-treated rat liver tissues, as determined by a decrease in the expression of γ-H2AX and an increase in GPX4 and SLC7A11 levels. Overexpression of TP53 weakened the protective effects of baicalin.

Conclusions

Baicalin can alleviate AFB1-induced OS and DNA damage in liver cells via the TP53-mediated ferroptosis pathway. In this study, a theoretical foundation was established for the use of baicalin in protecting the liver from the toxic effects of AFB1.

Keywords: Baicalin; Aflatoxin B1; Hepatocytes; TP53; Ferroptosis

24. **Effects of aflatoxin B1 on metabolism- and immunity-related gene expression in Hermetia illucens L. (Diptera: Stratiomyidae),** Parth N. Shah, Kelly Niermans, Elise F. Hoek- van den Hil, Marcel Dicke, Joop J.A. van Loon, Pesticide Biochemistry and Physiology, Volume 202, 2024, 105944, https://doi.org/10.1016/j.pestbp.2024.105944.

Abstract:

Contamination of food products with mycotoxins such as aflatoxin B1 (AFB1) poses a severe risk to human health. Larvae of the black soldier fly (BSFL), Hermetia illucens (Diptera: Stratiomyidae), can successfully metabolize AFB1 without any negative consequences on their survival or growth. Organic waste streams contaminated with mycotoxins can be upcycled into protein-rich BSFL as an alternative feed for livestock and the left-over feed residue into nutrient-rich crop fertilizers. However, the underlying mechanisms that allow BSFL to metabolize AFB1 are unknown. In this study, five-day-old BSFL were fed with either a control or an AFB1-spiked (20 μg/kg) diet to elucidate the underlying mechanisms. Larval samples were collected

at three timepoints (6 h, 24 h and 72 h) and subjected to RNA-Seq analysis to determine gene expression patterns. Provision of an AFB1-spiked diet resulted in an up-regulation of 357 and a down-regulation of 929 unique genes. Upregulated genes include multiple genes involved in AFB1 metabolism in other (insect) species. Downregulated genes were generally involved in the insects' growth, development, and immunity. BSFL possesses a diverse genetic arsenal that encodes for enzymes capable of metabolizing AFB1 without trade-offs on larval survival. In conclusion, the adverse impact of AFB1 exposure on immunity-related processes is observed in the transcriptomic response, and is indicative of a trade-off between detoxification and immune responses.

Keywords: Aflatoxin B1; Hermetia illucens; Cytochrome P450; Glutathione S-transferase; Detoxification

25. **The effect of dielectric barrier discharge (DBD) cold plasma treatment on the reduction of aflatoxin B1 and the physicochemical properties of oat,**Mandana Dousti, Moein Bashiry, Parvin Zohrabi, Vahid Siahpoush, Asma Ghaani, Khadije Abdolmaleki,Applied Food Research,Volume 4, Issue 2,2024,100515,https://doi.org/10.1016/j.afres.2024.100515.

Abstract:

Cold plasma is an emerging technology that has increased in demand because of consumers' desire for fresh, safe and minimally processed food products. Aflatoxin B1 is one of the main mycotoxins that is usually found in cereals, especially oats. This study was conducted to determine the effect of DBD cold plasma on aflatoxin B1 and the physicochemical characteristics of oats. Whole oat grains were subjected to DBD plasma treatment for 1, 3, and 6 min at powers of 32, 37, and 42 W. Untreated samples were also evaluated as controls. The results showed that DBD treatment had the greatest reduction in 6 min and power 37, which reduced AFB1 concentration by 82%. Furthermore, there was no significant difference for some qualitative parameters such as protein and pH compared with the control sample, whereas the peroxide value and β-glucan increased and alpha-tocopherol decreased. The farinograph test showed that the improvement in water absorption capacity, dough stability, dough development time, and the degree of softening decreased. In addition, the viscosity of treated sample was reduced compared with that of the control sample. This study showed that DBD plasma had an effective reduction in aflatoxin AFB1, and it also improved the technological properties.

Keywords: Mycotoxin; Novel food processing; Cereal grains; Rheological properties

26. **Chronic exposure to aflatoxin B1 increases hippocampal microglial pyroptosis and vulnerability to stress in mice,** Dapeng Su, Weike Jiang, Qingsong Yuan, Lanping Guo, Qin Liu, Mengmeng Zhang, Chuangzhi Kang, Chenghong Xiao, Changgui Yang, Liangyuan Li, Chunyun Xu, Tao Zhou, Jinqiang Zhang,Ecotoxicology and Environmental Safety,Volume 258,2023,114991,https://doi.org/10.1016/j.ecoenv.2023.114991.

Abstract:

Chronic aflatoxin B1 (AFB1) exposure may increase the risk of multiple neuropsychiatric disorders. Stress is considered one of the main contributors to major depressive disorder. Whether and how chronic AFB1 exposure affects vulnerability to stress is unclear.

Methods

Mice were exposed for three weeks to AFB1 (100 μg/kg/d) and/or chronic mild stress (CMS). The vulnerability behaviors in response to stress were assessed in the forced swimming test (FST), sucrose preference test (SPT), and tail suspension test (TST). Microglial pyroptosis was investigated using immunofluorescence, enzyme-linked immunosorbent assays, and western blot assay in the hippocampus of mice. Hippocampal neurogenesis and the effects of AFB1-treated microglia on proliferation and differentiation of neural stem/precursor cells (NSPCs) were assessed via immunofluorescence in the hippocampus of mice.

Results

Mice exposed to CMS in the presence of AFB1 exhibited markedly greater vulnerability to stress than mice treated with CMS or AFB1 alone, as indicated by reduced sucrose preference and longer immobility time in the forced swimming test. Chronic aflatoxin B1 exposure resulted in changes in the microglial morphology and increase in TUNEL+ microglia and GSDMD+ microglia in the hippocampal dentate gyrus. When mice were exposed to both CMS and AFB1, pyroptosis-related molecules (such as NLRP3, caspase-1, GSDMD-N, and interleukin-1β) were significantly upregulated in the hippocampus. These molecules were also significantly enhanced by AFB1 in primary microglial cultures. AFB1-treated mice showed decrease in the numbers of BrdU+, BrdU-DCX+, and BrdU-NeuN+ cells in the hippocampal dentate gyrus, as well as the percentages of BrdU+ cells that were NeuN+ in the presence or absence of CMS when compared with vehicle-treated mice. The combination of AFB1 and CMS exacerbated these effects to an even greater extent. The number of DCX+ cells correlated negatively with the percentage of ameboid microglia, TUNEL+ microglia and GSDMD+ microglia in the hippocampal dentate gyrus. AFB1-treated microglia suppressed the proliferation and neuronal differentiation of NSPCs in vitro.

Conclusion

Chronic AFB1 exposure induces microglial pyroptosis, promoting an adverse neurogenic microenvironment that impairs hippocampal neurogenesis, which may render mice more vulnerable to stress.

Keywords: Stress vulnerability; Aflatoxin B1; Microglial pyroptosis; NLRP3; Neurogenesis

27. **Vitamin C-reduced graphene oxide/Fe3O4 composite for simultaneous removal of aflatoxin B1 and benzo(a)pyrene in vegetable oils,**Fei Ma, Fen Tang, Boyi Yang, Qi Guo, Peiwu Li, Li Yu,LWT,Volume 203,2024,116342,https://doi.org/10.1016/j.lwt.2024.116342.

Abstract:

Vegetable oils are essential constituent of the daily dietary intake. Nevertheless, they often contain aflatoxin B1 (AFB1) and benzo(a)pyrene (BAP). Physical adsorption is the most commonly used detoxification method in the vegetable oils industry. Regrettably, the previously reported adsorbents have exhibited limited efficacy, rendering them inadequate for meeting the requirements of large-scale production of edible oils. In this work, a superparamagnetic Fe3O4/reduced graphene oxide (rGO) composite was synthesized by a one-step hydrothermal method using vitamin C (VC), and systematically characterized. The Vitamin C-reduced graphene oxide/Fe3O4 composite (VC-rGO/Fe3O4) could serve as an efficient adsorbent in edible vegetable oils for the simultaneous removal of AFB1 and BAP. The results of the batch adsorption studies indicated that the pseudo-second order model and Sips model, respectively, were the best fit kinetic model and adsorption isotherm model. The adsorption of AFB1 and BAP on VC-rGO/Fe3O4 was spontaneous, which included both physical and chemical adsorption processes. The removal efficiencies of VC-rGO/Fe3O4 in 13 vegetable oils were evaluated, revealing a wide range of practical applications. Moreover, the quality and safety of detoxified vegetable oils is acceptable. Therefore, VC-rGO/Fe3O4 have been tested as promising candidates for the simultaneous removal of AFB1 and BAP in edible vegetable oils.

Keywords: Aflatoxin B1; benzo[a]pyrene; Detoxification; Ascorbic acid; Reduced graphene oxide

28. **A new insight on alleviating the inhibitory effect of aflatoxin B1 on muscle development in grass carp (Ctenopharyngodon idella): the effect of 4-methylesculetin in vivo and in vitro,**Xiangning He, Jiajia Zhang, Weidan Jiang, Pei Wu, Yang Liu, Hongmei Ren, Xiaowan Jin, Hequn Shi, Xiaoqiu Zhou, Lin Feng,Animal Nutrition,2024,https://doi.org/10.1016/j.aninu.2024.08.001.

Abstract:

Aflatoxin B1 (AFB1), an important fungal toxin, exists mainly in plant feed ingredients and animals consuming feed contaminated with AFB1 will have reduced growth and impaired health condition mainly due to oxidative stress and reduced immunity. Our previous study found that AFB1 caused oxidative damage and inhibited muscle development of zebrafish. 4-methylesculetin (4-ME), a coumarin derivative, is now used in biochemistry and medicine widely because of its antioxidant function. Whether 4-ME could alleviate the inhibition of muscle development in grass carp induced by AFB1 has not been reported. In this experiment, 720 healthy grass carp (11.40 ± 0.01 g) were randomly divided into 4 groups with 3 replicates of 60 fish each, including control group, AFB1 group (60 μg/kg diet AFB1), 4-ME group (10 mg/kg diet 4-ME), and AFB1+4-ME group (60 μg/kg diet AFB1 + 10 mg/kg 4-ME diet), for a 60 d growth experiment. In vitro, we also set up 4

treatment groups in grass carp primary myoblast, including control group, AFB1 group (15 μmol/L AFB1), 4-ME group (0.5 μmol/L 4-ME) and AFB1+4-ME group (15 μmol/L AFB1+0.5 μmol/L 4-ME). The results showed that dietary AFB1 decreased growth performance of grass carp, damaged the ultrastructure and induced oxidative damage in grass carp muscle, and significantly decreased the mRNA and protein expression levels of myogenin (MyoG), myogenic differentiation (MyoD), myosin heavy chain (MYHC), as well as the protein expression levels of laminin β1, fibronectin and collagen Ⅰ ($P < 0.05$), significantly activated the protein expression levels of urokinase-type plasminogen activator (uPA), matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9) and phosphorylate-38mitogen-activated protein kinase (p38 MAPK) both in grass carp muscle and grass carp primary myoblast ($P < 0.05$). Supplementation of AFB1 with 4-ME significantly improved the growth performance inhibition and alleviated the muscle fiber development inhibition and extracellular matrix (ECM) degradation in grass carp induced by AFB1. The present results revealed that supplementation of AFB1 contaminated feed with 4-ME reduced the inhibition of growth and muscle development by alleviating AFB1-induced ECM degradation in grass carp, which might be related to the p38 MAPK/uPA/MMP/ECM pathway. The results implied that 4-ME could be used as a valuable mycotoxin scavenger in animal feed.

Keywords: 4-methylesculetin; Aflatoxin B1; Muscle development; Grass carp

29. **Pharmacokinetics, dynamics, toxicology and molecular docking of bioactive alkaloid vasicine from Adhatoda vasica: a promising toxin binder against aflatoxin B1 and ochratoxin A,** Sakthi Priya Muthusamy, Appusamy Jagadeeswaran, Amirthalingam Natarajan,Poultry Science,Volume 103, Issue 2,2024,103272,https://doi.org/10.1016/j.psj.2023.103272.

Abstract:

Vasicine from Adhatoda vasica was investigated in the management of aflatoxicosis and ochratoxicosis by in silico molecular docking approach. The computational analysis was carried out using Discovery Studio Autodock 4.5 tool. Absorption, distribution, metabolism, and excretion (ADME), pharmacodynamics and toxicity studies were also carried out using Swiss ADME and PASS online server, respectively. The standard drug compound used was silymarin and the structure were retrieved from the protein data bank for both the test compound vasicine and the standard drug. Vasicine interacted with aflatoxin B1 at 10 different poses and the maximum dock score was found to be 83.04 and the binding energy was -37.54 kcal/mol. Silymarin interacted with aflatoxin B1 at 10 different poses and the maximum dock score was found to be 143.578 and the binding energy was -67.32 kcal/mol. Vasicine interacted with ochratoxin A at 10 different poses and the maximum dock score was found to be 73.75 and the binding energy was -56.20 kcal/mol. Silymarin interacted with ochratoxin A at 10 different poses and the maximum dock score was found to be 89.23 and the binding energy was -98.86 kcal/mol. The compounds possess good gastro intestinal absorption with antioxidant property and exhibits minimum adverse effects. The obtained results support the toxin mitigating potential of the test compound with minimum adverse effects and hence vasicine can

be regarded as a potential toxin binder of aflatoxin B1 and ochratoxin A, wherein it can be implemented for alleviating aflatoxicosis and ochratoxicosis.

Keywords: Drug discovery; Vasicine; Aflatoxin B1; Ochratoxin A; Silymarin; In silico studies

30. **From food toxins to biomarkers: Multiplexed detection of aflatoxin B1 and aflatoxin M1 in milk and human serum using PEGylated ternary transition metal sulfides,**Kanchan Yadav, Kalimuthu Moovendaran, Namasivayam Dhenadhayalan, Shang-Fan Lee, Man-Kit Leung, Raman Sankar,Sensors and Actuators Reports,Volume 5,2023,100156,https://doi.org/10.1016/j.snr.2023.100156.

Abstract:

Aflatoxins (AF) are fungal toxins which not only contaminate food, but also adversely impact human health if consumed and serve as biomarkers for deadly diseases like liver cancer. Therefore, there is a strong need to develop ultra-sensitive AF detection method in physiological environments. In the present study, ternary transition metal sulfides single crystals (Mn0.02Ta3S6 and Fe0.65Ta3S6) were grown, exfoliated to thin layered nanosheets (NSs) followed by polyethylene-glycol modification (PEG@NSs). High fluorescence quenching abilities towards the aptamers for AF biosensing were demonstrated using PEGylated NSs. It has been observed that PEGylated NSs improves the detection sensitivity ∼ 248 times better than non-PEGylated NSs. Subsequently, multiplexed detections of aflatoxin B1 (AFB1) and M1 (AFM1) in diverse mediums including PBS buffer (1 ×), milk and human serum were performed by employing PEG@Mn0.02Ta3S6 NSs with TAMRA dye-labelled AFB1 aptamer and FAM dye-labelled AFM1 aptamer, respectively. The fluorescence intensity of designed sensor exhibited ultrasensitive detections (∼ pM) and a wide linear range (≥ 5 orders). Hence, the present study can serve as a unique platform for facile, ultra-sensitive, selective, cost-effective and quick multiplexed detection of food toxins and disease biomarkers in complex-matrix.

Keywords: Aflatoxin; Aptamer; Complex-matrix; Mn0.02Ta3S6-nanosheet; Multiplexed biosensing; Polyethylene-glycol

31. **A fluorescent aptasensor based on functional graphene oxide and FRET strategy simultaneously detects aflatoxins B1 and aflatoxins M1,**Yuzheng CAI, Ge GUO, Yankun FU, Xianqing HUANG, Tianlin WANG, Tiange LI,Chinese Journal of Analytical Chemistry,Volume 52, Issue 6,2024,100408,https://doi.org/10.1016/j.cjac.2024.100408.

Abstract:

Simultaneous and rapid detection of various mycotoxins in food holds significant practical importance in the field of food processing and safety. In this study, a fluorescent aptasensor based on functionalized graphene oxide (FGO) is developed for simultaneous detection of aflatoxin B1 (AFB1) and aflatoxin M1

(AFM1). The two aptamers specific to AFB1 and AFM1 are labeled with Cy3 and Cy5 respectively. Both the aptamers can be adsorbed onto the surface of FGO through π-π stacking, resulting in fluorescence resonance energy transfer (FRET) between the fluorophore and FGO. The absence of target leads to quenching of fluorescence while presence of either aflatoxin causes interaction between corresponding aptamer and target, leading to release from FGO surface thereby turning on fluorescence signal. The limit of detection (LOD) for AFB1 is determined as 8.7 pg/mL whereas for AFM1 it is found to be 20.1 pg/mL, demonstrating fast and sensitive detection capability using this approach. Furthermore, the aptasensor exhibits good specificity and selectivity even under influence from other common interfering toxins. With its simplicity in operation and portability features, this sensor has potential applications for establishing sensitive and portable on-site detection methods for various mycotoxins.

Keywords: Functionalized graphene oxide; Fluorescence resonance energy transfer; Aptasensor; Aflatoxin; Simultaneous detection

32. **Colorimetric detection of aflatoxins B1 and M1 using aptamers and gold and silver nanoparticles,**Fiona Ebanks, Hadi Nasrallah, Timothy M. Garant, Erin M. McConnell, Maria C. DeRosa,Advanced Agrochem,Volume 2, Issue 3,2023,Pages 221-230,https://doi.org/10.1016/j.aac.2023.07.003.

Abstract:

Mycotoxins are small molecules produced by fungi that contaminate crops and cause notable health effects for humans, owing to their inherent toxicity. Aflatoxins are among the most potent mycotoxins, with aflatoxin B1 and M1 being the most concerning. Due to their negative impact on human health and agro-economics, developing cost-effective, rapid, highly sensitive and specific detection tools is urgently needed. Nucleic acid–based synthetic receptors, aptamers, have been successfully selected for aflatoxin with high binding affinity and selectivity, and have been incorporated into a wide array of sensor platforms. By exploiting the optical properties of metallic nanoparticles, aptasensors have been developed to achieve low-cost, rapid, and sensitive tests to detect contaminated foods. Herein, we describe the use of functional nucleic acids, specifically DNA aptamers with metallic nanoparticles such as gold and silver for detecting aflatoxin B1 and M1. This review highlights various aptamer-nanoparticle assay types designed for colorimetric aflatoxin detection (i.e., solution and paper-based) along with their associated detection limits, as well as their strengths and areas for further development.

Keywords: DNA Aptamer; Aflatoxin; Agricultural monitoring; Gold nanoparticle; Biosensor

33. **Aflatoxin B1 impairs porcine oocyte quality via disturbing intracellular membrane system and ATP production,** Lin-Lin Hu, Shun Chen, Meng-Ying Shen, Qiu-Yan Huang, Hong-Ge Li, Shao-Chen Sun, Jun-Li Wang, Xiao-Qiong Luo,Ecotoxicology and Environmental Safety,Volume 263,2023,115213,https://doi.org/10.1016/j.ecoenv.2023.115213.

Abstract:

Aflatoxin is the most common type of mycotoxins in contaminated corn, peanuts and rice, which affects the livestock and ultimately endangers human health. Aflatoxin is reported to have carcinogenicity, mutation, growth retardation, immunosuppression and reproductive toxicity. In present study we reported the causes for the declined porcine oocyte quality under aflatoxin exposure. We set up an in vitro exposure model and showed that aflatoxin B1 disturbed cumulus cell expansion and oocyte polar body extrusion. We found that aflatoxin B1 exposure disrupted ER distribution and elevated the expression of GRP78, indicating the occurrence of ER stress, and the increased calcium storage also confirmed this. Besides, the structure of cis-Golgi apparatus, another intracellular membrane system was also affected, showing with decreased GM130 expression. The oocytes under aflatoxin B1 exposure showed aberrant lysosome accumulation and higher LAMP2 expression, a marker for lysosome membrane protection, and this might be due to the aberrant mitochondria function with low ATP production and the increase of apoptosis, since we found that BAX expression increased, and ribosomal protein which is also an apoptosis-related factor RPS3 decreased. Taken together, our study revealed that aflatoxin B1 impairs intracellular membrane system ER, Golgi apparatus, lysosome and mitochondria function to affect porcine oocyte maturation quality.

Keywords: Oocyte; Aflatoxin; Organelle; Apoptosis

34. **Dietary garden cress (Lepidium sativum) seeds mitigate the effect of aflatoxin B1 contamination on growth, antioxidant status, AFB1 residues, immune response, and tissue architecture of Oreochromis niloticus,** Walaa El-Houseiny, Abd Elhakeem El-Murr, Noura A. Abd-Allah, Yasmina M. Abd-Elhakim, Abdel-Wahab A. Abdel-Warith, Elsayed M. Younis, Simon J. Davies, Mohamed M.M. Metwally, Mai E. Nasr, Adham A. Al-Sagheer, Bayan A. Hassan, Basma A. Elkhadrawey,Aquaculture Reports,Volume 36,2024,102040,https://doi.org/10.1016/j.aqrep.2024.102040.

Abstract:

The contamination of fish diets with aflatoxin B1 (AFB1) poses a significant hazard to the aquaculture sector. This study investigates the efficacy of supplementing Nile tilapia (Oreochromis niloticus) diets with garden cress (Lepidium sativum) seeds (GCS) to mitigate the adverse effects of AFB1. For this purpose, a factorial design was utilized, consisting of a 2×3 arrangement of treatments. This included two contamination levels of AFB1: (0 and 2 mg kg−1 diet). Within each contamination level, three dietary levels of GCS were used (0 %, 1 %, and 2 %). The study involved 270 healthy O. niloticus with an average weight of 25.14 ± 0.39 g (45 fish/group and 15 fish/replicate). The duration of the trial was 60 days. The

study evaluated growth performance, hematological indices, hepatorenal function, immune and antioxidant indicators, histomorphology of organs, AFB1 residues in muscles, and fish resistance to Aeromonas hydrophila. The results indicated that GCS dietary supplementation significantly counteracted AFB1-induced growth retardation and reduced fish survival. Additionally, GCS effectively reversed the anemia, leukopenia, hypoproteinemia, hypoalbuminemia, and hypoglobulinemia that were produced by AFB1. Moreover, GCS dietary supplementation significantly suppressed the AFB1-induced increase in serum bilirubin, hepatic enzymes, kidney damage products, cortisol, and glucose. Additionally, the inclusion of GCS in the fish's diet effectively eliminated the accumulation of AFB1 in their muscles. Moreover, GCS supplementation demonstrated significant restoration of depleted antioxidant and innate immune components induced by AFB1. Furthermore, GCS significantly reduced the degenerative changes in renal, hepatic, splenic, and small intestine tissues caused by AFB1. Fish fed a GCS-fortified diet and exposed to AFB1 displayed restored disease resistance when challenged with A. hydrophila. In conclusion, supplementing Nile tilapia diets with GCS at 2 %, even in the presence the AFB1 contamination, significantly enhanced the fish growth, improved health, and increased disease resistance while reducing AFB1 residues, thereby benefiting consumer health.

Keywords: Foodborne toxin; Nile tilapia; Herbal seeds; Pathology; Bacterial challenge

35. **Exonuclease III assisted electrochemical aptasensor simultaneous detection of aflatoxin B1 and ochratoxin a in grains,**Xiaoran Liu, Yunzhe Zhang, Yu Wang, Hui Xu, Xin Lu, Xiaoyan Ma, Wei Zhang,LWT,Volume 201,2024,116211,https://doi.org/10.1016/j.lwt.2024.116211.

Abstract:

The synergistic effect of multiple mycotoxins in cereals increases their toxicity. Therefore, the simultaneous detection of multiple mycotoxins in cereals is of great importance. Exonuclease III (Exo III) assisted electrochemical aptasensor has been used for mycotoxin detection, but simultaneous detection of two mycotoxins has not been previously reported. By utilizing Exo III assisted strand displacement, this study developed an electrochemical aptasensor strategy for the simultaneous detection of aflatoxin B1 (AFB1) and ochratoxin A (OTA). This method demonstrates excellent detection of AFB1 and OTA within the range of 0.001–500 ng mL−1, with detection limits as low as 0.229 pg mL−1 for AFB1 and 0.564 pg mL−1 for OTA. The electrochemical aptasensor was successfully applied to analyze grain samples, and the sensor has good specificity, stability and repeatability. The adapter provides simultaneous detection of AFB1 and OTA in food in a fast, simple and highly sensitive platform.

Keywords: Electrochemical aptasensor; Simultaneous determination; Exonuclease III; Aflatoxin B1; Ochratoxin a

36. **Analysis and evaluation of in vitro bioaccessibility of aflatoxins B1, B2, G1 and G2 in plant-based milks,**Iván Romero-Sánchez, Irene Alonso-Núñez, Emma Gracia-Lor, Yolanda Madrid-Albarrán,Food Chemistry,Volume 460, Part 1,2024,140538,https://doi.org/10.1016/j.foodchem.2024.140538.

Abstract:

Plant-based milks emerge as a healthy and vegan alternative for human diet, but these foodstuffs are susceptible to be contaminated by aflatoxins. A new method based on SPE and HPLC-MS/MS analysis was optimized and validated to test the presence of aflatoxins B1, B2, G1 and G2 analysis in almond, oat, rice and soy commercial milks. Moreover, aflatoxin bioaccessibility was evaluated through an in vitro digestion assay applied to each type of spiked milk. Aflatoxins B1, B2 and G1 were detected in one soy milk sample below the LOQ, fulfilling the limits stablished by the European Legislation. The final bioaccessibility percentages were highly dependent on the type of mycotoxin and sample matrix, the highest and the lowest values were obtained for AFB2 (82%–92%) and AFG1 (15%–30%), whereas AFB1 (28%–50%) and AFG2 (32%–76%) values resulted more influenced by the plant-based milk matrix.

Keywords: Aflatoxins; Plant-based milks; In vitro digestion; Bioaccessibility; HPLC-MS; MS

37. **Aflatoxin B1 decreased flesh flavor and inhibited muscle development in grass carp (Ctenopharyngodon idella),**Xiang-Ning He, Zhen-Zhen Zeng, Wei-Dan Jiang, Pei Wu, Yang Liu, Sheng-Yao Kuang, Ling Tang, Shu-Wei Li, Lin Feng, Xiao-Qiu Zhou,Animal Nutrition,Volume 18,2024,Pages 27-38,https://doi.org/10.1016/j.aninu.2024.03.012.

Abstract:

In nature, aflatoxins, especially aflatoxin B1 (AFB1), are the common mycotoxins, which cause serious health problems for humans and animals. This paper aimed to study the effects of AFB1 on flesh flavor and muscle development of grass carp (Ctenopharyngodon idella) and its mechanism. There were 1440 individual fish in total, with 6 treatments and each treatment replicated 3 times. The 6 treatments were fed a control diet with different doses of AFB1 (0.04, 29.48, 58.66, 85.94, 110.43 and 146.92 μg/kg diet) for 60 d. AFB1 increased myofiber diameter, as well as decreased myofiber density of grass carp muscle ($P < 0.05$). The contents of free amino acid decreased gradually ($P < 0.05$) as dietary AFB1 increased in the muscle of grass carp. The levels of reactive oxygen species, malonaldehyde and protein carbonyl (PC) were increased ($P < 0.05$) with the dietary AFB1 increased. The levels of antioxidant enzyme (glutathione peroxidase, glutathione, glutathione reductase, total antioxidant capacity, anti-superoxide anion, and anti-hydroxyl radical) were decreased ($P < 0.05$) with the dietary AFB1 increased. In addition, dietary AFB1 decreased the content of collagen, and downregulated the mRNA and protein levels of transforming growth factor-β (TGF-β)/Smads signaling pathway in grass carp muscle ($P < 0.05$). The mRNA and protein levels of myogenic regulatory factors were downregulated in grass carp muscle ($P < 0.05$). Furthermore, the activities of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) were increased ($P < 0.05$), and the protein levels of phosphorylate-38 mitogen-activated protein kinase (p-

p38MAPK), phosphorylate-c-Jun N-terminal kinase, urokinase-type plasminogen activator (uPA), MMP-2 and MMP-9 were upregulated ($P < 0.05$), but collagen Ⅰ, laminin β1 and fibronectin were downregulated ($P < 0.05$) with the dietary AFB1 increased in the muscle of grass carp. Based on the results of this study, we can draw the following conclusion: dietary AFB1 might damage flesh flavor and inhibit the muscle development through MAPK/uPA/MMP/extracellular matrix (ECM) signaling pathway in grass carp. Moreover, the recommended safe limit of AFB1 in feed is no more than 26.77 μg/kg diet according to the PC levels in grass carp muscle.

Keywords: Aflatoxin B1; Flesh flavor; Muscle development; Grass carp

38. **Integrated network toxicology, molecular docking, and in vivo experiments to elucidate molecular mechanism of aflatoxin B1 hepatotoxicity,** Bingjie Ge, Kexin Yan, Rui Sang, Wei Wang, Xinman Liu, Minghong Yu, Xiaotong Liu, Qian Qiu, Xuemei Zhang,Ecotoxicology and Environmental Safety,Volume 275,2024,116278,https://doi.org/10.1016/j.ecoenv.2024.116278.

Abstract:

Due to the rise in temperature and sea level caused by climate change, the detection rate of aflatoxin B1 (AFB1) in food crops has increased dramatically, and the frequency and severity of aflatoxicosis in humans and animals are also increasing. AFB1 has strong hepatotoxicity, causing severe liver damage and even cancer. However, the mechanism of AFB1 hepatotoxicity remains unclear. By integrating network toxicology, molecular docking and in vivo experiments, this research was designed to explore the potential hepatotoxicity mechanisms of AFB1. Thirty-three intersection targets for AFB1-induced liver damage were identified using online databases. PI3K/AKT1, MAPK, FOXO1 signaling pathways, and apoptosis were significantly enriched. In addition, the proteins of ALB, AKT1, PIK3CG, MAPK8, HSP90AA1, PPARA, MAPK1, EGFR, FOXO1, and IGF1 exhibited good affinity with AFB1. In vivo experiments, significant pathological changes occurred in the liver of mice. AFB1 induction increased the expression levels of EGFR, ERK, and FOXO1, and decreased the expression levsls of PI3K and AKT1. Moreover, AFB1 treatment caused an increase in Caspase3 expression, and a decrease in Bcl2/Bax ratio. By combining network toxicology with in vivo experiments, this study confirms for the first time that AFB1 promotes the FOXO1 signaling pathway by inactivating PI3K/AKT1 and activating EGFR/ERK signaling pathways, hence aggravating hepatocyte apoptosis. This research provides new strategies for studying the toxicity of environmental pollutants and new possible targets for the development of hepatoprotective drugs.

Keywords: Aflatoxin B1; Network toxicology; Liver damage; Mice; Signaling pathway

39. **Effect of zearalenone on aflatoxin B1-induced intestinal and ovarian toxicity in pregnant and lactating rats,**Kuntan Wu, Minjie Liu, Huanbin Wang, Shahid Ali Rajput, Omar Mahmoud Al Zoubi, Shuai Wang, Desheng Qi,Ecotoxicology and Environmental Safety,Volume 258,2023,114976,https://doi.org/10.1016/j.ecoenv.2023.114976.

Abstract:

Aflatoxin B1 (AFB1) and zearalenone (ZEN) cause serious damage to mammals, but few studies have investigated the impacts of these toxins on pregnant and lactating mammals. This study investigated the effects of ZEN on AFB1-induced intestinal and ovarian toxicity in pregnant and lactating rats. Based on the results, AFB1 reduces the digestion, absorption, and antioxidant capacity in the intestine, increases intestinal mucosal permeability, destroys intestinal mechanical barriers, and increases pathogenic bacteria' relative abundances. Simultaneously, ZEN can exacerbate the intestinal injury caused by AFB1. The intestines of the offspring were also damaged, but the damage was less severe than that observed for the dams. While AFB1 activates various signalling pathways in the ovary and affects genes related to endoplasmic reticulum stress, apoptosis, and inflammation, ZEN may exacerbate or antagonize the AFB1 toxicity on gene expression in the ovary through key node genes and abnormally expressed genes. Our study found that mycotoxins can not only directly damage the ovaries and affect gene expression in the ovaries but can also impact ovarian health by disrupting intestinal microbes. Mycotoxins are an important environmental pathogenic factor for intestinal and ovarian disease in pregnancy and lactation mammals.

Keywords: Aflatoxin B1; Zearalenone; Rat; Intestinal; Ovarian

40. **In silico approximation to aflatoxin B1 metabolism and sensitivity in commercial poultry species based on empirical mathematical equations,**Hansen W. Murcia, Gonzalo Diaz, Rubén Darío Acosta,Toxicology Reports,2024,101752,https://doi.org/10.1016/j.toxrep.2024.101752.

Abstract:

Enzyme kinetic parameters for aflatoxin B1 metabolism have been reported for chicken, quail, turkey and duck, but an integrated in silico model has not been proposed. Both enzyme-catalyzed reactions and spontaneous reactions were modeled in the CellDesigner software and results were adjusted to Hill, Rational and Hoerl models. Results revealed that the higher amount of aflatoxin B1 epoxide produced in a short lapse of time and a low production of epoxide conjugated to glutathione explains the severe genotoxic effect of aflatoxin B1 in duck. Also, the higher amount of aflatoxicol produced is time-associated to aflatoxin B1 resistance in chicken. Finally, the cytotoxic effects in quail and duck are caused by a large aflatoxin B1 dialdehyde production in a short period of time.

Keywords: Xenobiotic metabolism simulation; in silico simulation; aflatoxin B1 metabolism; cytotoxic pathway; genotoxic pathway

41. **Aflatoxin B1 inhibited the development of primary myoblasts of grass carp (Ctenopharyngodon idella) by degrading extracellular matrix,**Xiang-Ning He, Wei-Dan Jiang, Pei Wu, Yang Liu, Hong-Mei Ren, Xiao-Wan Jin, Sheng-Yao Kuang, Ling Tang, Shu-Wei Li, Lin Feng, Xiao-Qiu Zhou,Ecotoxicology and Environmental Safety,Volume 276,2024,116332,https://doi.org/10.1016/j.ecoenv.2024.116332.

Abstract:

According to the International Agency for Research on Cancer (IARC), aflatoxin B1 (AFB1) has been recognized as a major contaminant in food and animal feed and which is a common mycotoxin with high toxicity. Previous research has found that AFB1 inhibited zebrafish muscle development. However, the potential mechanism of AFB1 on fish muscle development is unknown, so it is necessary to conduct further investigation. In the present research, the primary myoblast of grass carp was used as a model, we treated myoblasts with AFB1 for 24 h. Our results found that 5 μM AFB1 significantly inhibited cell proliferation and migration ($P < 0.05$), and 10 μM AFB1 promoted lactate dehydrogenase (LDH) release ($P < 0.05$). Reactive oxygen species (ROS), protein carbonyl (PC) and malondialdehyde (MDA) levels were increased in 15, 5 and 10 μM AFB1 ($P < 0.05$), respectively. Catalase (CAT), glutathione peroxidase (GPx) and total superoxide dismutase (T-SOD) activities were decreased in 10, 10 and 15 μM AFB1 ($P < 0.05$), respectively. Furthermore, 15 μM AFB1 induced oxidative damage by Nrf2 pathway, also induced apoptosis in primary myoblast of grass carp. Meanwhile, 15 μM AFB1 decreased MyoD gene and protein expression ($P < 0.05$). Importantly, 15 μM AFB1 decreased the protein expression of collagen Ⅰ and fibronectin ($P < 0.05$), and increased the protein levels of urokinase plasminogen activator (uPA), matrix metalloproteinase 9 (MMP-9), matrix metalloproteinase 2 (MMP-2), and p38 mitogen-activated protein kinase (p38MAPK) ($P < 0.05$). As a result, our findings suggested that AFB1 damaged the cell morphology, induced oxidative damage and apoptosis, degraded ECM components, in turn inhibiting myoblast development by activating the p38MAPK/urokinase-type plasminogen activator (uPA)/matrix metalloproteinase (MMPs)/extracellular matrix (ECM) signaling pathway.

Keywords: Aflatoxin B1; Primary myoblasts; Extracellular matrix; Grass carp

42. **Comprehensive and sensitive validation of the method and determination of measurement uncertainty for simultaneous specification of aflatoxin B1, B2, G1 and G2 in nuts,**Hacer Sibel Karapinar, Numan Eczacioglu, Faruk Dogan,Measurement: Food,Volume 13,2024,100124,https://doi.org/10.1016/j.meafoo.2023.100124.

Abstract:

In this study, a comprehensive and sensitive validation study of the legally used method was conducted to simultaneously determine the levels of aflatoxin B1, B2, G1, and G2 in nuts. The applied method has been extensively evaluated in terms of in-laboratory detection limit and quantification limit, linearity, selectivity, precision, recovery, accuracy, adequacy, and measurement uncertainty. Levels of aflatoxins were examined by a validated immunoaffinity column-high performance liquid chromatography-post

column derivatization-fluorescence detection (IAC-HPLC-PCA-FLD) analytical method. Detection limits (LOD) for aflatoxin G2, G1, B2, and B1 were found to be 0.05 µg/kg, 0.13 µg/kg, 0.06 µg/kg, and 0.15 µg/kg, respectively. The regression coefficient (R2) was greater than 0.999 according to constructed calibration curves for all aflatoxin components. Thus, the curves exhibited satisfactory linearity within the analyzed range. The combined standard uncertainty values for precision (RSD%) studies were below 10%. It showed a robust and reliable sensitivity with <10% RSD with the test procedure. Average recovery values were detected as 67-84% for hazelnut and 60-89% pistachio. All aflatoxin components in hazelnut paste, which is the proficiency test material, were determined within an appropriate |Z|-score range (≤ 2). The relative expanded uncertainty of measurement was 0.80 µg/kg for AFB1 and AFG1, 0.83 µg/kg for AFB2 and AFG2, and 0.78 µg/kg for total AFs. The results showed that the approved method for the investigation and monitoring of aflatoxins in hazelnuts and pistachios is highly sensitive, robust, reliable, selective, and reproducible. Validation parameters can be successfully used in routine analysis to monitor aflatoxins in nuts for compliance with current regulations.

Keywords: Aflatoxin; Nuts; Validation; Uncertain measurement; Proficiency test performance; High pressure liquid chromatography

43. **Establishment of an amplification strategy - specific binding - convenient processing integrated aflatoxin B1 detection method based on Fe3O4-NH4/AuNPs/apt-S1,** Hua Zheng, Linlin Feng, Zheng Huang, Ziwei Zou, Xiaolong Ma, Ziping Pan, Jinfeng Li, Jinxia Wu, Mei Li, Zhiheng Su,Food Chemistry: X,Volume 23,2024,101605,https://doi.org/10.1016/j.fochx.2024.101605.

Abstract:

Aflatoxin B1 (AFB1) is a potent toxin in food, necessitating rapid, instant, and sensitive detection. We have engineered an electrochemical sensor to monitor AFB1 using a system composed of Fe3O4-NH4/AuNPs/apt-S1. The aptamer specifically recognizes AFB1, while 'S1' is functionalized with methylene blue to enhance the current. The RecJf exonuclease promotes the formation of the electrochemical strategy. The Fe3O4 component, with its magnet properties, enables a rapid separation of solids and liquids without the need for instrumentation. The sensor exhibits a linear range for AFB1 ranging from 1 ng to 10 µg. The regression equation is I(nA) = 446.8 × logc+2085 (where I and c represent the peak current and AFB1 concentration, respectively). The correlation coefficient is 0.9508, and the detection limit is 3.447 nM. The relative standard deviation of AFB1 in peanut oil ranges from 4.80% to 6.80%. These results demonstrate that the sensor has high sensitivity, stability, repeatability, and specificity for AFB1 detection.

Keywords: Aflatoxin B1; Electrochemistry; Amplification strategy; Peanut oil

44. **Licochalcone A mitigates aflatoxin B1-induced immunotoxicity via ferroptosis in bursa of broilers and macrophages,**Shijie Xia, Yuxi He, Songya Yang, Lihan Zhang, Xiaoqing Yu, Li Zhen, Chunren Wang, Hongming Lv,Poultry Science,Volume 103, Issue 10,2024,104080,https://doi.org/10.1016/j.psj.2024.104080.

Abstract:

Aflatoxin B1 (AFB1) is a mycotoxin which is responsible for severe damage to the immune system of humans and livestock. Licochalcone A (Lico A), a polyphenol derived from turmeric, has attracted great attention due to its wonderful antioxidant properties. Ferroptosis, an iron-dependent cell death related to oxidative stress, which plays a crucial role in the resistance of phytochemical to immune-associated injury. Nevertheless, effects of Lico A on the bursa of broilers exposed to AFB1 remain unclear. In this work, broilers were fed diets supplemented with 2 mg/kg of AFB1 and 50 mg/kg of Lico A. Meanwhile, various concentrations of Lico A and AFB1 (15 μM) were used to stimulate macrophages. These results revealed that AFB1 resulted in more severe bursa atrophy and relative weight reduction; the expression of pro-ferroptosis protein ACSL4 and the content of malondialdehyde (MDA) were significantly elevated, while the expression of anti-ferroptosis proteins GPX4, xCT, FSP1 and the content of Glutathione (GSH) was obviously reduced. However, Lico A treatment effectively reversed these effects in the bursa of broilers. Meanwhile, in bursa and macrophages, Lico A mitigated the expression of AFB1-induced apoptosis-associated protein (Caspase-3, Bax, Bcl-2) as well as antioxidant protein (Nrf2, GCLM, HO-1). Importantly, ferroptosis was also observed in macrophages induced by AFB1. Lico A efficaciously alleviated AFB1-induced mitochondrial membrane potential decrease and reactive oxygen species (ROS) production in macrophages; in contrast, Lico A evidently inhibited AFB1-triggered ROS generation and cytotoxicity, which was disabled by the addition of Erastin. Moreover, Liproxstatin-1 significantly inhibited ROS generation induced by AFB1. In summary, the present study elucidates that the main mechanism by which Lico A attenuates AFB1-induced immunotoxicity is through the suppression of ferroptosis, apoptosis, mitochondrial damage and oxidative stress, which is promising for the improvement of immunotoxic effects of AFB1.

Keywords: aflatoxin B1; licochalcone A; immunotoxicity; ferroptosis; oxidative stress

45. **Smartphone-based magneto-immunosensor on carbon black modified screen-printed electrodes for point-of-need detection of aflatoxin B1 in cereals,**Safiye Jafari, Loïc Burr, Davide Migliorelli, Roger Galve, M.-Pilar Marco, Katrina Campbell, Chris Elliott, Michele Suman, Shana J. Sturla, Silvia Generelli,Analytica Chimica Acta,Volume 1221,2022,340118,https://doi.org/10.1016/j.aca.2022.340118.

Abstract:

Considering the complexities and speed of modern food chains, there is an increasing demand for point-of-need detection of food contaminants, particularly highly regulated chemicals and carcinogens such as aflatoxin B1. We report a user-friendly smartphone-based magneto-immunosensor on carbon black

modified electrodes for point-of-need detection of aflatoxin B1 in cereals. For buffered analyte solutions and a corn extract sample, the assay demonstrated a low limit of detection of 13 and 24 pg/mL, respectively. The assay was also highly reproducible, exhibiting mean relative standard deviations of 3.7% and 4.0% for the buffered analyte and corn extract samples. The applicability of the assay was validated on the basis of EU guidelines and the detection capability was lower than or equal to 2 μg/kg, which is the EU maximum residue limit for aflatoxin B1 in cereals. False-positive and false-negative rates were less than 5%. Additionally, an open-source android application, AflaESense, was designed to provide a simple interface that displays the result in a traffic-light-type format, thus minimizing user training and time for data analysis. AflaESense was used for smartphone-based screening of spiked corn samples containing aflatoxin B1 (0.1, 2, and 10 ng/mL), and naturally contaminated corn containing 0.15 ng aflatoxin B1/mL. The measured values were in close agreement with spiked concentrations ($r2 = 0.99$), with recovery values ranging between 80 and 120%. Finally, contaminated samples correctly triggered a red alert while the non-contaminated samples led to the display of a green color of AflaESense. To the best of our knowledge, this is the first smartphone-based electrochemical system effective for screening samples for contamination with aflatoxin B1.

Keywords: Smartphone-based immunosensor; Carbon black; Electrochemical biosensor; Point-of-need detection; Aflatoxin B1; Validation

46. **Curcumin alleviates Aflatoxin B1-triggered chicken liver necroptosis by targeting the LOC769044/miR-1679/STAT1 axis,**Sihong Li, Yixin Zhang, Muhammad Ishfaq, Ruimeng Liu, Gaoqiang Wei, Xiuying Zhang,Poultry Science,Volume 103, Issue 8,2024,103883,https://doi.org/10.1016/j.psj.2024.103883.

Abstract:

Aflatoxin B1 (AFB1) is an unavoidable environmental toxin. The accumulation of AFB1 and its metabolites in the liver poses a threat to both human and animal health. Curcumin exhibits anti-oxidative, anti-tumor, and anti-inflammatory properties. There is no report on the mechanism regarding how curcumin relived liver necroptosis in chickens induced by AFB1 based on the regulatory network of ceRNA. To explore this, we performed transmission electron microscopy and sequenced lncRNA and mRNA in chicken livers treated with AFB1 and/or curcumin for 28 d in vivo. We observed substantial alterations in the lncRNA and mRNA expression profiles within the chicken liver, indicating that curcumin can mitigate AFB1-induced necroptosis both in vivo and in vitro. Further analysis, including the establishment of an lncRNA-miRNA-mRNA network and the utilization of a dual luciferase reporter assay, revealed that LOC769044 acts as a competing endogenous RNA (ceRNA) for miR-1679. In addition, STAT1 was identified as a direct target of miR-1679. Modulating miR-1679 levels through overexpression, and silencing LOC769044 and STAT1, effectively reversed the necroptotic effects induced by AFB1, a reversal that was also observed with curcumin supplementation. In conclusion, our data demonstrate that curcumin alleviates AFB1-induced liver necroptosis through the LOC769044/miR-1679/STAT1 signaling axis. This study suggests that LOC769044 may serve as a novel therapeutic target for managing AFB1-mediated liver toxicity.

Keywords: Aflatoxin B1; curcumin; chicken liver; ceRNA; necroptosis

47. **Effects of supplemented multicomponent mycotoxin detoxifying agent in laying hens fed aflatoxin B1 and T2-toxin contaminated feeds,**Jog Raj, Hunor Farkaš, Zdenka Jakovčević, Marko Vasiljević, Rakesh Kumar, Rajesh Kumar Asrani,Poultry Science,Volume 102, Issue 8,2023,102795,https://doi.org/10.1016/j.psj.2023.102795.

Abstract:

The present study was conducted to determine the ability of multicomponent mycotoxin detoxifying agent (MMDA) in feed to prevent the gastrointestinal absorption of aflatoxin B1 (AFB1) and T2-toxin supplemented via spiked maize. For comparisons, hens were fed with uncontaminated basal diet without or with addition of MMDA at 2 g/kg feed. The trial consisted of 105 laying hens (Lohmann Brown) without obvious signs of disease allocated to 7 treatment groups in 35 pens. Responses were demonstrated on laying performance and health status throughout the 42 d experimental period. The results of laying performance indicated significantly decreased egg mass with increasing mycotoxin (AFB1 and T2-toxin) levels up to the maximum tolerated dosage, however simultaneous presence of MMDA laying performance was slightly modified linearly to increasing application. Dose-dependent pathological changes in liver and kidneys and their relative weights, changes in blood parameters and reduced eggshell weights were observed in the hens fed AFB1 and T2-toxin. The pathological changes in the hens fed with diets containing AFB1 and T2-toxin without MMDA were significantly higher as compared with the control group, but eggshell stability was not affected. The contents of AFB1, T2-toxin and their metabolites in liver and kidney tissues were significantly decreased in the hens supplemented with MMDA at 2 and 3 g/kg in feed. MMDA supplementation significantly reduced the deposition of AFB1, T2-toxin and their metabolites in liver and kidneys at the maximum tolerated dosage (2 and 3 g/kg) indicating specific binding to AFB1 and T2-toxin in the digestive tract as compared to the corresponding diets without MMDA. Exposure of AFB1 and T2-toxin indicated significantly decreased egg mass with increasing mycotoxin levels up to the maximum tolerated dosage because of the significantly reduced egg production. Therefore, in this study, MMDA could reduce negative effects of feeding AFB1 and T-2 to laying hens.

Keywords: mycotoxin; laying hen; aflatoxin B1; MMDA; T2-toxin

48. **Determination of uncertainty in the measurement of Aflatoxins B1 in pistachio nuts by the HPLC-FLD method,**Rodríguez Ana María, Gutiérrez Aida, Giménez Myriam, Martínez Nora, Mamaní Arminda, Journal of Chromatography Open,Volume 2,2022,100044,https://doi.org/10.1016/j.jcoa.2022.100044.

Abstract:

The present study defined uncertainties during Aflatoxin B1 measurements in pistachio nuts using HPLC equipped with fluorescence detector and derivatizing cell (Kobra® Cell) to increase the detector signal.

The uncertainty of a method is one of the parameters required by EURACHEM/CITAC Guide, and forms part of the validation of analytical methods. Six variables affected the final value of the measured concentration, which were included in the mathematical model. The contribution index of each variable to the combined uncertainty (uc) was also calculated and analysis of the Pareto diagram showed that 80.47% of the variability was the result of the calculation and execution of the calibration curve of the Aflatoxin B1 standard, 19% was determined by the recovery rate of the pistachio sample and volumetric measurements affected the concentration to a very lesser extent. The value of expanded uncertainty in the determination of Aflatoxin B1 for pistachios was Ucx = 0.83 µg/kg.

Keywords: Uncertainty; Aflatoxin B1; Pistachio nuts; HPLC

49. **Omics analysis revealed the intestinal toxicity induced by aflatoxin B1 and aflatoxin M1**,Ya-Nan Gao, Zi-Wei Wang, Chuan-You Su, Jia-Qi Wang, Nan Zheng,Ecotoxicology and Environmental Safety,Volume 278,2024,116336,https://doi.org/10.1016/j.ecoenv.2024.116336.

Abstract:

Aflatoxin B1 (AFB1), a common mycotoxin, can occur in agricultural products. As a metabolite of AFB1, aflatoxin M1 (AFM1) mainly exist in dairy products. These two mycotoxins threaten human health, although it is unclear how they affect the function of the intestinal barrier. In this study, mice were exposed to AFB1 (0.3 mg/kg body b.w.) and AFM1(3.0 mg/kg b.w.) either individually or in combination for 28 days to explore the main differentially expressed proteins (DEPs) and the associated enriched pathways. These findings were preliminarily verified by the transcriptomic and proteomic analyses in differentiated Caco-2 cells. The results revealed that AFB1 and AFM1 exposure in mice disrupted the function of the intestinal barrier, and the combined toxicity was greater than that of each toxin alone. Further proteomic analysis in mice demonstrated that the mechanisms underlying these differences could be explained as follows: (i) lipid metabolism was enriched by AFB1-induced DEPs. (ii) protein export pathway was stimulated by AFM1-induced DEPs. (iii) cell metabolic ability was inhibited (as evidenced by changes in UDP-GT1, UDP-GT2, and Gatm6), apoptosis was induced (MAP4K3), and epithelial cell integrity was disrupted (Claudin7 and IQGAP2), resulting in more extensive intestinal damage after combined treatment. In conclusion, the hazardous impact of co-exposure to AFB1 and AFM1 from proteomic perspectives was demonstrated in the present study.

Keywords: Aflatoxin B1; Aflatoxin M1; Intestinal toxicity; Proteomics; Co-exposure

50. **The role of larvae of black soldier fly and house fly and of feed substrate microbes in biotransformation of aflatoxin B1**,K. Niermans, E.F. Hoek- van den Hil, H.J. van der Fels-Klerx, J.J.A. van Loon,Ecotoxicology and Environmental Safety,Volume 279,2024,116449,https://doi.org/10.1016/j.ecoenv.2024.116449.

Abstract:

Over the past few years, there has been growing interest in the ability of insect larvae to convert various organic side-streams containing mycotoxins into insect biomass that can be used as animal feed. Various studies have examined the effects of exposure to aflatoxin B1 (AFB1) on a variety of insect species, including the larvae of the black soldier fly (BSFL; Hermetia illucens L.; Diptera: Stratiomyidae) and the housefly (HFL; Musca domestica L.; Diptera: Muscidae). Most of these studies demonstrated that AFB1 degradation takes place, either enzymatic and/or non-enzymatic. The possible role of feed substrate microorganisms (MOs) in this process has thus far not been investigated. The main objective of this study was therefore to investigate whether biotransformation of AFB1 occurred and whether it is caused by insect-enzymes and/or by microbial enzymes of MOs in the feed substrate. In order to investigate this, sterile and non-sterile feed substrates were spiked with AFB1 and incubated either with or without insect larvae (BSFL or HFL). The AFB1 concentration was determined via LC-MS/MS analyses and recorded over time. Approximately 50% of the initially present AFB1 was recovered in the treatment involving BSFL, which was comparable to the treatment without BSFL (60%). Similar patterns were observed for HFL. The molar mass balance of AFB1 for the sterile feed substrates with BSFL and HFL was 73% and 78%, respectively. We could not establish whether non-enzymatic degradation of AFB1 in the feed substrates occurred. The results showed that both BSFL and substrate-specific MOs play a role in the biotransformation of AFB1 as well as in conversion of AFB1 into aflatoxin P1 and aflatoxicol, respectively. In contrast, HFL did not seem to contribute to AFB1 degradation. The obtained results contribute to our understanding of aflatoxin metabolism by different insect species. This information is crucial for assessing the safety of feeding fly larvae with feed substrates contaminated with AFB1 with the purpose of subsequent use as animal feed.

Keywords: Aflatoxin B1; Hermetia illucens; Musca domestica; Degradation