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Toxic effects and residue of aflatoxin B1 in tilapia (*Oreochromis niloticus* × *O. aureus*) during long-term dietary exposure

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ABSTRACT

As a fish farmed widely in the tropical and subtropical regions where aflatoxin contamination has been generally detected, tilapia has been studied for aflatoxicosis evaluation in Asia. However, relatively short-term aflatoxin B1 (AFB1) exposure in previous studies resulted in some contradictory conclusions. Therefore, this work was designed to investigate the toxic effects and residue of AFB1 in tilapia during a long-term trial of 20 weeks, during which the tilapia obtained more than 1900% weight gain and grew to a commercial size (around 500 g). Tilapia were fed six diets containing different levels of AFB1 (19, 85, 245, 638, 793 and 1641 μg/kg), which were prepared with AFB1-contaminated peanut meal. AFB1-related physiological and toxicological properties in fish were determined during the 20-week period. The results indicated that dietary AFB1 led to aflatoxicosis effects in tilapia in a dose- and duration-dependent manner. No toxic effects of AFB1 were found during the first 10 weeks, but by 20 weeks, the diet with 245 μg AFB1/kg or higher doses reduced the growth and induced hepatic disorder, resulting in decreased lipid content, hepatosomatic index, cytochrome P450 A1 activity, elevated plasma alanine aminotransferase activity and abnormal hepatic morphology, but such dietary AFB1 doses did not affect the survival rate of experimental fish. The AFB1 residue was only detected in liver, in a dose-dependent manner, but not in edible flesh. Taken together, under good culture conditions, tilapia is a rather tolerant species for dietary AFB1 exposure up to 1641 μg/kg diet during 20 weeks. Long-term exposure for more than 15 weeks is necessary to evaluate aflatoxicosis in tilapia. Consuming only tilapia flesh would not increase the risk of exposure to AFB1 for human consumers.

1. Introduction

Aflatoxins are a group of well-known toxic metabolites produced primarily by certain strains of fungi *Aspergillus flavus* and *A. parasiticus* under favorable temperature and humidity. The contamination of aflatoxins in food crops is common in subtropical and tropical areas, especially for the crops containing high starch and lipid content, such as peanut, cottonseed, maize, wheat, sunflower and soybean (Ostrowski-Messner et al., 1995). Chen and Rawlings (2008) reported that aflatoxins could be detected in 96.1% of the 334 tested commercial feeds and raw materials collected from Asia.

In the family of aflatoxins, aflatoxin B1 (AFB1) is the most prevalent and toxic for human, land animals and aquatic organisms, mostly by its strong carcinogenic, mutagenic and teratogenic effects (IARC, 1993; Santacroce et al., 2008; Han et al., 2008). In addition, AFB1 and its metabolites can accumulate in animal tissues after AFB1 exposure, and then be taken up by human customs through the food chain. Bintvihok et al. (2002) reported that quails fed a diet with 3 mg AFB1/kg feed for 8 days had mean concentrations of 7.83 μg AFB1/kg in liver and 0.38 μg AFB1/kg in muscle. Other studies (Wu, 1998; Boonyaratpalin et al., 2001; El-Sayed and Khalil, 2009) also revealed that AFB1 residues can be retained in aquatic animal tissues, and impair the health of humans who consume these tissues. Therefore, aflatoxin contamination has been a potential threat to the health of humans and animals (Agag, 2004; Messonnier et al., 2007), which is extensively aggravated by the currently increasing use of plant ingredients in feeds of animals.

As a fish farmed widely in the tropical and subtropical regions where aflatoxin contamination has been generally detected, tilapia is frequently studied to investigate the toxic effects of AFB1 on its physiological properties. However, the mechanism of the toxic effects of AFB1 is still poorly understood, and some contradictory results exist in different studies. El-Banna et al. (1992) revealed that a diet with 100 μg AFB1/kg of feed significantly reduced the growth of

Abbreviations: AFB1, aflatoxin B1; AFBO, AFB1-8,9-Epoxide; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, asparitic aminotransferase; CF, condition factor; CYP1A, cytochrome P450 1A; EROD, ethoxyresorufin-o-deethylase; FE, feed efficiency; GSH, glutathione; HSI, hepatosomatic index; ROS, reactive oxygen species; VI, viscera index; WG, weight gain.

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Nile tilapia for 10 weeks, and a 200 μg/kg dose led to 16.7% mortality. Cagauan et al. (2004) showed in a 90-day trial, that the survival rate of tilapia exposed to 5–38.62 μg AFB1/kg feed was 67% less than that of the control group, and yellowing of the tilapia surface was observed in the groups given more than 29 μg AFB1/kg feed. However, Tuan et al. (2002) demonstrated that there was no adverse effect when tilapia was fed a diet containing 250 μg AFB1/kg. They also considered that only a high dose as 2.5 mg AFB1/kg feed would affect the hematocrit and growth performance of tilapia. Moreover, Chávez-Sánchez et al. (1994) suggested that tilapia can sustain dietary AFB1 as high as 30 mg/kg of feed without mortality.

In fact, the toxic effect of AFB1 is not only dependent on the AFB1 content in the feed, but also depends on the duration of exposure, as well as animal species, sex and age. However, the majority of previous studies of aflatoxicosis in aquatic animals are relatively short (less than 12 weeks), and only growth performance but not AFB1 residue was monitored. Therefore, we designed a 20-week exposure trial with tilapia (from juvenile to adult) to determine the long-term toxic effects of AFB1-contaminated diets on physiological properties. In this study, not only growth but also the residue of AFB1 in tissues was measured to evaluate the potential threat of consuming AFB1-feeding fish to human health.

2. Materials and methods

2.1. Preparation of AFB1-contaminated peanut meal

The method of preparation of AFB1-contamintated peanut meal was modified from previous studies (Lim et al., 2001; Casado et al., 2001; Bintvihok and Kositcharoenkul, 2006). *A. flavus* (NRRL 3357) was first cultured on potato dextrose agar and incubated for 7 days. The spores of *A. flavus* were suspended in distilled water, and collected to mix in the sterilized fresh peanut meal with 20% moisture. The mixture was kept in pallets for 7 days in a sterile environment at 27 °C with manual stirring regularly to allow AFB1 production. Afterwards, the AFB1-contaminated peanut meal (moldy peanut meal) was dried at 60 °C and kept in –80 °C. The AFB1 contents of all materials used to formulate diets were shown in Table 1. AFB1 concentration of materials was determined using ELISA test kits (Detection limit 0.1 ppb, Brins-live pro Biotechnology Co., China).

2.2. Experimental diets and fish

Six experimental diets, in which fresh peanut meal was replaced by 0, 1, 5, 10, 15, or 25% moldy peanut meal respectively, were formulated for the 20-week experiment. The feeds were pelleted at 5-week intervals, and the diameter of pellets was regulated as follows, 1.5, 2, 3 and 3.5 mm, to fit the growth of experimental fish. The resultant pellets were air dried, and stored at −80 °C. The formulation and mycotoxin content of the diets are given in Table 2. All mycotoxins in the control diet were lower than the regulatory limits of U.S. FDA (Henry, 2006).

About 2000 juvenile tilapia (*Oreochromis niloticus*×*O. aureus*) were obtained from a local hatchery. Prior to the present study, the fish were acclimated to a commercial diet for 3 weeks in 18 aquariums (3 m×2 m×1 m) with flowing water. Afterwards, fish were sorted by weight (initial body weight of 20 g) and divided into 6 groups in triplicate, with 60 fish per aquarium. Continuous aeration was provided by an air blower and natural light cycle was maintained during the trial. Fish were fed to apparent satiation twice per day (09:00 and 16:00) for 20 weeks. After a 24 h-fast, fish were group weighed and counted at 5-week intervals, and weight gain (WG) and feed consumption were determined at these intervals. During the whole trial, the temperature, oxygen, ammonia and pH were 23.93 ± 3.5 °C, 8.17 ± 0.7 mg/L, 0.54 ± 0.1 mg/L, 7.43 ± 0.4, respectively.

Table 1
AFB1 content in dietary ingredients (by dry weight).

Materials	AFB1 (μg/kg)
Rapeseed meal	3.4
Soybean meal	13.3
Fish meal	1.6
Peanut meal	62.6
Moldy peanut meal	6264.0

Table 2
Formulation and analysis of experimental diets.

	Diets					
	1	2	3	4	5	6
Formulation (g/100 g diet)						
Peanut meal	25	24	20	15	10	0
Moldy peanut meal	0	1	5	10	15	25
Vitamin mix[a]	0.2	0.2	0.2	0.2	0.2	0.2
Mineral mix[b]	0.6	0.6	0.6	0.6	0.6	0.6
Others[c]	74.2	74.2	74.2	74.2	74.2	74.2
Proximate composition (%)						
Crude protein	33.0	32.9	33.1	33.2	33.4	33.5
Crude lipid	7.2	6.3	7.0	6.5	6.8	6.7
Ash	5.2	5.5	5.5	5.1	5.4	5.4
Moisture	7.5	7.7	8.0	9.0	9.1	9.1
Mycotoxin analysis(μg/kg)						
AFB1	19	85	245	638	792	1641
Ochratoxin	8.43	6.16	6.46	9.77	9.39	15.3
T-2 toxin	<75	<75	<75	78	<75	89.7
Zearalenone	<40	<40	<40	<40	<40	<40
Vomitoxin	<250	340	<250	360	270	340
Fumonisin	<250	<250	<250	<250	<250	<250

[a] Vitamin mix (mg/kg feed): VB1, 20;VB2, 20; VB6, 10;VB3, 100; VB5, 50; VH, 1; folic acid, 5; inositol, 500;VE, 50; VA, 2; VB12, 0.02; VK3, 10; VD3, 0.05; V means Vitamin.
[b] Mineral mix (mg/kg feed): $ZnSO_4 \cdot 7H_2O$, 525.46; $MnSO_4 \cdot H_2O$, 49.22; KI, 5.23; $FeSO_4 \cdot 7H_2O$, 238.83; $MgSO_4 \cdot 7H_2O$, 4061.50; $CuSO_4 \cdot 5H_2O$, 11.82; $CoCl2 \cdot 6\ H_2O$, 0.20; Na_2SeO_3, 0.66; KCl, 600; NaCl, 400; cellulose, 107.08.
[c] Others: fish meal, 5%; soybean meal, 15%; rapeseed meal, 25%; flour, 22.5%; soybean oil, 3%; soybean lecithin, 1%; choline chloride, 0.3%; VC phosphate, 0.1%; calcium biphosphate, 2%; MHA-Ca, 0.3%.

2.3. Sample collection

At the end of each 5-week interval, two fish from each aquarium were randomly collected. The fish were anaesthetized by MS-222 (Sigma Chemical Co.), and their individual body weights, viscera weights and body lengths were determined (n = 6 for each treatment). At the end of 20 weeks, two fish per aquarium were used for analysis of body compositions (n = 6 for each treatment), and another two fish were used for blood, liver and dorsal flesh collection (n = 6 for each treatment). Liver and muscle were weighed and stored at −80 °C for further measurement.

2.4. Biochemical analysis

Blood samples were collected and the plasma was separated by centrifugation with 2000×*g* for 5 min at 4 °C. The concentrations of plasma albumin, total protein, and activities of asparitic amino-transferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined using automatic biochemical analyzer (Hitachi 7170, Tokyo, Japan) and attached kits (Daiichi Pure Chemicals Co., Ltd, Tokyo, Japan). The activity of liver cytochrome P450 1A (CYP1A) ethoxyresorufin-o-deethylase (EROD) was measured in the microsomal fraction using fluorescence kit (Genmed Scientific Inc., USA). Pure liver microsomal fraction was obtained by