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Research Article

Effects of Dietary Aflatoxin B1 on Hybrid Striped Bass (*Morone chrysops* × *M. saxatilis*) and Assessment of Supplemental Arginine as a Potential Aflatoxicosis Alleviator

Yufan Zhang[1,2,3] **Waldemar Rossi Jr.**[1,4] **Fernando Y. Yamamoto**[1,5] **Alejandro M. Velasquez**[1] **Anli Wang**[3] **and Delbert M. Gatlin III**[1]

[1]*Department of Wildlife and Fisheries Sciences, Texas A&M University System, College Station, TX, USA*
[2]*Alltech Biological Products Co., Beijing, China*
[3]*College of Life Science, South China Normal University, Guangzhou, China*
[4]*School of Aquaculture and Aquatic Sciences, College of Agriculture, Communities and the Sciences, Kentucky State University, Frankfort, KY, USA*
[5]*Thad Cochran National Warmwater Aquaculture Center, Mississippi Agriculture and Forestry Experiment Station, Mississippi State University, Stoneville, MS, USA*

Correspondence should be addressed to Fernando Y. Yamamoto; f.yamamoto@msstate.edu
and Delbert M. Gatlin III; d-gatlin@tamu.edu

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Aflatoxin contamination of feeds poses a significant threat to aquaculture. This six-week study was conducted to evaluate the impact of aflatoxin B1 (AFB1) in diets with and without supplemental arginine (Arg) on the production performance, somatic indices, body composition, and immune responses of hybrid striped bass (HSB). Six experimental diets were formulated to contain different levels of AFB1 (0, 1, or 2 mg/kg) and supplemental Arg (0 or 2%) following a 3 × 2 factorial arrangement. Triplicate groups of HSB (~5.44 g/fish) stocked in 18 glass aquaria were randomly assigned each diet and fed at a rate approaching apparent satiation twice daily. At the conclusion of the feeding trial, no interactions among dietary AFB1 and Arg were found. However, both medium (~1 mg/kg) and high (~2 mg/kg) levels of dietary AFB1 caused severe toxic effects on HSB directly affecting performance. Relative to control groups without AFB1 supplementation, steep reductions in weight gain (from 553% to 120% of initial weight), feed efficiency ratio (from 0.96 to 0.36), and protein retention (from 43% to 12%) were found in fish fed diets containing medium AFB1, and further reductions were observed in groups exposed to high AFB1. Dietary AFB1 also negatively affected condition factor and somatic indices and reduced whole-body protein and lipid contents relative to control groups. Significantly lower plasma hemolytic and lysozyme activities were found in fish exposed to dietary AFB1. Despite no significant protective effect of supplemental Arg being observed for most response parameters, reduced levels of Arg in plasma and muscle of AFB1-fed HSB indicated increased catabolism under nutritional stress. In conclusion, this study demonstrated that exposure to dietary AFB1 concentrations of ~1 and 2 mg/kg severely affected HSB and that supplemental Arg above requirement levels may be ineffective in alleviating aflatoxicosis.

1. Introduction

Aflatoxins are secondary metabolites of the fungi *Aspergillus flavus* and *A. parasiticus*, which can grow in soil, hay, decaying vegetation, and grains. They can be produced by fungi during the production, harvesting, storage, and processing of food or feed [1]. The principal aflatoxins commonly found in raw feed materials are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) [2]. Aflatoxins are recognized as inhibitors of nucleic acid and protein synthesis and can modify lipid metabolism and mitochondrial respiratory pathways

depending on exposure levels [3]. They are the most potent naturally occurring liver carcinogens identified to date. Among all aflatoxins, AFB1 is considered the most toxic [4]. The toxicity of AFB1 to economically important land animals has been well investigated and is known to lead to poor growth, behavioral abnormalities, immunity inhibition, liver necrosis, reproductive damage, and aflatoxin residues in the liver, milk, and other edible tissues [5–8].

A. flavus contamination of feedstuffs may occur in aquaculture because high concentrations of aflatoxins can be produced in raw materials containing elevated starch and lipid contents, such as corn and peanut [2, 9]. Almeida et al. [10] found six kinds of molds in 35 of 87 samples of European sea bass, *Dicentrarchux labrax*, feeds collected in Portugal, and among the contaminants, aflatoxin was the most frequent metabolite, occurring in all samples. Aflatoxin exposure can suppress the immune system of fish, hence increasing their susceptibility to pathogenic insults [10]. Thus, aflatoxin contamination has been identified as a major problem in aquaculture, causing economic losses and fish health complications [9]. Furthermore, the consumption of fish fed diets contaminated with AFB1 could potentially be hazardous for human health [11].

As an abundant amino acid in tissue proteins, arginine (Arg) plays a fundamental role in nitrogen metabolism including creatine and polyamine synthesis. This amino acid is also the major substrate for nitric oxide (NO) production [12, 13], a reactive molecule with bactericidal properties. Therefore, Arg plays a vital role in regulating the immune response and may enhance immune function in response to immunological challenges [14, 15]. Previous research has shown that dietary Arg supplementation to channel catfish, *Ictalurus punctatus*, plays a significant role in improving the ability of neutrophils and macrophages to produce NO and enhance resistance against *Edwardsiella ictaluri* [16, 17]. Because aflatoxin has been reported to directly damage the gastrointestinal tract causing pathological changes and reducing nutrient digestibility [18–20], supplemental Arg may increase protection through the enhancement of substrate-driven biosynthesis of polyamines. Increasing evidence supports the role of polyamines (putrescine, spermidine, and spermine) in enhancing gastrointestinal mucosa growth in various animals, including fish [21–23].

The hybrid striped bass (HSB) is a cross between the striped bass, *Morone saxatilis*, and white bass, *M. chrysops*. In terms of growth, survival, environmental tolerance, and stress resistance, it has been found to perform better in captivity than its parents [24, 25]. It has been produced in the United States for both stock enhancement and as seafood, and in recent years, HSB introduction in China has been widely accepted owing to its characteristics of strong disease resistance and high meat quality [26]. Although the relatively high acceptance of plant-based diets [27, 28] and digestible carbohydrate as dietary energy [29, 30] makes HSB an attractive candidate for modern aquaculture, it also increases its likelihood of exposure to mycotoxin-contaminated ingredients/feeds.

To the best of our knowledge, no previous studies on the effects of aflatoxins have been published with HSB although thoroughly assessed in various other fish species [1]. Furthermore, it has been reported that the addition of Arg to diets improves weight gain, feed efficiency, and immunological responses of various fish species including HSB [31–33]. Therefore, the present study investigated the effects of dietary AFB1 and supplemental Arg on production performance, somatic indices, body composition, and immune responses of HSB.

2. Materials and Methods

A feeding trial was conducted to evaluate physiological responses of HSB to dietary AFB1 and the potential protective effect of supplemental Arg against AFB1 toxicity. The experiment followed a 3×2 factorial design comprised of three targeted AFB1 levels (zero = undetectable, medium = 1 mg/kg (MAF), and high = 2 mg/kg (HAF)) and two Arg levels (0 and 2%). Given the lack of information on mycotoxin tolerance of HSB, these levels of AFB1 were chosen based on the reported higher tolerance of warmwater fish (*e.g.*, Channel catfish, *Ictalurus punctatus*, Jantrarotai and Lovell [34]; and hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, Deng et al. [7]) to AFB1 when compared to rainbow trout, *Onchorhyncus mykiss*, Halver [35]. In addition, dietary Arg above the minimum requirement established to support normal growth [36] was previously evaluated in HSB and found to have beneficial effects when supplemented at either 1% or 2% [32]. The latter level was chosen for this study.

2.1. Diet Preparation. Six experimental diets were evaluated in this study (Table 1) and formulated to contain 45% crude protein and 10% lipid, with an estimated digestible energy of 14.5 MJ/kg to attend the protein/energy requirement previously established for this fish [29]. A basal diet (0AF) designed to contain AFB1 below detection levels was formulated using ingredients including menhaden fishmeal and fish oil, dehulled and solvent-extracted soybean meal, and dextrinized starch. The 0AF diet served as the overall control for both dietary AFB1 and Arg inclusions and met all established nutrient requirements of hybrid striped bass (NRC 2011). For the dietary inclusion of AFB1, purified AFB1 (Sigma Chemicals, St. Louis, MO, USA) diluted in 100% alcohol was pre-mixed with dietary cellulose to obtain four test diets (MAF, HAF, MAF+Arg, and HAF+Arg) designed to contain approximately 1 mg/kg (MAF diets) and 2 mg/kg (HAF diets) of AFB1. For the evaluation of supplemental Arg, crystalline L-Arg was included in MAF+Arg and HAF +Arg at 2% replacing glycine on an isonitrogenous basis.

All ingredients were weighed and mixed for 15 min in a V-mixer (Blend Master, Buflovak, NY, USA). The mixture was then transferred to an industrial food processor (A-200 Hobart Meat Grinder, Hobart Corporation, OH, USA) wherein fish oil followed by deionized water (250 mL/kg) was gradually incorporated into the mixture for an additional 15 min of homogenization. The wet dough was pelleted through a 3-mm die plate using the single-screw barrel of the food processor. Next, pelleted diets were air-dried for 24 h and stored at -20°C until fed. The AFB1-free

Table 1: Formulation and analyzed composition of the experimental diets fed to the hybrid striped bass for 6 weeks. Results for the analyzed proximate composition are reported as g/100 g on a dry-matter basis.

Ingredients	Diet designation					
	0AF	MAF	HAF	0AF + Arg	MAF + Arg	HAF + Arg
Menhaden fishmeal[1]	38.50	38.50	38.50	38.50	38.50	38.50
Dehulled soybean meal[2]	28.00	28.00	28.00	28.00	28.00	28.00
Dextrinized starch[3]	18.00	18.00	18.00	18.00	18.00	18.00
Menhaden fish oil[1]	4.00	4.00	4.00	4.00	4.00	4.00
Arginine[4]	0.00	0.00	0.00	2.00	2.00	2.00
Glycine[4]	3.50	3.50	3.50	0.00	0.00	0.00
Cellulose[3] + Aflatoxin B1∗[5]	1.00	1.00	1.00	2.50	2.50	2.50
Mineral premix[6]	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin premix[6]	3.00	3.00	3.00	3.00	3.00	3.00
Analyzed composition						
Dry matter	87.2	87.4	87.6	88.4	88.5	88.8
Crude protein	46.6	46.6	46.9	46.2	46.1	46.1
Crude lipid	10.3	10.6	10.8	10.6	10.5	10.6
Ash	13.4	13.5	13.5	13.2	13.2	13.2
Aflatoxin B1 (mg/kg)	0	1.2	1.7	0	0.7	2.4

Abbreviations: AF: AFB1; 0AF: no supplemental AFL; MAF: medium AFL; HAF: high AF; Arg: Arginine∗Only included in AFB1-containing diets.[1]Omega Protein Corporation, Abbeville, LA, USA.[2]Producers Cooperative Association, Bryan, TX, USA.[3]MP Biomedicals, Solon, OH, USA.[4]Ajinomoto North America Inc., Itasca, IL, USA.[5]Sigma Aldrich Co., St. Louis, MO, USA.[6]Same as in Moon and Gatlin III (1991).

diets (0AF and 0AF+Arg) were manufactured prior to the remaining diets to avoid potential contamination.

2.2. Analysis of Dietary AFB1 and Proximate Composition. The concentration of AFB1 in the diet was measured using an established methodology [37]. Briefly, 250 mL of 70% diluted methanol was mixed with 50 g of the diet from three replicate samples and blended by mixing. The mixture was filtered and transferred to a clean plastic centrifuge tube to which NaCl (1 g) and HPLC water (20 mL) were added and thoroughly stirred. The filtrate was filtered through a microfiber filter and transferred into a plastic syringe barrel reservoir. The filtrate was slowly injected into the column at a rate of 2 drops/s. Deionized water was slowly added to the syringe barrel at the same injection rate to drain the remaining filtrate. Aflatoxin B1 was eluted from the column into a glass tube with 1 mL methanol, and the solution was filtered. Using a miscible liquid comprising water, acetonitrile, and methanol in a 3 : 1 : 1 ratio, the samples were injected into an HPLC system. Quantification of AFB1 was performed using the HPLC software. Proximate composition analyses of dry matter, crude protein, crude lipid, and ash contents were conducted according to standard methods [38].

The analyzed composition of the experimental diets is presented in Table 1. No AFB1 was detected in the control diets 0AF and 0AF+Arg. In the test diets, AFB1 levels were variable but close to the targeted levels of 1 and 2 mg/kg. Proximate composition values (mean ± standard deviation, SD) of dry matter (88.0 ± 0.2%), crude protein (46.4 ± 0.4%), crude lipid (10.6 ± 0.2%), and ash (13.3 ± 0.2%) were consistent across diets.

2.3. Fish Husbandry, Experimental Design, and Rearing Conditions. Hybrid striped bass fingerlings were kindly donated by Keo Fish Farm, Inc. (Keo, AR, USA) and maintained under quarantine at the Texas A&M University Aquacultural Research and Teaching Facility until the commencement of the feeding trial. After grown to adequate size (mean weight ± SD = 5.44 ± 0.05 g) using a commercial feed, 360 HSB juveniles were evenly distributed into 18 glass aquaria (38 L) connected as a recirculating aquaculture system as previously described [31]. Each of the six experimental diets was randomly assigned to three aquaria ($n = 3$) following a completely randomized design. A 12 : 12 light/dark photoperiod was kept constant by timer-controlled fluorescent lighting and the water quality was maintained suitable for HSB culture (mean ± SD): temperature = 27 ± 0.5°C; total ammonia nitrogen = 0.02 ± 0.01 mg/L; nitrite nitrogen = 0.007 ± 0.003 mg/L. In addition, dissolved oxygen and pH were maintained at near air saturation and between 7 and 8, respectively. Occasional additions of sodium bicarbonate ($NaHCO_3$) to the system's sump tank were performed as needed to adjust pH. This study was conducted in compliance with the Institutional Animal Care and Use Committee at Texas A&M University (IACUC 2016-0368).

2.4. Experimental Procedures. Fish were acclimated to the culture system for one week prior to the beginning of the study. During the feeding trial, fish were fed daily at fixed rates (4-5% of body weight), which approached apparent satiation without overfeeding. The feed rations were offered twice daily (at ~9 : 00 am and 3 : 00 pm) for 6 weeks. Fish in each aquarium were group weighed every week to monitor

weight gain and survival as well as adjust daily rations. At the end of the feeding trial, total weight and counts of fish in each aquarium were recorded. Seven fish per aquarium were collected randomly 36 h after the last feeding and anesthetized with tricaine methane-sulphonate (MS-222, Western Chemicals Inc., Ferndale, WA, USA) at 50 mg/L for blood collection. Blood samples were collected from the caudal vasculature of four fish using sterile heparinized syringes. A portion of the whole blood was immediately used for reactive oxygen production analysis (see Section 2.5), and the remainder was centrifuged for 10 min at 10,000 × *g* for plasma separation. The resulting plasma was aliquoted and stored at -20°C to measure the plasma immune assays (see Section 2.6), and amino acid analysis. Following blood collection, fish were euthanized with an overdose of MS-222 (300 mg/L). Total lengths and weights were recorded for these four bled fish per aquarium for computing condition factor, and the viscera, liver, and intraperitoneal fat were excised and weighed for computing somatic indices including viscerosomatic, hepatosomatic, and intraperitoneal fat indices, respectively. Next, these fish were filleted to compute muscle yield and the muscle were also stored at -20°C if the dietary treatments impacted their amino acid profile. The remaining three fish were euthanized and stored at -20°C for whole-body proximate composition analyses [38]. Metrics for production performance, protein utilization efficiency, and somatic indices of HSB were calculated as follows:

Survival (%) = 100 × (final number of fish ÷ initial number of fish)

Weight gain (%) = 100 × (final body weight − initial body weight) ÷ (initial body weight)

Feed efficiency (FE) = wet weight gain (g) ÷ dry feed fed (g)

Protein retention (PR, %) = 100 × fish protein gain (g) ÷ total protein intake (g)

Protein efficiency ratio (PER) = weight gain (g) ÷ total protein intake (g)

Survival (%) = 100 × (number of surviving fish/initial number of fish)

Condition factor (CF) = 100 × final body weight (g) ÷ (body length)^3 (cm)

Viscerosomatic index (VSI, %) = 100 × visceral weight (g)/body weight (g)

Hepatosomatic index (HSI, %) = 100 × liver weight (g) ÷ body weight (g)

Intraperitoneal fat index (IPFI) = 100 × intraperitoneal fat weight ÷ body weight (g)

Muscle yield (%) = 100 × [fillet muscle weight] ÷ [whole body weight]

2.5. Reactive Oxygen Species from Blood Phagocytes. Reactive oxygen species production from whole-blood phagocytes was measured with the reduction of nitroblue tetrazolium suspension (NBT, 2 mg mL^{-1} in PBS) using the methods established by Siwicki et al. [39] and modified by Yamamoto et al. [40]. Briefly, a 50 μL of heparinized whole blood from each fish was incubated for 30 min with the NBT solution in a U-bottom 96-well microplate sheltered from light. After incubation, formazan granules were resuspended in 1 mL of dimethyl formamide, and absorbance was read at 545 nm. Concentration of NBT was expressed by a previously established standard curve, as follows:

mg of NBT diformazan mL^{-1} of blood = 80 × (Absorbance at 545 nm − 0.0245) ÷ 5.8564

2.6. Plasma Hemolytic and Lysozyme Activity. Total complement hemolytic activity was measured by the procedure described by Sutili et al. [41] with minor modifications (Yamamoto et al., [42]). Briefly, diluted goat red blood cells (GRBCs) in PBS (26 μL) were added to 100 μL of plasma in a U-shaped microtiter plate and incubated at 20°C for 1 h with regular shaking. The reaction was terminated by adding 150 μL ice-cold saline and centrifuging at 500 × *g* for 10 minutes. Absorbance of the supernatant was measured at 405 nm using a microplate reader. One hundred percent hemolysis was determined by measuring a mixture of 26 μL of GRBC with 176 μL of deionized water. Meanwhile, a negative control was considered for spontaneous lysis by measuring a mixture of 26 μL GRBC with PBS. Hemolytic activity of complement proteins against GRBC by the HSB plasma was calculated using the following formula:

Percent hemolysis = (A405 absorbance of sample − A405 absorbance of spontaneous lysis)/(A405 absorbance of 100% hemolysis − A405 absorbance of spontaneous lysis) ×100.

The lysozyme activity of the plasma was measured turbidimetrically using a *Micrococcus lysodeikticus* (cat# M3770, Sigma Aldrich Co, St Louis, MO, USA) suspension in PBS buffer [43]. Briefly, 10 μL of plasma from each fish was incubated with 190 μL of Micrococcus suspension in a flat bottom 96-well microplate for 4 min at 27°C. Absorbance was recorded at times 0 and 4 min, and lysozyme activity was expressed as the decrease of absorbance at 450 nm using the following formula:

Lysozyme activity (U mL^{-1} of plasma) = [(ΔAbsorbance ÷ 4 min) ÷ 0.001] ×100.

2.7. Plasma and Muscle Amino Acid Profile. Muscle samples were subjected to acid hydrolysis (HCl 6N) for 24 h at 110°C. Hydrolyzed samples were resuspended in distilled water and evaporated in a water bath at 60°C and forced N_2 flow. Plasma and hydrolyzed muscle samples were deproteinized prior to derivatization with perchloric acid solution (1.5 M $HClO_4$) and the dilution was neutralized with potassium bicarbonate (2 M K_2CO_3). Precipitated proteins and salt were centrifuged at 12,000 × *g* for 5 min and supernatant was filtered through 0.2-μm syringe filters. Amino acid profile of the samples was determined using a high-pressure liquid chromatography (UPLC - Acquity system, Waters, Milford, WA, USA) where the retention of derivatized amino acids was estimated by an integrated TUV detector [44].

2.8. Statistical Analyses. All data were subjected to a two-way ANOVA with dietary AFB1 (zero, medium, and high) and supplemental Arg (0 and 2%) as the main effects. When significant ($P \leq 0.05$) main effect or interactions were detected, Duncan's multiple range test was performed for the comparison of means. All statistical analyses were carried out using SPSS version 18.0 (SPSS, IL, USA).

Table 2: Impact of dietary AFB1 and supplemental arginine on weight gain (WG), feed efficiency (FE), protein retention (PR), protein efficiency ratio (PER), and survival of hybrid striped bass after 6 weeks of feeding.

	WG (%)	FER	PR (%)	PER	Survival (%)
Treatment means[1]					
0AF	545	0.96	41.6	2.06	100
MAF	113	0.35	11.3	0.76	100
HAF	52	0.19	5.7	0.4	94.9
0AF+Arg	561	0.97	44.2	2.1	100
VMAF+Arg	122	0.36	12.1	0.79	100
HAF+Arg	40	0.15	4.9	0.33	97.4
Pooled SE	54	0.08	4.0	0.17	0.02
Main effect means					
AFB1[2]					
0	553[a]	0.96[a]	42.9[a]	2.08[a]	100
M	118[b]	0.36[b]	11.7[b]	0.77[b]	100
H	46[c]	0.17[c]	5.3[c]	0.36[c]	96.1
Arg (%)[3]					
0	236	0.5	19.5	1.07	98.3
2	241	0.5	20.4	1.07	99.1
Two-way ANOVA (Pr > F)					
AFB1	<0.001	<0.001	<0.001	<0.001	0.20
Arg	0.61	0.78	0.14	0.94	0.66
AFB1×Arg	0.44	0.34	0.07	0.29	0.82

[1, 2, 3] Values represent means of three (n =3), six (n =6), and nine (n =9) replicate aquaria, respectively. Different letters within a column indicate significant differences by Duncan's multiple range test. SE = standard error.

3. Results

No acute responses of the fish to the different dietary levels of AFB1 were observed during the 6-week feeding trial with mean survival of fish fed all diets being 95% or higher (Table 2). Additionally, no significant interactions were found between AFB1 and supplemental Arg for all response parameters evaluated.

3.1. Production Performance. At the end of the 6-week feeding period, although survival of HSB was unaffected by diet, significant dose-dependent responses in weight gain, feed efficiency, and nutrient utilization of HSB exposed to AFB1 were observed (Table 2). Regardless of Arg supplementation, fish fed MAF diets displayed reductions in weight gain from 553% to 118%, FE from ~1.0 to 0.17, PR from 43% to ~12%, and PER from ~2 to 0.8. Similarly, when HSB were fed the HAF diets, further reductions in performance were observed. No significant effects of supplemental Arg on the above response metrics were observed.

3.2. Conditional Factor and Somatic Indices. Inclusion of AFB1 in the diets significantly affected the CF and somatic indices of HSB (Table 3). Dose-dependent reductions ($P < 0.05$) in CF (from 1.65 to 1.17) and IPFI (from 4.91 to 1.78%) were observed in AFB1 exposed fish. Similarly, lower VSI and HSI were observed in all AFB1 exposed groups with no differences between MAF- and HAF-fed groups, while significantly lower muscle yield was found in groups fed HAF diets. No significant effects of supplemental Arg on conditional factor and somatic indices were observed.

3.3. Whole-Body Composition. All metrics of whole-body composition showed significant changes with AFB1 exposure (Table 4). Whole-body moisture and ash content of HSB increased from 69.2 and 3.6%, respectively, in groups fed AF0 diets to 73.9 and 4.1% in HSB fed MAF diets, and further increased to 74.8 and 4.5% in groups fed HAF diets. Meanwhile, reductions in whole-body protein (from 20.6 to ~15%) and whole-body lipid (from 2.9 to 1.4%) were observed in fish fed MAF and HAF diets. No significant effects of supplemental Arg on the whole-body composition of HSB were observed.

3.4. Immune Responses. The immune responses of HSB exposed to AFB1 were determined by assessing whole-blood neutrophil oxidative radical production (NBT), plasma hemolytic activity, and plasma lysozyme activity (Table 5). Blood NBT of HSB ranged from 0.95 to 0.98 mg/mL and as unaffected by AFB1 or Arg supplementation. Meanwhile, steep reductions in hemolytic activity (from 82.4 to 22.4) and lysozyme activity (from 588 to 181 Units/mL) between groups fed 0AFs and HAFs were observed ($P < 0.05$), while neither of these parameters were influenced by supplemental Arg.

3.5. Amino Acids in Plasma and Muscle. The levels of histidine (His) and Arg in plasma and muscle were measured

Table 3: Impact of dietary AFB1 and supplemental arginine on condition factor (CF), visceral somatic index (VSI), hepatosomatic index (HSI), intraperitoneal fat index (IPFI), and muscle yield of hybrid striped bass after 6 weeks of feeding.

Dietary treatments	CF	VSI (%)	HSI (%)	IPFI (%)	Muscle yield (%)
Treatment means[1]					
0AF	1.69	11.9	3.26	5.08	37.3
MAF	1.27	6.8	1.07	3.33	36.9
HAF	1.17	6.4	0.93	1.91	35.9
0AF+Arg	1.61	11.5	2.98	4.75	39.0
MAF+Arg	1.3	6.8	0.9	2.4	36.9
HAF+Arg	1.19	6.5	0.97	1.65	35.1
Pooled SE	0.03	0.35	0.15	0.26	0.34
Main effect means					
AFB1[2]					
0	1.65[a]	11.8[a]	3.12[a]	4.91[a]	38.3[a]
M	1.28[b]	6.8[b]	0.98[b]	2.87[b]	36.9[ab]
H	1.17[c]	6.5[b]	0.95[b]	1.78[c]	35.5[b]
Arg (%)[3]					
0	1.38	8.43	1.75	3.44	36.7
2	1.37	8.28	1.62	2.93	37.0
Two-way ANOVA (Pr > F)					
AFB1	<0.001	<0.001	<0.001	<0.001	0.005
Arg	0.72	0.57	0.20	0.13	0.63
AFB1 × Arg	0.22	0.66	0.74	0.33	0.26

[1, 2, 3] Values represent means of three (n =3), six (n =6), and nine (n =9) replicate aquaria, respectively. Different letters within a column indicate significant differences by Duncan's multiple range test. SE = standard error.

Table 4: Impact of dietary AFB1 and supplemental arginine on whole-body proximate composition (% of fresh weight) of hybrid striped bass after 6 weeks of feeding.

	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Treatment means[1]				
0AF	69.2	20.2	3.2	3.6
MAF	74.2	15.1	1.7	4.2
HAF	74.8	14.3	1.4	4.6
0AF+Arg	69.2	21	2.6	3.8
MAF+Arg	73.7	15.4	1.1	4.2
HAF+Arg	74.9	14.9	1.5	4.4
Pooled SE	0.4	0.6	0.2	0.07
Main effect means				
AFB1[2]				
0	69.2[a]	20.6[a]	2.9[a]	3.6[a]
M	73.9[b]	15.2[b]	1.4[b]	4.1[b]
H	74.8[c]	14.6[b]	1.4[b]	4.5[c]
Arg (%)[3]				
0	72.7	16.9	2.1	4.0
2	72.6	17.5	1.7	4.1
Two-way ANOVA (Pr > F)				
AFB1	<0.001	<0.001	<0.001	<0.001
Arg	0.06	0.24	0.07	0.61
AFB1 × Arg	0.06	0.90	0.25	0.32

[1, 2, 3] Values represent means of three (n =3), six (n =6), and nine (n =9) replicate aquaria, respectively. Different letters within a column indicate significant differences by Duncan's multiple range test. SE = standard error.

Table 5: Impact of dietary AFB1 and supplemental arginine on whole-blood reactive oxygen species production (NBT), and plasma hemolytic and lysozyme activity of hybrid striped bass after 6 weeks of feeding.

	NBT (mg/mL)	Hemolytic activity (%)	Lysozyme (units/mL)
Treatment means[1]			
0AF	0.95	70.6	616
MAF	0.96	39.4	287
HAF	0.96	24.8	181
0AF+Arg	0.98	95.2	560
MAF+Arg	0.94	45.8	214
HAF+Arg	0.97	20.0	179
Pooled SE	0.01	3.6	19
Main effect means			
AFB1[2]			
0	0.95	82.4[a]	588[a]
M	0.97	42.6[b]	251[b]
H	0.97	22.4[c]	181[c]
Arg (%)[3]			
0	0.96	44.6	362
2	0.96	53.7	321
Two-way ANOVA (Pr > F)			
AFB1	0.73	0.001	0.001
Arg	0.84	0.15	0.08
AFB1 × Arg	0.90	0.15	0.41

[1, 2, 3] Values represent means of three (n =3), six (n =6), and nine (n =9) replicate aquaria, respectively. Different letters within a column indicate significant differences by Duncan's multiple range test. SE = standard error.

Table 6: Impact of dietary AFB1 and supplemental arginine on plasma and muscle concentrations of histidine and arginine in hybrid striped bass after 6 weeks of feeding.

	Plasma		Muscle	
	His (nmol/mL)	Arg (nmol/mL)	His (ng/nmol)	Arg (ng/nmol)
Treatment means[1]				
0AF	13.0	51.0	2.9	3.0
MAF	12.4	27.7	5.0	1.63
HAF	10.1	24.3	4.6	1.19
0AF+Arg	13.2	56.0	3.5	3.94
MAF+Arg	11.2	40.3	5.6	2.14
HAF+Arg	8.5	33.3	5.1	1.41
Pooled SE	0.97	4.2	0.33	0.31
Main effect means				
AFB1[2]				
0	13.1	54.0[a]	3.17[a]	3.47[a]
M	11.2	34.0[b]	5.33[b]	1.89[b]
H	9.3	29.7[b]	4.85[b]	1.30[b]
Arg (%)[3]				
0	11.8	33.4	4.17	1.94
2	11	43.2	4.74	2.5
Two-way ANOVA (Pr > F)				
AFB1	0.36	0.25	0.02	0.01
Arg	0.70	0.05	0.32	0.28
AFB1 × Arg	0.94	0.91	0.99	0.83

[1, 2, 3] Values represent means of three (n =3), six (n =6), and nine (n =9) replicate aquaria, respectively. Different letters within a column indicate significant differences by Duncan's multiple range test. SE = standard error.

to determine the impact of dietary AFB1 on free amino acid content (Table 6). Plasma His was unaffected by dietary AFB1 or supplemental Arg ($P > 0.05$). Supplementation of Arg in the diets significantly elevated free Arg in plasma (from 33.4 to 43.2 nmol/mL) but did not affect plasma and muscle His or muscle Arg concentrations. Meanwhile, plasma Arg decreased from 54.0 mmol/mL in HSB fed the 0AF diets to 29.7 mmol/mL in fish fed the HAF diets ($P < 0.05$). In the muscle, His content increased while that of Arg decreased in fish fed the MAF and HAF diets ($P < 0.05$).

4. Discussion

Previous studies have demonstrated that AFB1 exposure can adversely affect growth performance in various fish species, such as Nile tilapia, *Oreochromis niloticus* [37], and matrinxã, *Brycon cephalus* [45]. The biological effects of AFB1 in fish are influenced by its concentration in the feed and animal species [46]. Some fish species are more sensitive to AFB1 than others. For instance, growth and feed efficiency of juvenile red drum, *Sciaenops ocellatus*, were negatively affected by 0.1 mg/kg of dietary AFB1 [47], whereas the addition of up to 1.65 mg/kg of AFB1 in gibel carp, *Carassius auratus gibelio*, diets did not significantly affect production performance including body weight gain and FE [48]. In the present study, the production performance of HSB was adversely affected from the lowest inclusion level of AFB1 in the diet (~1.0 mg/kg), with respective reductions in weight gain, FER, and PR of 80, 60, and 70% in groups fed the MAF diets. It is common for fish growth to be inhibited in a dose-dependent manner by AFB1 as observed in Nile tilapia by Zychowski et al. [37]. Our findings corroborate previous observations and indicate that HSB is sensitive to AFB1.

The liver is the main impacted organ for aflatoxicosis in fish [49] and in the present study, HSI was reduced by 70% (3.12 to 1.0%) in fish exposed to dietary AFB1 (Table 3). Aflatoxins also have been reported to directly damage the anterior intestine and cause pathological changes, including decreased nutrient digestibility [18–20]. Decreases in other body condition indices including CF, VSI, IPFI, and muscle yield were also observed in HSB fed the diets containing AFB1. These results indicate that AFB1 severely affected growth of various tissues in HSB, and the decrease in muscle yield indicated loss of edible tissue.

Depending on the exposure level, mycotoxins also can decrease protein synthesis and modify lipid metabolism [3, 10]. After the influx of aflatoxins into the liver, aflatoxin metabolites inhibit carbohydrate and lipid metabolism and protein synthesis, eventually inducing liver necrosis, cell death, and/or tumorigenesis. Sahoo and Mukherjee [8] described reductions in total protein of rohu, *Labeo rohita*, exposed to AFB1 at 1.25 mg/kg of body weight. Deng et al. [7] reported a hepatic disorder in hybrid tilapia, *O. niloticus* × *O. aureus*, induced by AFB1, which was characterized by decreased lipid content. Analyses of the body composition of the HSB in this study showed that dietary AFB1 reduced organic components, including body protein and lipid, reflecting toxicity of AFB1 consistent with findings in other fish species.

Lysozyme, a mucolytic enzyme of leukocyte origin [50], is a vital defense molecule of the fish's innate immune system that mediates protection against microbial infections. Hemolysis consists of the breakdown of red blood cells with the release of hemoglobin [51], and complement hemolytic activity is an important indicator of immunity. In this study, both lysozyme and hemolytic activity of HSB exposed to AFB1 were reduced, indicating that immunity of HSB was weakened by dietary AFB1, corroborating previous findings in yellow catfish, *Pelteobagrus fulvidraco* [52] and rainbow trout [53].

Although oxidative radical production in blood is another widely used indicator of innate immunity, the whole-blood NBT of HSB was unaffected by AFB1 in this study. Decreased extracellular superoxide anion production has been previously reported in Labeo rohita fingerlings [54] and Nile tilapia [37]. Contrastingly, another study with AFB1-exposed Nile tilapia did not report a decrease in extracellular superoxide anion production but found a decrease in total superoxide anion [55]. Our findings mirror other reports showing inconsistent effects of AFB1 on oxidative radical production.

In this study, it was hypothesized that Arg supplementation in the diet would alleviate the toxic effects of AFB1 in HSB. However, our findings showed that supplemental Arg did not significantly improve growth performance, condition indices, body composition, or non-specific immune response of AFB1-exposed fish. This is contrary to previous findings in this laboratory when supplementation of Arg at 1 and 2% into a similar control formulation improved production performance and immune responses of HSB including lysozyme activity [32]. However, although Arg levels in plasma of HSB increased in groups fed Arg supplemented diets, the observed declines in both plasma and muscle Arg in AFB1-fed groups likely reflect increased amino acids utilization for energy needs during toxicity or stress-induced protein catabolism [56]. As Arg is a substrate for nitric oxide (NO) production [12, 13], it is possible that increased Arg catabolism occurred intracellularly for NO production in AFB1 exposed fish, despite the lack of significant positive effects on production performance.

In summary, dietary AFB1 at concentrations of ~1 and 2 mg/kg caused severe toxic effects in HSB, significantly affecting growth performance, feed efficiency, somatic indices, body composition, and plasma immune indices including lysozyme and hemolytic activity. Although dietary Arg in excess of the established requirement level for growth could not significantly alleviate AFB1 toxicity in HSB in this study, the observed decline in plasma and muscle Arg in AFB1-exposed fish indicates a potential role under nutritional stress. Given the sensitivity of HSB to AFB1 demonstrated in this study, upper-tolerance levels of HSB to AFB1 and other prominent mycotoxins should be evaluated in future studies.

Data Availability

The data that support the findings of this study are available upon reasonable request to the corresponding authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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