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The effect of Ochratoxin A on antimicrobial polypeptide expression and resistance to water mold infection in channel catfish (*Ictalurus punctatus*)

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ABSTRACT

Mycotoxin contamination of agricultural commodities poses a serious risk to animal health, including aquaculture species. Ochratoxin A (OA) is the most immunotoxic ochratoxin, yet little is known about its effect on immune function in fish. Antimicrobial polypeptides (AMPPs) are one of the most potent, innate, host defense factors, yet very little is known about what types of chronic stressors affect their expression. Among the most prevalent and potent AMPPs in fish are histone-like proteins (HLPs). In this study, fish were fed 2, 4, or 8 mg OA/kg diet. Skin antibacterial activity and HLP-1 levels were measured on Days 0, 28 and 56. Feeding 2, 4 or 8 mg OA/kg diet resulted in significant growth depression, but higher levels (4 or 8 mg OA/kg diet) resulted in lowering feed intake (FI) and impaired feed conversion ratio. In addition, feeding 8 mg OA/kg diet increased susceptibility to experimental water mold (*Saprolegnia*) challenge, suggesting that OA toxicity might contribute to some saprolegnosis outbreaks. However, there were no changes in AMPP expression in any treatment group. Our data suggests that the increased disease susceptibility of channel catfish due to OA is probably due to mechanisms other than a direct effect on antimicrobial polypeptide expression.

1. Introduction

Mycotoxins are secondary fungal metabolites, which contaminate various types of feed commodities such as corn, wheat, cottonseed meal, peanuts, and soybean meal [1,2]. Ochratoxin A (OA), produced by *Penicilliumand* and *Aspergillus* species, especially *Aspergillus ochraceous*, is one of the most important mycotoxins [3] and is associated with many signs such as reduced growth rate, feed efficiency ratio, reproductive performance, resistance to infectious diseases. It also causes damage to liver and other organs of fish and farm animals [4,5].

OA mediates its toxic effect by acting on cellular respiratory enzymes through competitive inhibition of ATPase, succinate dehydrogenase, and cytochrome C oxidase in mitochondria. Moreover, because of its phenylalanine moiety, it competitively inhibits phenylalonyl-tRNA synthase, thus disrupting protein synthesis. In addition, cellular damage is caused by hydroxyl radical formation and lipid peroxidation [6]. OA induces oxidative damage in vivo [7] and in vitro [8] that eventually, lead to mitochondrial dysfunction, apoptosis and DNA damage [9].

OA is toxic to fish, but susceptibility varies considerably among species. Sea bass (*Dicentrarchus labrax* L.), are highly sensitive, having a 96 h LC_{50} of 9.23 mg OA/kg diet [10]. The LD_{50} for rainbow trout (*Oncorhynchus mykiss*) by injection is 4.76 mg OA/kg [11]. Channel catfish are much more resistant, tolerating as high as 4 mg OA/kg diet for at least 8 weeks without mortalities, and 8 mg OA/kg diet with 80% survival [12].

However, the sublethal effects of OA intoxication are much more economically damaging [13]. OA is well-known to increase disease susceptibilty in homeotherms [14]. Pigs fed 3 mg OA/kg diet for up to three weeks spontaneously contracted salmonellosis, with *Salmonella choleraesuis* found in the faeces and liver, and deaths

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between days 15 and 17. Fewer pigs were affected at a lower dose (1 mg OA/kg diet) and none was affected in the control group [15]. Broiler chickens fed 2 mg OA/kg diet and challenged after 14 days with *Escherichia coli* O_{78} had increased mortality (by 21.4%) compared with chicks inoculated with *E. coli* alone [16]. Eleven-day-old chickens orally challenged with *Salmonella typhimurium* for 2 consecutive days (3 mg OA/kg) had increased *S. typhimurium* in both their duodenal and cecal contents [17].

OA also increases disease susceptibility to *Edwardsiella ictaluri*, one of the most important pathogens affecting channel catfish (*Ictalurus punctatus*) [18]. Channel catfish fed 2.0 or 4.0 mg OA/kg diet for 6 weeks and then challenged by immersion with a highly virulent isolate of *Edwardsiella ictaluri* had significantly greater mortality (80%) than the control (68%). This result together with the less weight gain in both doses compared to control, indicate that feeding intoxicated diet could be implicated in higher susceptibility to the infection.

One of the most prevalent innate defenses in animals is antimicrobial polypeptides (AMPPs) [19]. Studies that have shown that depressed AMPP levels can greatly increase disease susceptibility. For example, recurrent bacterial infections occur in cases where there is a deficiency of α-defensins in neutrophils. Morbus Kostmann syndrome, which is a severe congenital neutropenia in humans that is typified by low concentrations of AMPPs in the mouth, results in recurrent oral infections [20]. Depressed levels of histatins, AMPPs in human oral mucosa, have been associated with an increased risk of developing human immunodeficiency virus (HIV) infection [21]. Furthermore, Furci et al. [22] showed that α-defensin-5 (HD5) is a potent and broad-spectrum inhibitor of biologically diverse HIV-1 strains, through their interaction with the major HIV-1 envelope glycoprotein, gp120, and with its primary cellular receptor, CD4 interfering with their reciprocal binding. In the same context, immunomodulatory peptide (IDR-1002) enhanced in vitro chemokine induction activities and stronger in vivo anti-infective properties were confirmed. Additionally, it showed protection against the Gram-negative bacterial pathogen *Escherichia coli* [23].

Among the most common AMPPs in fish are histone-like proteins (HLPs), which have high homology to core nuclear histones. Originally isolated from the skin of channel catfish (*Ictalurus punctatus*) [24], HLPs were subsequently identified in skin, gill and/or spleen of hybrid striped bass (*Morone saxatilis* male x *M. chrysops* female) and rainbow trout (*Oncorhynchus mykiss*) [25,26]. HLP-1 is the most prevalent and potent HLP, with broad-spectrum activity against bacteria, parasites and water molds [24,26]. HLPs are also the predominant AMPP in normal healthy channel catfish [24].

Water molds (Oomycetes), one of the most important pathogens of freshwater fish [27], are classical opportunists that typically cause outbreaks when fish are exposed to some type of stress, such as adverse water temperature, poor water quality, handling or crowding. These factors may compromise immunity, increasing susceptibility to infection [28,29]. Members of the genus *Saprolegnia* are the most common and important water molds and thus the disease is often termed saprolegnosis. Saprolegniosis is associated with "winter kill" a syndrome in channel catfish in the southeastern United States that is associated with rapid temperature drop (below 15 °C) in fall and winter [30,31].

There have been no studies that have examined the effect of OA or any other mycotoxin on the expression of AMPPs in any animal. Thus, to better understand possible mechanisms underlying OA immunotoxicity, we assessed the effect of OA on the expression of AMPPs in channel catfish. We also determined if there was any relationship between AMPP tissue levels and resistance to challenge with the water mold *Saprolegnia*.

2. Materials and methods

2.1. Experimental fish

Channel catfish were obtained from a local producer and transported to North Carolina State University. Fish were acclimated in a 380 L fiberglass aquarium at 24 °C for 60 days prior to beginning the experiment. During that time, fish were fed ad lib with a 2 mm pellet (40% crude protein, 10% crude fat, and 4% crude fiber, Zeigler Bros, Inc., Gardners, PA). Ammonia, nitrite, and pH were monitored weekly. Two weeks before initiating the experiment, fish were sedated with 60 mgL^{-1} buffered tricane and transferred to the experimental aquaria.

2.2. Experimental design

Fifteen fish (7 month old and 17–21 cm length) were placed in each of twelve, 60 L freshwater aquaria (total N = 180). All aquaria were connected to a central filtration system having a conditioned biofilter (biocubes and bead filter [Aquadyne, Koi Camp Aquariology, Loganville, GA]) and a titanium heater (Process Technology Co., Mentor, OH). Fish began feeding normally almost immediately and were fed close to apparent satiation twice daily. After 7 days, the temperature was increased from 24 °C to 29 °C over 7 days. After 14 d at 29 °C (day 0), all fish were weighed, the mean weight of all fish on day 0 was 55 ± 15 g and the day 0 sampling was performed. At day 1, all fish were switched to semi-purified diets (Table 1) prepared as described previously (Manning et al., 2003), and having one of four concentrations of OA (0, 2.0, 4.0, or 8.0 mg OA/kg diet). "The experiment was conducted with a protocol approved by the North Carolina State University Institutional Animal Care and Use Committee. Triplicate aquaria were assigned to one of four treatments. During the experiment, 75% water changes were performed thrice weekly, and water quality was measured (ammonia, nitrite, nitrate, pH, and dissolved oxygen) twice weekly via water quality test kits (Aquarium Pharmaceuticals, Inc.).

2.3. Sample collection

Three fish from each aquarium were sampled at days 0, 28 and 56. Each aquarium was sampled one at a time; the three fish were

Table 1
Ingredient composition of the semipurified basal diet[a].

Ingredient	Amount (g/kg dry mixture)
Casein, vitamin free (USB#12866)[b]	290.0 g
Gelatin	80.0 g
Dexrin(USB#9004-53-9)	360.0 g
Cellulose	106.0 g
Fish meal, menhaden	20.0 g
Carboxymethyl cellulose	30.0 g
Corn oil	30.0 g
Menhaden oil (USB#8002-50-4)	30.0 g
Mineral premix[c]	40 g
Vitamin premix[d]	12.5 g
Vitamin C[e]	1.5 g

[a] This prepares 1000 g (1 kg) of diet with a calculated crude protein concentration of 32.7%. Prepare feed in 2–4 kg batches.
[b] USB: USB Corp., Cleveland, OH.
[c] Williams and Briggs mineral premix (Purina Mills Test Diets, Richmond, IN).
[d] Vitamin premix supplies per kg of diet the following: vitamin A, 5500 IU; vitamin D3, 1835 IU; vitamin E, 110 IU; vitamin K, 7.3 mg; thiamin, 8.4 mg; riboflavin, 22 mg; pyridoxine, 18.4 mg; pantothenic acid, 58.7 mg; niacin, 36.7 mg; biotin, 2 mg; folic acid, 3.7 mg; vitamin B12, 0.018 mg; choline, 2327 mg; selenium, 0.1 mg.
[e] L-ascorbyl 2-polyphosphate (Stay-C, Hoffman La Roche, Nutley, NJ, USA, 25% ascorbic acid active) (Manning et al., 2003.