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Role of fumonisin B_1 on the immune system, histopathology, and muscle proteins of white shrimp (*Litopenaeus vannamei*)

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Abstract

White shrimps, *Litopenaeus vannamei*, were tested in two indoor trials to determine the effect of fumonisin B_1 on (i) immune response, (ii) histopathology, and, (iii) muscle proteins. Trial 1: (0, 0.5, 0.75 and 1.0 μg/g of FB_1 levels, 18-day duration; shrimp 5–6 g) to evaluate the FB_1 effect on the immune system and histopathology response. Trial 2: (0.0, 0.5, 0.75 and 1.0 μg/g of FB_1 levels, 16-day duration; shrimp 5–6 g) to detect FB_1 effect on muscle proteins. Prophenoloxidase activity was affected by all FB_1 concentrations tested. Both, total haemocyte count and phenoloxidase activity decreased by the 18th day in shrimp exposed to FB_1. Marked histological changes in the hepatopancreas of shrimp fed on diet containing FB_1, at the all FB_1 levels tested, as well as a necrotic tissue were observed. Changes in both, electrophoretic patterns and thermodynamic properties of myosin extracted from shrimp exposed to FB_1 were also observed.

Keywords: Fumonitoxicosis; *Litopenaeus vannamei*; Histopathology; Muscle proteins

1. Introduction

Fumonisins are a group of fungal toxins that are commonly found on corn (Shephard, Thiel, Stockenstrom, & Sydenham, 1996) and other cereals grains used worldwide in animal feed and human foods (Pohland, 1996; Visconti, Boenke, Doko, Solfrizzo, & Pascale, 1991). Several studies have reported that *F. moniliforme* and FB_1 are hepatocarcinogenic in rats (Gelderblom, Kriek, Marasas, & Thiel, 1991). Fumonisin B_1 has been reported to cause morphological and functional changes in chicken macrophages in vitro which indicate an immunosuppressing effect (Qureshi & Hagler, 1992). Fumonisin have been found to disrupt sphingolipids metabolism in hepatocytes from Sprague–Dawley rats (Riley & Voss, 2006; Wang, Norred, Bacon, Riley, & Merrill, 1991), ducks (Tran et al., 2005), and mice (Voss et al., 2002).

Scarce information is available on the effects of *F. verticillioides* (=*Fusarium moniliforme* Sheld, Nirenberg) toxins on seafood products. Dietary levels of FB_1, at or above 20 mg/kg, have shown to be toxic to channel catfish (Lumlertdacha, Lovell, Shelby, Lenz, & Kemppainen, 1995). Rainbow trout liver was sensitive to FB_1-induced changes in sphingolipid metabolism (Meredith, Riley, Bacon, Williams, & Carlson, 1998), and a cancer promoter in the presence of a initiator such as aflatoxin B1 (Carlson et al., 2001).

Fumonisin B_1 is a mycotoxin that has not been extensively studied as a shrimp feed contaminant; however, FB_1 has been detected in shrimp feed used in Sonora, Mexico at levels above the FDA recommendations (Burgos-Hernández, Farias, Torres-Arreola, & Ezquerra-Brauer, 2005). In our laboratory, the inhibition of trypsin and the potentiation of collagenase, both extracted from the hepatopancreas of farmed white shrimp, were observed

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when these enzymes were exposed to 1 μg/g FB_1 (Burgos-Hernández et al., 2005). Activation of collagenases might have an impact on shrimp muscle protein and, therefore, on shrimp muscle texture which is considered a very important quality parameter for the consumer (Ezquerra-Brauer, Salazar-Leyva, Bringas-Alvarado, & Rouzaud-Sandez, 2003).

On the other hand, phenoloxidase, part of the immune system of penaeid shrimp, is regulated by digestive enzymes from the hepatopancreas. Phenoloxidase has been reported to cause a discoloration called melanosis or blackspot in crustacean species including shrimp; this is a problem that connotes spoilage and reduces consumer acceptability, shelf life, and market value of these highly prized and economically valuable products (Kim, Marshall, & Wei, 2000).

Based on the above, the present research work was carried out to determine the adverse effects of FB_1 on cultured white shrimp. Here are reported the results of the studies done on the immune system (mainly phenoloxidase), hepatopancreas histology, and muscle protein, on the pathogenesis of fumonitoxicosis in farmed white shrimp (*Litopenaeus vannamei*).

2. Materials and methods

2.1. Formulation of feed for shrimps

Fumonisin B1-contaminated shrimp feed at concentrations of 0.5, 0.75, and 1.0 μg/g of fumonisin B_1 were prepared. Feeds were formulated and prepared according to the methodology described in the scheme of Pierson for more than 6 ingredients (Houser & Akiyama, 1997). All prepared feeds were both, isoproteic and isolipidic (Table 1) according to the proximate analysis. Appropriate amounts of fumonisin B_1 were dissolved in water to achieve the desired concentrations and incorporated into the feed formulations. All formulations were processed in a meat mill (Molino TOR–REY, Model 19, San Nicolas de las Garzas, Nuevo León, México) used as an extruder to obtain the pellets. Feed pellets were oven-dried at 60 °C, packed in high density polyethylene bags, and stored at –20 °C until further use.

Table 1
Chemical composition and fumonisin B_1 concentration of the feed used during *in vivo* assay[A]

Analysis	Control	Feed I	Feed II	Feed III
Moisture (%)[B]	13.0[a]	11.0[b]	12.0[c]	11.0[d]
Crude protein (%)[B]	45.0[a]	45.0[a]	45.0[a]	45.0[a]
Ash (%)[B]	5.0[a]	5.0[a]	5.0[a]	5.0[a]
Crude fat (%)[B]	30.0[a]	30.0[a]	30.0[a]	31.0[a]
Fibre (%)[B]	0.9[a]	0.8[b]	0.9[a]	1.0[a]
Fumonisin B_1 (μg/g)[C]	0	0.5	0.75	1.0

[A] Values followed by the same letter in a row are not significantly different at 5% level.
[B] Data are average of at least triplicate determinations.
[C] Fumonitest® affinity chromatography column.

2.1.1. Determination of fumonisin B_1 concentration in shrimp feeds

Ground FB_1-spiked feed (50 g) was mixed with 5 g of NaCl, combined with 100 ml of extraction solvent (80% methanol/water), blended at high speed for 1 min and filtered through a No. 4 Whatman filter paper. A 5 ml aliquot of the filtrate was mixed with 20 ml of a wash buffer (25 g NaCl + 5 g bicarbonate + 0.1 ml Tween, dissolved in 1 l of water) and passed through a microfiber filter. Ten milliliters of this filtrate were passed (at a slow flow rate of about 1 drop per second) through a Fumonitest® affinity chromatography column which was attached to the outlet of 10 ml reservoir on a pump stand. The column was washed twice with 10 ml of the wash buffer, and once with 10 ml of water. Washes were discarded. The fumonisin B_1 was eluted from the column using 1.0 ml of HPLC grade methanol and collected into a cuvette to which 1.0 ml of Fumonitest® developer A and B solutions were also added. The cuvette was vortexed and the fumonisin B_1 concentration was determined using a calibrated Torbex FX-100 series 3 fluorometer (VICAM, Watertown, MA, USA).

2.2. Feeding trials

Two indoor feeding trials were conducted. In both trials the same range of FB_1 (0.0, 0.5, 0.75, and 1.0 μg/g) was used. Shrimp specimens were let to adjust to climate and fed during 24 h prior to the assay at experimental conditions (27–30 °C, 35‰, pH 6.8–7.2, controlled aeration using aquarium air pumps).

The first trial was done to evaluate the effect of FB_1-contaminated feed on shrimp immunological system and histopathological analysis. The experiment consisted as follows: white shrimp (5–6 g) were divided in four groups of 10 shrimps per group and fed on diets containing 0.0 (control), 0.5, 0.75 and 1.0 μg/g of FB_1 for 18 consecutive days. This study included two independent experiments and the analyses were carried out in quintuple. Hemolymph was extracted from specimens after 18 days. After 8- and 18-day feeding periods, five shrimps from each group were sampled, starved for 24 h (to reduce digestive enzymes activity due to feeding), sacrificed, and their hepatopancreas removed for immediate analyses.

The second study was done to observe the effect of FB_1 on the proteins behavior from the muscle of white shrimp. In this assay juvenile 5–6 g white shrimps were also used. They were randomly divided in four groups and fed on the diet previously mentioned for a period of 16 days. The analyses were carried out in triplicate.

2.3. Humoral and cellular analyses

2.3.1. Hemolymph extraction

The hemolymph was extracted from the zone located between the last pair of pereiopods and the first pair of pleopods of the shrimp specimens. The volume of hemolymph extracted from every organisms was combined with