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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

2 parts of previously cooled anticoagulant, a 10 mM EDTA (ethylenediaminetetraacetic acid) isotonic solution (450 mM NaCl, 10 mM KCl, 10 mM HEPES, pH 7.3) (Vargas-Albores, Guzmán-Murillo, & Ochoa, 1993).

2.3.2. Phenoloxidase (PO) activity

The PO activity was determined according to Hernandez-Lopez, Gollas-Galvan, and Vargas-Albores (1996). Briefly, 10 μl of hemolymph and 250 μl of a L-DOPA aqueous solution were combined, incubated for 20 min at 25–28 °C, and the A_{490nm} was read using a microplate reader (BioTech).

2.3.3. Total prophenoloxidase (totProPO) detection

In a microplate, 10 μl of hemolymph, 10 μl of a 1.0 mg/ml trypsin aqueous solution, and 250 μl of a L-DOPA aqueous solution were combined, incubated for 10 min at 25–28 °C, and the A_{490nm} was recorded using a microplate reader (Gollas-Galvan, Sotelo-Mundo, Yepiz-Plascencia, Vargas-Requena, & Vargas-Albores, 2003). The proPO activity was calculated subtracting the totPO value to the PO data.

2.3.4. Total count of haematocytes

The total number of haematocytes was determined microscopically by direct count using a Neubauer chamber and according to the technique described by Hernandez-Lopez and Vargas-Albores (2003). Briefly, 10 μl of hemolymph were combined with 90 μl of SIC EDTA (containing 20% formol). A 10 μl aliquot of this solution was placed in a Neubauer chamber, let to rest for 1 min, and the haematocytes were counted directly using a microscope (20X).

2.3.5. Reduction of NTB by haematocytes

To determine the amount of superoxide anion the reduction of nitro blue tetrazolium (NBT) by haematocytes was measured (Munoz et al., 2000). Hemolymph (100 μl) was placed in a microplate and incubated for 30 min at room temperature. The supernatant was discarded and 50 μl of 0.3% NBT were added and incubated for 2 h at room temperature. The supernatant was again discarded, and the haematocytes were fixed with 200 μl of absolute ethanol. Haematocytes were washed twice with 200 μl 70% methanol and let dry. The formazan deposits generated were dissolved in 120 ml 2 M KOH + 140 ml dimethyl sulfoxide (DMSO), and the A_{620nm} was recorded.

2.4. Histological study

After 8 and 18 days of feeding trial, the hepatopancreas of live shrimps were obtained and observed using an optical microscope. Briefly, shrimp was decapitated alive and the cephalotorax was fixed letting it rest for 24 h in Davidson solution (sea water – 95% ethanol – 40% formaldehyde – glycerol [3.3:3.3:2.2:1.2, v/v] in 10% glacial acetic acid) (World Organization for Animal Health, 2006). Then, 2 mm-thick slices were obtained and dehydrated using a tissue processor Tissue-TEK 11 (Mishawaka, IN) according to Lightner and Redman (1982). Dehydrated tissue samples were placed in epoxic resine (HistoEmbedder, Leica Nussloch, Germany) at 56 °C. Sections (5 μm-thick) were prepared using a microtome (AG, Scientific Instruments, Buffalo, NY) and stained following the hematoxiline and eosine standard technique (Prophet, 1992).

2.5. Muscle protein analysis

2.5.1. Muscle protein extraction

To extract myofibrillar proteins from the muscle of the FB_1-exposed shrimp, 1 g of shrimp muscle was homogenized for 2 min with 10 ml of urea (8 M urea, 0.1 mM phenylmethylsulphonyl fluoride, 0.1 M ethylenediaminetetraacetic acid (EDTA), 0.01% sodium azide, and 0.6 M KCl) solution, using a Biospec model 133 (Biospec Co., Bartlesville, OK, USA) tissue homogenizer. The extract was mixed (1:3) with a buffer solution (25% of 0.05 M TRIS–HCl pH 6.8, 20% glycerol, 40% of 10% sodium dodecyl sulphate, 15% deionized water, and 0.03% bromophenol blue) with denaturing characteristics (lack of mercaptoethanol). Triplicate samples from each group were extracted. The mixture was heated in boiling water for 4 min and was frozen-stored for further electrophoresis.

2.5.2. Electrophoresis

Electrophoresis was carried out according to Laemmli (1970) using a MiniProtean III equipment (BioRad Laboratories Chemical, Hercules, CA, USA). Stacking and separating gels were prepared with 4 and 10% acrylamide, respectively. Aliquots of 100 μg of ureic extracts from shrimp muscle were applied to the gel, and the electrophoresis was carried out at 4 °C and 120 V. Gels were developed using a 0.1% (w/v) Cooummassie Blue as a dying solution.

2.5.3. Differential scanning calorimetry

Three organisms from each group were taken and a muscle sample was obtained from the first abdominal segment from each one of them. The muscle samples (35–40 mg) were placed in stainless steel capsules hermetically sealed, and the enthalpies (J/g) and maximum temperature of transition (°C), were determined using a Perkin–Elmer DSC-7 calorimeter (Norwalk, NC, USA). Measurements were carried out at a scanning rate of 10 °C/min within a temperature range of 26–124 °C, using air as reference (Beas, Wagner, Anon, & Crupkin, 1991). Thermograms were obtained for three shrimp specimens sampled from each treatment at days 8 and 16, and analyses were done in triplicate.

2.6. Statistical analysis

Data from degree of hydrolysis, cellular counts, enzymatic activities, and calorimetric parameters, were analyzed applying a bifactorial design (Factor 1: FB_1-spiked feed, and Factor 2: period of sampling). Means were compared by analysis of variance. Where differences were detected, a Tukey test was applied with a significance level

of 5%, using the JMP 4.0 statistical software (Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Proximate analysis and fumonisin B_1 (FB_1) content of shrimp feed

Shrimp feed was spiked with pure FB_1 to achieve levels of 0, 0.5, 0.75, and 1.0 μg FB_1/g. Fumonisin B1 analysis of spiked feed indicated that the three levels were reached (Table 1).

3.2. Effect of FB_1 on white shrimp immune humoral and cellular system

Several fungal toxins are known to be immunosuppressive and most of the studies found in the literature suggest that these toxins can adversely affect components of the immune system (Qureshi & Hagler, 1992). Phenoloxidase (PO) plays a crucial role in shrimp immune response (Gollas-Galvan et al., 2003) because it is an enzyme involved in shrimp melanin synthesis, oxidizing phenols to quinones which polymerize melanin, a pigment with microbicide properties. PO is found as an inactive pro-enzyme known as prophenoloxidase (ProPO) (Gollas-Galvan et al., 2003).

The total prophenoloxidase (totProPO) activity in the hemolymph extracted from the white shrimp exposed to three feed exposed to FB_1 was statistically different compared to the control (Table 2). Similar results were observed when PO activity was assayed. Several studies have reported that the total phenoloxidase (totPO) activity increased when white shrimp and crab were exposed (via feed) to a variety of compounds (β-glucans and peptideglucans) commonly found in biological contaminants such as fungi and bacteria (Itami et al., 1998; Sung, Chang, Her, Chang, & Song, 1999). Also, Boonyaratpalin, Supamattaya, Verakunpiriya, and Suprasert (2001) reported an increase in PO when white shrimp was exposed to aflatoxin B_1 (AFB_1), a well known mycotoxin found in a wide variety of commodities, including feed for aquaculture (Burgos-Hernández et al., 2005). Therefore, the effect of FB_1 might be the decrease in phenoloxidase activity, as a response of the shrimp towards the foreign or toxic substances.

The activation of the humoral immune system (ProPO and PO activities) of white shrimp generates several factors that seem to stimulate the heamatocytes population (cellular immune system) that shrimp uses to eliminate the foreign material (Hernandez-Lopez et al., 1996). Based on the results obtained during the study of the humoral immune system described above, the effect of FB_1 exposure on the count of haematocytes in farmed white shrimp was investigated. White shrimp was exposed to FB_1-spiked feed during 18 days and the count of haematocytes per ml of hemolymph was obtained after 18 days. As shown in Table 2, a reduction in the number of haematocytes compared to the control was observed when white shrimp was exposed to FB_1 at all levels tested. Similar results were reported by Boonyaratpalin et al. (2001), they observed up to 19% decrease in the number of haematocytes per ml of hemolymph in white shrimp fed on feed spiked with AFB_1 levels ranging from 0 to 2500 ng/g. This response is noted as a decrease in the number of blood cells in the circulatory system.

The above results suggests that exposure to FB_1 might cause damage or even death of heamatocytes; therefore we proceeded to determine the amount of superoxide anion produced when these immune cells are exerting their protective role in the organisms. As shown in Table 2, the amount of superoxide anion found in white shrimp was always below the level observed in the control. The low level of superoxide anion detected is in accordance with the decreased level of haematocytes observed in white shrimp hemolymph, suggesting a depression of the cellular immune activity due to the amount of immune cells present. Similar results were reported by Rodríguez (1996) who reported that the amount of superoxide anion decreases in white shrimp fed with feed containing different levels of protein and different concentrations of β-glucans. Fumonisins can be regarded as foreign substances in shrimp that stimulate shrimp to activate a mechanism to excrete them. Fumonisin B_1 causes a drop in the activity of such immuno-competent cells, and, hence, a decline in the shrimp immune response.

3.3. Histological study

Figs. 1a and 2a show histological observations for the control organisms throughout the entire experiment. The

Table 2
Effects of fumonisin B_1 on humoral and cellular systems in juvenile white shrimp (after feeding for 18 days)

Parameter	Fumonisin B_1 (μg/g)			
	0[B]	0.5[B]	0.75[B]	1.0[B]
Prophenoloxidase (Units/min/mg protein)[A]	57.24 ± 6.4^{a}	15.97 ± 5.4^{c}	44.42 ± 8.3^{b}	32.6 ± 8.6^{bc}
Phenoloxidase (Units/min/mg protein)[A]	72.8 ± 14.7^{a}	24.74 ± 2.3^{b}	31.13 ± 9.8^{b}	16.89 ± 6.7^{b}
Total haemocyte ($\times 10^6$ cell/ml)[A]	36.0 ± 2.6^{a}	16.0 ± 3.5^{b}	21.0 ± 2.9^{b}	13.0 ± 2.9^{b}
Superoxide anion[C] (rate)[A]	0.31 ± 0.01^{a}	0.14 ± 0.01^{b}	0.08 ± 0.002^{c}	0.01 ± 0.001^{d}

[A] Values followed by the same letter in a row are not significantly different at 5% level.
[B] Data are average of five determinations.
[C] Reduction of NTB.

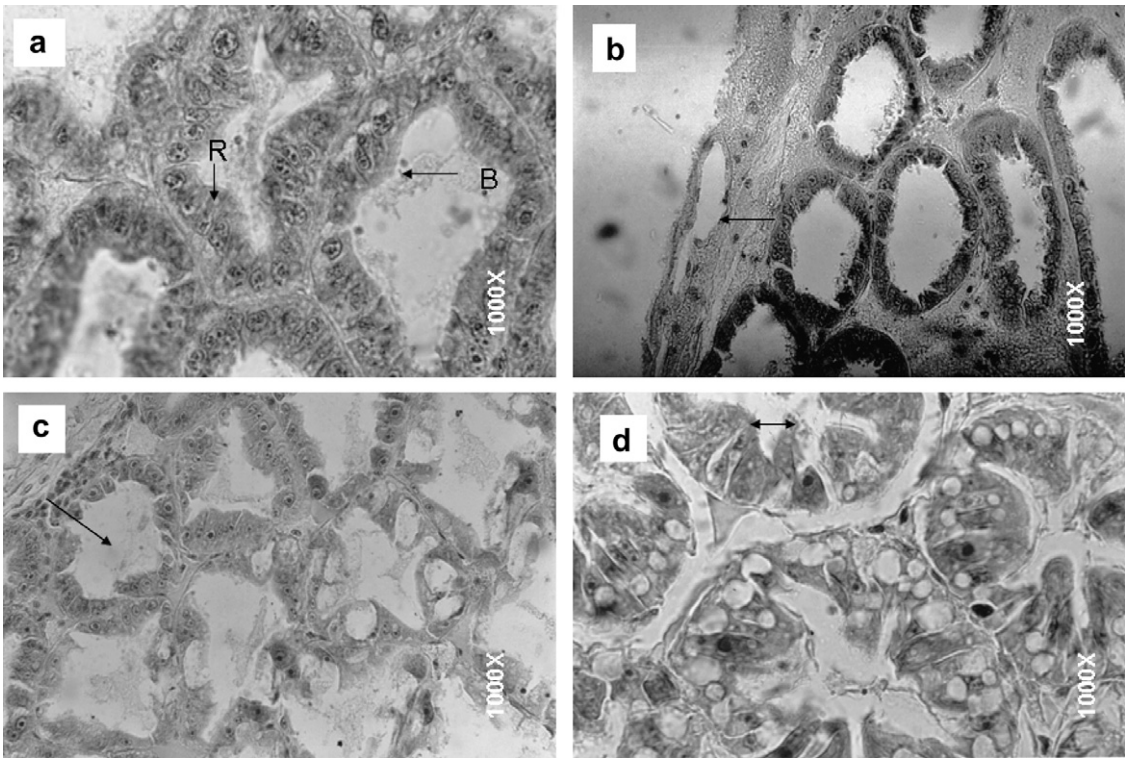

Fig. 1. Histological observations of hepatopancreas from white shrimp exposed during 8 days to (a) Hepatopancreas of healthy white shrimp (fumonisin 0.0 μg/g) showing complete structure of different cell. (b) Atrophic changes of hepatopancreas observed in shrimp fed with diet containing fumonisin 0.5 μg/g. Arrows indicates R-cell that was reduced in size. (c) Atrophic changes of hepatopancreas observed in shrimp fed with diet containing fumonisin 0.75 μg/g. Arrows indicates cell alteration. (d) Shrimp fed with fumonisin 1.0 μg/g, hepatopancreas exhibited atrophic and vacuolization. Arrows indicates vacuole alteration.

cellular structure of the tubule shows the disposition of normal cells.

After 8 days of exposure to 0.5 μg FB_1/g feed, a deformation of the hepatopancreas tubules was observed; they presented a retraction of the cells with a loss of normal anatomy compared to the control (Fig. 1b). After 18 days, a similar pattern was observed with presence of melanization (Fig. 2b). After 8 days of exposure to 0.75 μg FB_1/g feed (Fig. 1c), a severe vacuolization (a form of inflammatory reaction) on tubules was observed in addition to cellular retraction. These anomalies were also detected after 18 days of exposure (Fig. 2c). Finally, after 8 (Fig. 1d) and 18 days (Fig. 2d) of exposure to 1.0 μg FB_1/g feed, a severe damage characterized by an excessive vacuolization was evident. It is important to mention that at 1.0 μg FB_1/g feed, shrimp branches was severely damaged and it was characterized by deformities in the structure (Fig. 3).

Boonyaratpalin et al. (2001) reported severe atrophy of the hepatopancreatic cells in white shrimp after being fed with 500 ng/g AFB_1-spiked feed during 8 weeks. When these organisms were exposed to 1000 ng AFB_1/g, disperse necrosis of hepatopancreatic cells was observed. Hepatopancreatic cellular size reduction and hematocyte infiltration in *Penaeus monodon* exposed to 50 ng AFB_1/g during 60 days had been previously reported (Bautista, Lavilla-Pitogo, Subosa, & Begino, 1994). They also observed signs of melanization and hepatopancreatic cellular necrosis. Ostrowski-Meissner, LeaMaster, Duerr, and Wlash (1995) reported that exposure to 15 μg AFB_1/g for 21 days atrophied the epithelial cells and formation of fibrous tissue that replaced the normal anthenal gland architecture. The current experiment clearly shows that when high concentrations of FB1 were introduced for short periods, it led to the histological changes observed in the hepatopancreas of all the samples examined.

3.4. Effect of FB_1-spiked feed on muscle protein of farmed white shrimp

To determine the effects of FB_1 on the proteins from the muscle of white shrimp, an electrophoretical and thermic properties analysis were performed. Proteins from the muscle of white shrimp after 16 days of exposure to FB_1-spiked feed were extracted.

Fig. 4 shows the electrophoretic pattern of: (A) molecular weight markers, (B) protein extract from control, and (C, D, and E) extracts from white shrimp exposed to 0.5, 0.75, and 1.0 μg FB_1/g of FB1 contaminated-feed, respectively. In lane B, several protein fractions are presented, identified by their theorical relative mobilities (Srinivasan, Xiong, Blanchard, & Tidwell, 1997), myosin within the molecular weight range of 200 kDa; 97 kDa paramyosin

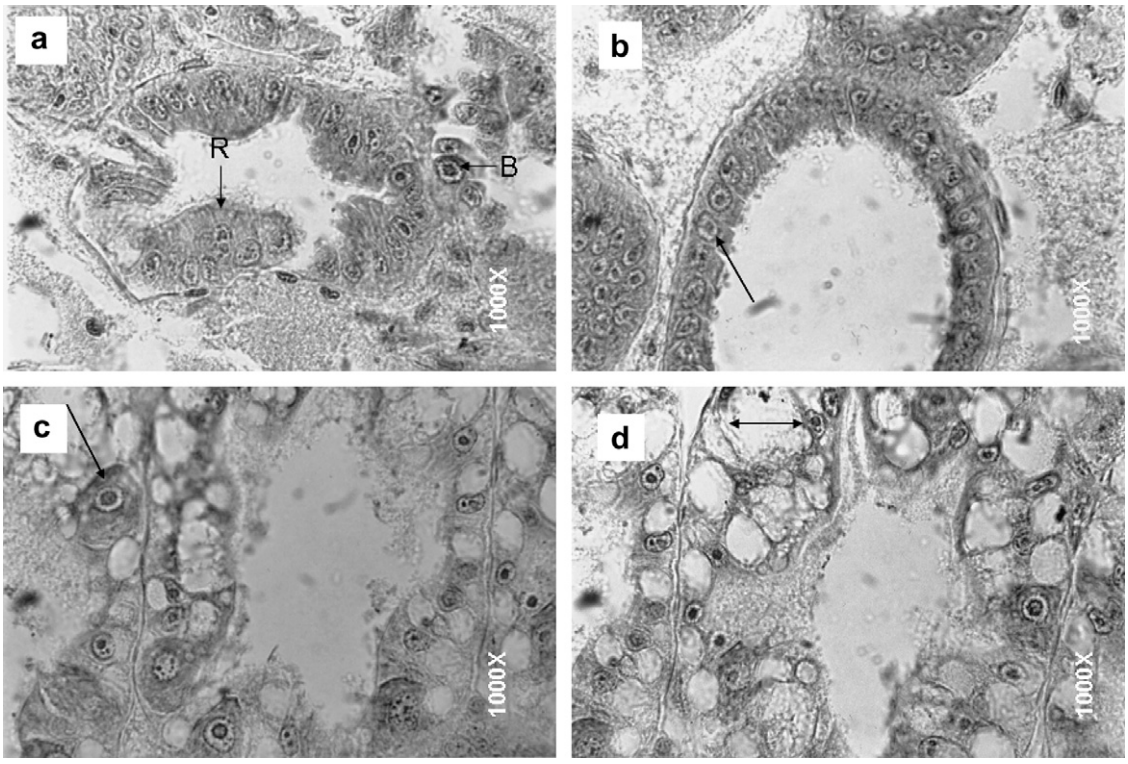

Fig. 2. Histological observations of hepatopancreas from white shrimp exposed during 18 days to (a) Hepatopancreas of healthy white shrimp (fumonisin B_1 0.0 μg/g) showing complete structure of different cell. (b) Atrophic changes of hepatopancreas observed in shrimp fed with diet containing fumonisin 0.5 μg/g. Arrows indicates R-cell that was reduced in size. (c) Severe atrophic changes of hepatopancreas observed in shrimp fed with diet containing fumonisin B_1 0.75 μg/g. Arrows indicates cell alteration. (d) Shrimp fed with fumonisin B_1 1.0 μg/g, hepatopancreas exhibited severe atrophic and excessive vacuolization. Arrows indicates vacuole alteration.

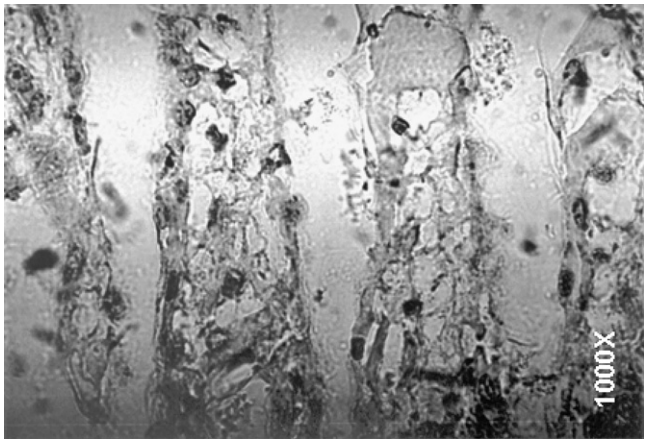

Fig. 3. Histological observations of branches from white shrimp exposed during 18 days to 1.0 μg FB_1/g of feed.

fractions; 45 kDa actin fraction; and under 45 kDa the myosin light polypeptidic chains. Comparing lane B to the rest of the lanes, a variation in color intensity was first detected (Fig. 4, lanes D and E). These observations might suggest that the muscular tissue of muscle fiber had probably been damaged due to exposure shrimp to FB_1.

It is important to mention that the fraction corresponding to molecular weights under the 200 kDa is not observed when shrimp was exposed to 1.0 μg FB_1 contaminated-feed

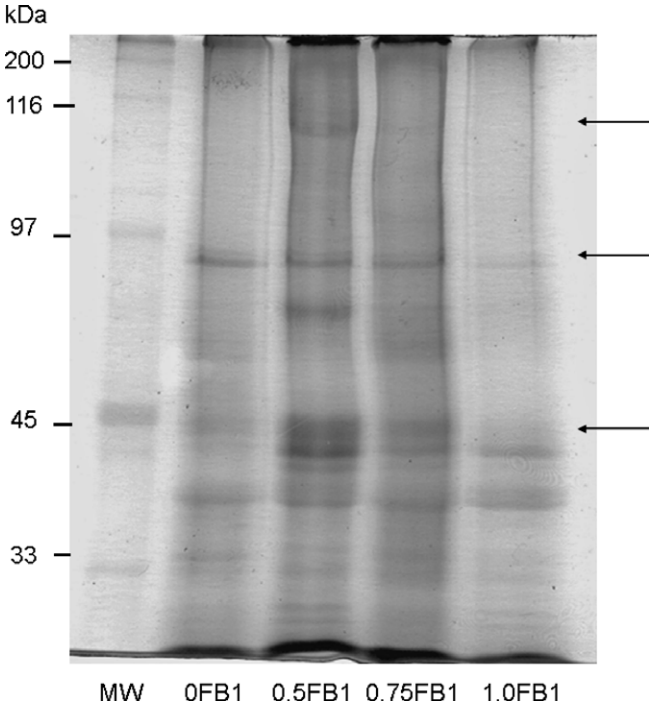

Fig. 4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) of the myofibrillar proteins extracted from white shrimp muscle. Lane A, molecular weight markers, Lane B, protein extract from control, and Lane C, D, and E, extracts from white shrimp exposed to 0.5, 0.75, and 1.0 μg FB_1/g of feed, respectively.

Table 3
Enthalpies, and temperatures of transition of muscle from farmed white shrimp (*Litopenaeus vanammei*) after 8 and 16 days of being exposed to FB_1-spiked feed.

Treatment	Peak I[D]			Peak II[D]			Peak III[D]		
	Enthalpy (J/g)[A]	T_0 (°C)[B]	T_{max} (°C)[C]	Enthalpy (J/g)[A]	T_0 (°C)[B]	T_{max} (°C)[C]	Enthalpy (J/g)[A]	T_0 (°C)[B]	T_{max} (°C)[C]
After 8 days of exposure									
Control	1.3[a]	44.3[a]	47.8[a]	0.7[a]	57.5[a]	64.8[a]	0.3[a]	79.5[a]	84.1[a]
0.5 μg/g	1.1[b]	44.1[a]	47.1[a]	0.5[b]	57.8[a]	66.9[a]	0.4[a]	79.1[a]	82.6[a]
0.75 μg/g	1.1[b]	43.1[a]	43.1[a]	0.2[b]	58.4[a]	65.4[a]	0.3[a]	76.6[a]	80.9[a]
1.0 μg/g	0.9[b]	44.5[a]	44.5[a]	0.4[b]	61.7[a]	68.2[a]	0.3[a]	78.5[a]	83.2[a]
After 16 days of exposure									
Control	1.3[a]	42.8[a]	47.8[a]	0.7[a]	57.8[a]	61.1[a]	0.4[a]	88.9[a]	83.7[a]
0.5 μg/g	0.9[b]	44.4[a]	45.9[a]	0.6[a]	59.2[a]	64.6[a]	0.3[a]	79.4[a]	82.2[a]
0.75 μg/g	0.7[b]	45.4[a]	49.6[a]	0.4[b]	62.2[a]	66.9[a]	0.4[a]	77.0[a]	84.3[a]
1.0 μg/g	0.9[b]	44.8[a]	48.1[a]	0.4[b]	60.2[a]	65.2[a]	0.3[a]	78.9[a]	83.9[a]

[A] Enthalpy: change in total enthalpy in shrimp muscle denaturation.
[B] T_0 (°C): Initial temperature.
[C] T_{max} (°C): Maximum temperature of heat flow.
[D] Values followed by the same letter in a column are not significantly different at 5% level.

(Fig. 4, lane E) suggesting a possible hydrolysis of myosin. These results suggest that the texture of muscle of white shrimp fed with 1.0 μg FB_1/g of feed might be affected. Regarding to actin, no changes were apparently detected; this implies that actin is more stable, even under the presence of FB_1.

It is known that functional and texture characteristics of meat mainly depend on their myofibrillar proteins, therefore, the study of the thermal behaviour of myofibrillar proteins is of technological importance to determine and predict the final quality of meat products. Differential scanning calorimetry (DSC) offers a direct means to study the thermal transition of muscle proteins *in situ*. A rapid and simple way to follow the muscle proteins behaviour before shrimp harvest might be the study of the thermal of its myofibrillar proteins. Therefore, the effect of FB_1 exposure on the thermal behaviour of muscle protein of farmed white shrimp was studied measuring both, maximum peak temperature (T_{max}) and denaturation enthalpies by DSC.

DSC thermograms of shrimp muscle from the treated groups and the control showed (Table 3) three endothermic transitions peaks, with T_{max} values of 52, 72, and 85 °C. Two of those transition peaks corresponded to myosin (Peak I: 57–60 °C) and actin (Peak III: 80–90 °C) denaturation (Srinivasan et al., 1997). Peak II (74–80 °C) could be attributed to either sarcoplasmatic proteins or connective tissue (Srinivasan et al., 1997). As shown in Table 3, the onset temperature (T_0) and T_{max} for the three peaks were not affected by the FB_1. However, the total enthalpy (ΔH) value of whole shrimp was different for each treatment. During the trial in shrimp fed on FB_1contaminated-feed, the lowest values for total enthalpy were observed mainly on peak I and peak II, whereas in the ΔH of peak III, corresponding to actin, no changes were recorded (Table 3). The low change in actin denaturation enthalpy due to FB_1 might be related to actin behaviour observed in the electrophoretic analysis. This confirms that the actin was not affected by the FB_1.

Results from the electrophoretic pattern and DSC analysis of muscle shrimp exposed to FB_1 suggested disruption of muscle cells, mainly on myosin heavy chain. Disruption of muscle cells might be caused by excessive muscle protease activity. Many endogenous proteases are considered important in postmortem degradation of muscle proteins (Jiang, 2000). Among them are collagenases that cleave the collagen molecule (Jiang, 2000) and have been implicated in postharvest deterioration in marine invertebrates (Simpson, 2000). Previously we reported that the collagenase-like activity was stimulated by FB_1 (Burgos-Hernández et al., 2005). Therefore considering collagen as a major component of the basement membrane, it may weaken the connective tissue and consequently cause disintegration of tissue. Based on evidences of protein hydrolysis in exposed FB_1 shrimp muscle when analyzed by electrophoresis, it is speculated that the activation of proteases, if any, occurred because of FB_1. However, more studies are needed to determine the real effects of FB_1 on muscle proteins shrimp.

4. Conclusion

Histopathological findings indicated hepatopancreatic damage by FB_1 with changes in hemolymph, suggesting immunosuppressing effect. The effects observed on histological changes were directly correlated with the concentration of FB_1 present in formulated feeds and the period of feeding. Muscle protein analysis suggested hydrolysis of myofibrillar muscle proteins by FB_1. The knowledge of thermal stability of muscle proteins of *L. vannamei* provide important information to try to understand how the muscle will behave during FB_1 exposure. The current experiments were developed in a short period; therefore, since no tumor development and mortality were observed, and growth

performance was not evaluated, a chronic exposure type of study would be appropriate to determine the response of this specie to an actual exposure to FB_1 in the farming ponds. Although consumer's health risk due to the consumption of mycotoxin-contaminated shrimp appears to remain low (Bintvihok et al., 2003), the presence of FB_1 in feed indeed might affect shrimp production as well as shrimp muscle texture, which could affect its acceptability by consumer. FB_1-contamination feeds are preventable if the raw materials for feed formulation are carefully inspected and properly managed.

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