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Histological alterations in the hepatopancreas of *Penaeus monodon* Fabricius (1798) given aflatoxin B_1-incorporated diets

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Abstract

Aflatoxin is a toxic contaminant produced by toxigenic fungi of the genus *Aspergillus* during the processing and storage of feeds and feed ingredients. Aflatoxins can cause abnormalities such as poor growth, physiological imbalances and histological changes that result in a reduction in the yield and profitability of shrimp culture. Histological changes in *Penaeus monodon* sub-adults fed different doses of aflatoxin B_1 were studied. The doses of aflatoxin B_1 administered in the diets were 50, 100, 150, 500, 1000 and 2000 ppb. At the end of the fourth and the eighth weeks of the experiment, the shrimps were sampled and the cephalothorax was observed for histological changes. Significant changes were observed in the different treatment groups at the fourth and eighth weeks. The severity of pathological changes was proportional to the increase in the concentration of aflatoxin fed to the shrimps. Histological changes in the hepatopancreas were loss of structure of the cells and tubules, nodule formation, cell elongation, desquamation, rounding of cells, fibrosis, necrosis, haemocytic infiltration and cellular inflammation.

Keywords: aflatoxin B_1, AFB_1 incorporated feed, *Penaeus monodon*, hepatopancreas, histological changes

Introduction

Aflatoxins are particularly important in aquaculture because their presence exerts a negative economic impact on relevant commerce as well as severe health problems after exposure to contaminated food and feed. Among the mycotoxins, aflatoxins are the most toxic and are of considerable interest in the fields of agriculture, livestock and aquaculture. Aflatoxins are extremely biologically active secondary metabolites produced by the fungi, *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are receiving increasing attention from researchers, the food industry and the general public – firstly because they reduce production and secondly they remain as residues in animal tissues, which in turn affects the human metabolic system on being consumed.

To date, only three species of the fungi have been reported to produce aflatoxins. These are *A. flavus*, *A. parasiticus* and *Pencillium tuberculum*. The toxins produced by moulds are broadly classified as nephrotoxins, hepatotoxins and neurotoxins depending on the haematological effects and general digestive disorders they cause. Aflatoxin comes under the category of hepatotoxins and targets its activities mainly on the liver (Spensley 1963). Aflatoxins are polycyclic unsaturated compounds with a coumarin molecule flanked on one side by a bisfuran moiety and on the other by either a pentanone for B series or a six-membered lactone for G series (Coulombe 1991).

The culture of *Penaeus monodon* (Fabricius 1798) is constantly hampered by outbreaks of bacterial, viral and/or parasitic diseases and also by environmental and nutrition-related diseases. One such constraint is the disease caused by fungal contamination of feed that often causes secondary infections. Experimental studies of aflatoxicosis in shrimps were restricted to *Peneaus vannamei* (Lightner, Redman, Price & Wiseman 1982), *P. stylirostris* (Wiseman, Price, Lightner & Williams 1982; Ostrowski-Meissner, LeaMaster, Duerr & Walsh 1995) and *P. monodon*, the most cultivated penaeid shrimp in India and

elsewhere (Boonyaratpalin, Supamattaya, Verakunpiriya & Suprasert 2001).

Only a few species of crustaceans like Brine shrimp, *Artemia salina* (Harwig & Scott 1971), copepod, *Cyclops fuscus* (Reiss 1972), and water flea, *Daphnia pulex* (Sinnhuber & Wales 1978), have been tested for aflatoxin sensitivity. Red disease or discolouration, the prominent diagnosis of aflatoxicosis, was first reported in *P. monodon* by Liao (1977) in Taiwan; the principal lesion types observed were marked atrophy and necrosis of the hepatopancreas, accompanied by an intense cellular inflammatory response. Histological observations in shrimps fed with a diet containing aflatoxin (150 and 200 g g^{-1} of feed) indicated severe damage to the hepatopancreas (Cruz & Tendencia 1989). The histopathological alterations in *P. stylirostris* and *P. vannamei* were time and dose dependent in the hepatopancreas, mandibular organ and in the haematopoietic organs (Lightner *et al.* 1982). Juvenile *P. vannamei* fed 50–300 ppm aflatoxin B_1 showed lesions in the hepatopancreas, mandibular organ and haematopoietic organ. Lavilla-Pitogo, Bautista and Subosa (1994) observed histopathological changes in *P. monodon* juvenile fed AFB_1 (26.5–202.8 ppb for 60 days).

Ostrowski-Meissner *et al.* (1995) observed growth rate and sub-lethal effects in *P. vannamei* juveniles fed AFB (3–15 ppm). Boonyaratpalin *et al.* (2001) observed atrophic changes and necrosis in hepatopancreas tubules in *P. monodon* fed 50–2500 ppb AFB_1 for 60 days. Kalaimani, Ali, Shanmugasundaram and Sarathchandra (1998) have reported the presence of aflatoxin in imported and indigenous shrimp feeds in the range 10–130 ppb collected from shrimp farms in Andhra Pradesh in India. The USFDA has regulated the levels of AFB_1 in food commodities to be processed into foods and has established an action guideline of 20 ppb for total aflatoxin (FDA 1989). The objective of the present study was to determine the histological changes caused by feeding different doses of AFB_1-incorporated diets in *P. monodon*.

Materials and methods

Experiment protocol

Two hundred sub-adult *P. monodon* of size 7.5 ± 0.72 g brought from a farm at Narakkal, Ernakulam district, Kerala, were acclimatized to 20 ± 0.5 g L^{-1} salinity for 1 week in holding tanks of 2-tonne capacity. One control and six treatment groups were selected for the experiment of 60 days duration. The doses of aflatoxin selected were 0, 50, 100 ppb, 150, 500, 1000 and 2000 ppb (Table 1). Shrimps were weighed and about 26 were segregated into separate 1-tonne fibreglass-reinforced plastic tanks of 2 m length, 1 m width and 0.5 m depth. The shrimps stocked in one tank were taken as one treatment, and after 4 weeks of feeding AFB_1, 13 shrimps were sacrificed for analysis and the rest of the 13 were sacrificed at the end of the experiment. Three shrimps from each group were taken for normal histological analysis at the start of the experiment, and samples were made in triplicate at the fourth and the eighth weeks for individuals exposed to each dose.

Table 1 Dosage of AFB_1 in shrimp feeds for study on histological changes in *Penaeus monodon*

Doses of AFB_1 (ppb)	Working solution of AFB_1 (mL)	AFB_1 in feed per 500 g (μg)
50	0.125	25
100	0.25	50
150	0.375	75
500	1.25	250
1000	2.5	500
2000	5	1000

Experimental diets

Preparation of a stock solution and a working solution of aflatoxin B_1

A pure crystalline powder of aflatoxin B_1 was obtained from Sigma chemical company, St Louis, MO, USA (Product Name A6636). Aflatoxin B_1 (50 mg) was dissolved in 5 mL of chloroform to form a stock solution containing 10 mg AFB_1/mL of chloroform. From this, a working solution was prepared by adding 1 mL of the stock solution to 49 mL of chloroform (10 mg aflatoxin in 50 mL of chloroform). The working solution was stored in amber-coloured bottles sealed tightly with teflon and cellotape and stored under refrigeration. Before addition of the toxin to experimental feeds, the required amount of toxin dissolved in chloroform from the working solution was taken in a glass beaker, evaporated in a water bath and replaced with equal volumes of ethanol.

Feed formulation and preparation

The feed ingredients chosen for preparing the experimental diets were fish meal, shrimp meal, clam meal, soyabean meal as protein sources, wheat flour as a

Table 2 Feed formulations of shrimp feeds for sub-adults of *Penaeus monodon*

Ingredients	Weight (g)
Fish meal	14
Shrimp meal	14
Clam meal	12
Soyabean meal	20
Wheat flour	24
Vitamins*	2
Minerals†	3
Oil‡	5
Lecithin	2
Gelatin	4
Total	100

*Vitamin premix (mg or IU kg^{-1} in diet): Vit. A, 6000 IU; Cholecalciferol, 1500 IU; Tocopherol acetate, 150 IU; Menadione, 20 mg; Ascorbic acid, 200 mg; Thiamine hydrochloride, 60 mg; Riboflavin, 40 mg; Calcium pantothenate, 60 mg; Pyridoxine hydrochloride, 40 mg; Nicotinic acid, 200 mg; D-Biotin, 1 mg; Choline chloride, 500 mg; Inositol, 250 mg.

†Mineral premix (g Kg^{-1} in diet) $CaHPO_4.2H_2O$, 8 g; $Mg\ SO_4.7H_2O$, 5 g; KH_2PO_4, 4 g; $Na_2\ H_2PO_4.2H_2O$, 2 g; $MnSO_4.H_2O$, 0.6 g; $FeSO_4$, 0.6 g; $ZnSO_4.7H_2O$, 0.6 g; $CO(NO_3)_3$, 0.1 g; $CuSO_4.5H_2O$, 0.1 g.

‡A combination of 1:1 cod liver oil and vegetable oil.

carbohydrate source and codliver oil as a lipid source. Moisture, crude protein and crude fibre levels in the feed and feed ingredients were determined as per AOAC (1990). Feed formulation was carried out using the standard protocol of AOAC (1990). Shrimp feed formulation containing 38% crude protein was used to prepare experimental diets (Table 2). All the feed ingredients were taken to prepare 500 g each of seven types of feed viz, control diet, and six test diets: 50, 100, 150, 500, 1000 and 2000 ppb.

Data collection

The tanks were labelled with a clipboard indicating details of the experiment such as dose of aflatoxin and day of experiment for record maintenance. The tanks were monitored daily for any unusual behaviour and feeding activity. The feeding rate was 4.2% for the first 30 days and 3.5% for next 30 days. Small amounts of feed were added at a time to ensure complete feeding to avoid feed wastage and contamination. The data collected from each group and replicate tanks included feed given and consumption, mortality and salinity, temperature and dissolved oxygen in the water. Water exchange was carried out daily at a 50% level. Water quality, temperature, dissolved oxygen and salinity were monitored daily. The salinity was maintained at $20 \pm 0.5\ g\ L^{-1}$, the temperature at $26 \pm 2\ ^{\circ}C$ and the dissolved oxygen at $5 \pm 2\ mL\ L^{-1}$ for the entire experimental duration. The shrimps were observed for any unusual behaviour and morphological changes. The cephalothorax region was used for histological study.

Histology

Three cephalothoracic regions from each treatment were fixed in Davidson's fixative overnight at the fourth and the eighth weeks of the treatment. Tissues were processed for paraffin embedding in a Leica TP 1020 automatic tissue processor (Leica Microsystems, Nussloch, Germany) and sections of 5–6 μm thickness were cut in a Leica RM 2145 semi-automatic rotary microtome. Longitudinal and transverse sections of the tissues were taken. The paraffin sections taken on glass slides were cleared in xylene, hydrated with descending grades of alcohol, stained in haematoxylin, passed through acid alcohol, Scott's top water and then stained by eosin. The stained sections were dehydrated in ascending grades of alcohol, cleared in xylene before mounting with DPX and observed under a light microscope (Leica DMLS).

Results

The cephalothorax of the control shrimp revealed normal architecture. Hepatopancreas sections alone showed drastic changes in the treatment groups. The lymphoid organ, mandibular organ and antennal gland revealed only mild lysis. The hepatopancreas was selected for detailed examination because it was the most affected target organ.

In the control group, the hepatopancreas was normal (Fig. 1) along with the proximal and the apical regions, specific components of the stomach and midgut namely, the gastric sieve and the lappets. The lumen contained a granular material and the lumen-facing surface of the tubule was covered with a microvillus border. The tubular apex contained undifferentiated embryonic cells (E cells). Proceeding away from the apex, the cells began to differentiate into storage cells (R cells/Restzellen). In the median region, R cells and F cells (Fibrillazellen) were observed. Those F cells farthest from the tubular apex were more basophilic and larger than those nearest the apex. F cell nuclei were larger than those of R cells and each typically contained one prominent nucleus. The cytoplasm of R cells characteristically contained numerous nuclei. The proximal region of the tubule

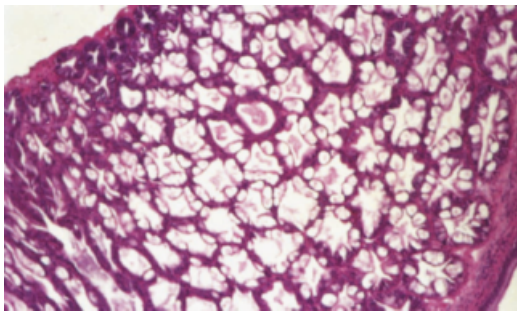

Figure 1 Tranverse section (TS) of the hepatopancreas of the shrimp in the control group. Note the normal structure of tubules, lumen and cells. H&E × 100. H&E, haemotoxylin and eosin.

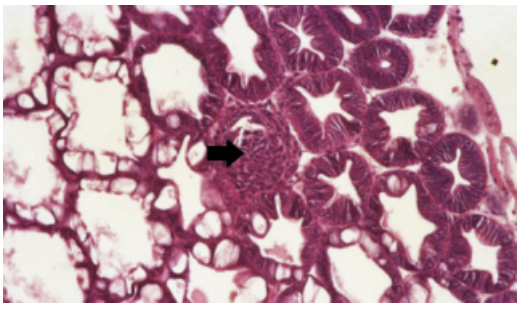

Figure 3 TS of hepatopancreas of 50 ppb aflatoxin B_1-fed shrimp at 4 weeks. Note the haemocytic nodule formation in the tubules (arrow). H&E × 200. TS, tranverse section.

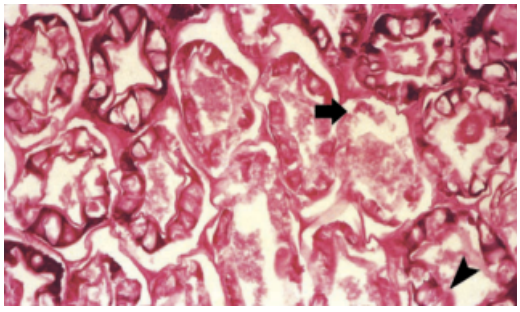

Figure 2 TS of hepatopancreas of the shrimp at 4 weeks given 50 ppb aflatoxin B_1, revealing a change in structure of tubules (arrow) and loss of brush-border appearance (arrow head). H&E × 200. TS, tranverse section; H&E, haemotoxylin and eosin.

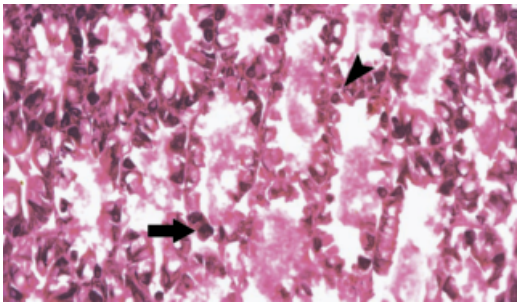

Figure 4 TS of hepatopancreas of shrimp given 50 ppb aflatoxin B_1 at 8 weeks, showing desquamation of tubules (arrow) and loss of cells (arrow head). H&E × 200. TS, tranverse section; H&E, haemotoxylin and eosin.

contained large distinctive secretory cells (B cells/ Blasenzellen), each of which contained one larger vacuole and a convex luminal surface. The lymphoid organ and the antennal gland in the control group revealed normal structures.

In the 50-ppb treatment group, at 4 weeks, there was lysis of tubules and loss of architecture in a few areas, a change in the structure of tubules, loss of brush border appearance (Fig. 2) and haemocytic nodule formation in the tubules (Fig. 3); more lysis was noticed in the apical and middle regions of the hepatopancreas. No significant changes were observed in the lymphoid organ and the antennal gland. After 8 weeks, necrosis, fibrosis, desquamation and cell destruction were observed in some regions of the hepatopancreas (Fig. 4). No change was observed in the lymphoid organ. Mild necrosis was noticed in the antennal gland. After 4 weeks of treatment, the hepatopancreas of shrimps in the 100-ppb AFB_1 group showed more fibrosis around the tubules, cellular inflammation, (Fig. 5) melanized nodules

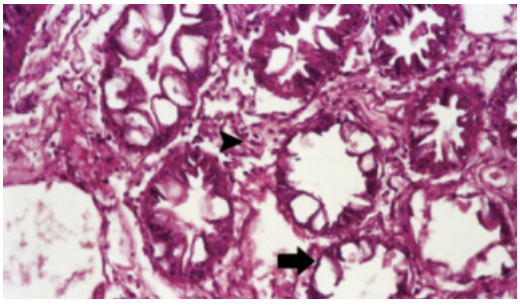

Figure 5 TS of hepatopancreas of 100 ppb aflatoxin B_1-treated shrimp at 4 weeks revealing cellular inflammatory response (arrow head) and loss of cells (arrow). H&E × 200. TS, tranverse section; H&E, haemotoxylin and eosin.

and necrotic cells in the lumen. There was desquamation, thickening of intertubular tissue and haemocytic infiltration in the hepatopancreas after 4 weeks. Formation of peculiar elongated cells and destruction of E cells were observed. R cells had almost disappeared in some areas. After 8 weeks, severe necrosis,

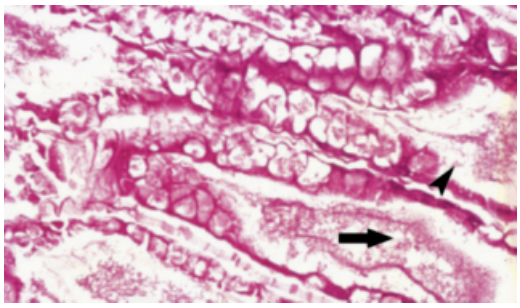

Figure 6 Longitudinal section (LS) of hepatopancreas of 100 ppb aflatoxin B_1-treated shrimp at 8 weeks revealing necrotic changes (arrow) and loss of tubules (arrow head). H&E × 200. H&E, haemotoxylin and eosin.

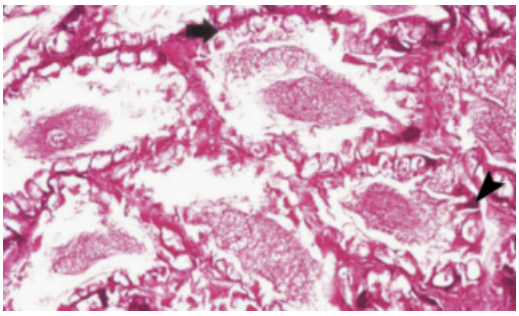

Figure 8 TS of hepatopancreas of shrimp fed 150 ppb aflatoxin B_1 at 8 weeks. Note the replacement of cells by fibrous growth (arrow head) and desquamated cells (arrow). H&E × 200. TS, tranverse section; H&E, haemotoxylin and eosin.

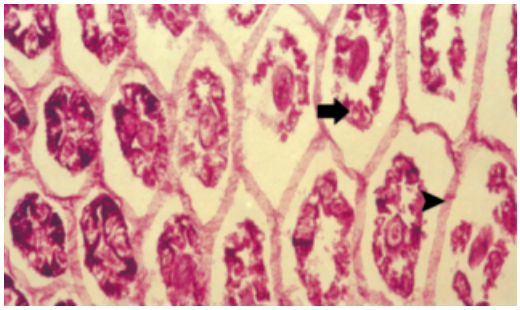

Figure 7 TS of hepatopancreas of shrimp fed 150 ppb aflatoxin B_1 at 4 weeks showing fibrous growth (arrow head) and desquamated cells (arrow) H&E × 200. TS, tranverse section; H&E, haemotoxylin and eosin.

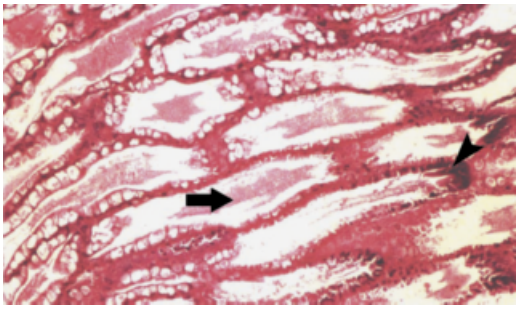

Figure 9 Longitudinal section (LS) of hepatopancreas of shrimp at 4 weeks fed 500 ppb aflatoxin B_1 showing extensive fibrosis (arrow head) and cell elongation (arrow). H&E × 100. H&E, haemotoxylin and eosin.

loss of tubules and fibrosis were found (Fig. 6). There was reduction in the number of R cells and B cells and rounding of cells in the hepatopancreas.

In the shrimps treated with 150 ppb AFB_1, after 4 weeks, there was complete loss of the structure of cells and tubules of the hepatopancreas. Necrotic cells were seen in the lumen and loss of brush-bordered appearance. Complete detachment of cells was observed. There was degeneration of focal areas and beginning of fibrous tissue growth (Fig. 7). After 8 weeks, necrosis and lysis became extensive; more fibrous growth (Fig. 8) and desquamated cells were noticed in the lumen; and an inflammatory reaction was observed in between the lobules.

Shrimps fed with 500 ppb AFB_1 after 4 weeks showed extensive fibrosis, degeneration, cell elongation and loss of cells in the distal end of the hepatopancreas (Fig. 9). After 8 weeks, the hepatopancreas showed destruction of the tubular structure in the distal region and necrosis (Fig. 10). In the shrimps dosed with 1000 ppb AFB_1, fibrosis, necrosis and degeneration were intense (Fig. 11). Inflammatory reaction and cell elongation were the peculiar features observed in the hepatopancreas. After 8 weeks, many lumen interconnections, cystic hyperplasia and dilation were noticed. There was necrosis of the antennal gland

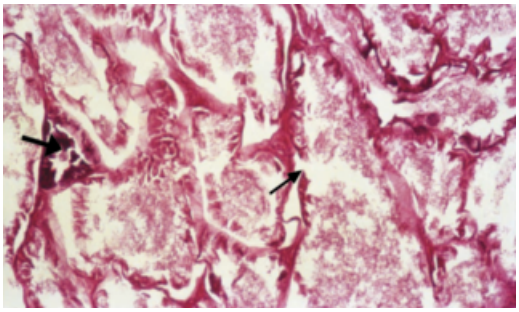

Figure 10 Tranverse section (TS) revealing a completely necrosed area (big arrow) and cell detachment (small arrow) in the hepatopancreas of 500 ppb aflatoxin-treated group at 8 weeks. H&E × 200. H&E, haemotoxylin and eosin.

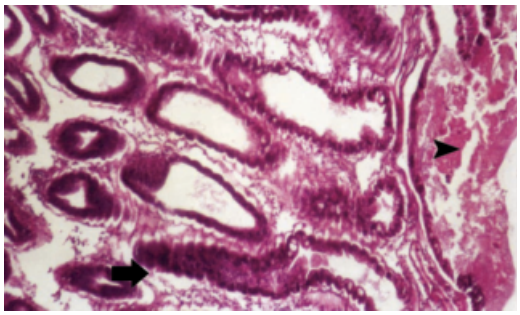

Figure 11 Necrosis (arrow head) and fibrosis (arrow) in the transverse section of hepatopancreas of shrimp fed 1000 ppb aflatoxin B_1 at 4 weeks. H&E × 200. H&E, haemotoxylin and eosin.

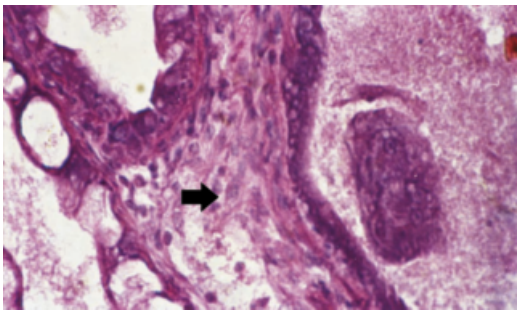

Figure 13 Severe necrosis and cellular inflammatory response (arrow) in shrimp at 4 weeks fed 2000 ppb aflatoxin B_1 in the TS of hepatopancreas. H&E × 400. TS, tranverse section; H&E, haemotoxylin and eosin.

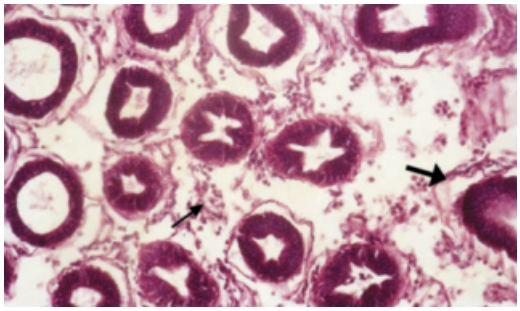

Figure 12 TS of hepatopancreas of 1000 ppb aflatoxin B_1-treated shrimp at 8 weeks revealing inflammatory response (small arrow) and cell detachment (big arrow). H&E × 200. TS, tranverse section; H&E, haemotoxylin and eosin.

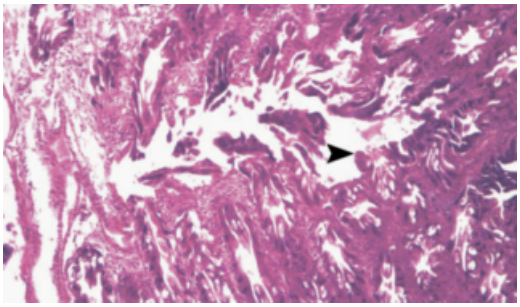

Figure 14 LS of hepatopancreas of shrimp given 2000 ppb aflatoxin B_1 at 8 weeks showing atrophy of the tubules. H&E × 100. LS, longitude section; H&E, haemotoxylin and eosin.

and an inflammatory reaction in the lymphoid organ. Other changes observed in the hepatopancreas were fibrous tissue growth around the tubules, haemocytic infiltration and cell detachment (Fig. 12).

Shrimps fed with 2000 ppb AFB_1 histologically showed severe necrosis, extensive fibrosis, fibrous tissue growth, haemocytic infiltration (Fig. 13) and intense papillomatous growth in the hepatopancreas after 4 weeks. After 8 weeks, a cellular inflammatory response was observed. There was severe necrosis and complete loss of architecture of the entire focal area (Fig. 14). Fibrous tissue growth had replaced the tubules and cells. Apoptosis or rounding of cells was also observed in a few areas.

Discussion

The histological analysis of the cephalothoracic region of the control shrimps conformed to the structure described by Bell and Lightner (1988). Histological study of the cephalothoracic region of the AFB_1 treatment groups revealed progressive damage to the hepatopancreas with increasing concentration of aflatoxin B_1, with only mild changes in the lymphoid organ and the antennal gland. The midgut gland or the hepatopancreas is considered to be the central organ of digestion in crustaceans. It is a system of blind-ending tubules consisting of four cell types (Loizzi 1971). The E cells at the summit of the tubules develop into R cells (absorption and storage of nutrients), F cells (production of digestive enzymes) and B cells (presumed to be secretory in function).

Besides, the effect of aflatoxins on the hepatopancreas appears to be directly correlated with the concentration of aflatoxins and the duration of feeding. The experimental study clearly shows that high doses of AFB_1 are detrimental to the shrimps as the changes in the hepatopancreas were severe and intense like complete fragmentation, apoptosis,

inflammation and desquamation. Smaller doses of the toxin (50 and 100 ppb) induced the onset of necrosis and fibrosis around the tubules of the hepatopancreas, and slight necrosis in the antennal gland. It is evident that even with mild doses of AFB_1, hepatopancreas becomes damaged.

The first sign of toxicity observed in the present investigation was the atrophy of hepatopancreatic tubules, followed by the destruction of E, R and B cells, desquamation, cellular inflammation, papillomatous growth, apoptosis in a few areas, necrosis and infiltration of fibroblastic tissue between the tubules of the hepatopancreas. Only mild necrosis was observed in the antennal gland, the gills and the lymphoid organ. Similar changes were reported in penaeid shrimps fed AFB_1 (Lavilla-Pitogo *et al.* 1994; Boonyaratpalin *et al.* 2001). As observed by Boonyaratpalin *et al.* (2001), AFB_1 levels above 100 ppb caused inflammation, necrosis, severe degeneration of tubules and infiltration of haemocytes. By the end of 4 weeks, there were histological changes in the hepatopancreatic tissues in all the shrimp fed over 50 ppb AFB_1. In the shrimps given the doses of 150 and 500 ppb, the distinct changes were necrotic cells in the lumen, loss of architecture of cells and tubules, extensive necrosis and an inflammatory reaction in between tubules. In the 1000 and 2000 ppb groups, cyst-like and papillomatous growth was observed, along with severe necrosis, cell elongation, inflammatory cells, cystic hyperplasia, haemocytic infiltration, complete loss of architecture of tubules, rounding of cells or apoptosis, desquamation and a cellular inflammatory response. Lightner *et al.* (1982) reported that the smallest dosage of 2 ppm in *P. stylirostris* and *P. vannamei* resulted in a low detectable hepatopancreatic lesion and doses higher than 2 ppm (2000 ppb) resulted in distinctive histopathologic alterations in the hepatopancreas and mandibular organs.

In *P. monodon* fed 26.5–202.8 ppb AFB_1 for 60 days, the first response was in the hepatopancreas and atrophy of R cells (Lavilla-Pitogo *et al.* 1994). Penaeid shrimps fed 50–300 ppm AFB_1 showed primary lesions in the hepatopancreas, mandibular organ and haemopoieitic organs (Wiseman *et al.* 1982). In the present study, the doses ranging from 50 to 2000 ppb were much lower than the LD_{50} value of AFB_1 reported for *P. stylirostris* and *P. vannamei* (Lightner *et al.* 1982); hence, the histological changes were targeted mainly on the hepatopancreas, while mild necrosis was noticed in the mandibular organ, lymphoid organ, antennal gland and haemopoietic organ.

Aflatoxin-related histopathologies were apparent in the hepatopancreas and antennal gland, when experimental diets containing 0–15 ppm AFB_1 were given to juvenile *P. vannamei* for 8 weeks (Ostrowski-Meissner *et al.* 1995). In contrast to the findings of Boonyaratpalin *et al.* (2001) that no histological changes were noted in *P. monodon* fed 50 ppb for 4 weeks, the present study, shrimps fed the 50 ppb AFB_1 diet at 4 weeks showed mild necrosis and a change in the structure of cells and tubules. The general histopathological changes observed in this study are consistent with the previous findings in penaeids (Lightner *et al.* 1982; Wiseman *et al.* 1982; Lavilla-Pitogo *et al.* 1994; Ostrowski-Meissner *et al.* 1995; Boonyaratpalin *et al.* 2001). Jantrarotai and Lovell (1990) and Jantrarotai, Lovell & Grizzle (1990) have reported necrotic foci in livers of channel catfish due to acute and subacute aflatoxicosis. Aflatoxin resulted in fatty liver, nuclear hypertrophy, cellular atrophy and leucocytic infiltration in the liver of *Oreochromis niloticus* (Chavez, Palacios & Moreno 1994).

The level of aflatoxin B_1 above 50 ppb caused significant damage to the hepatopancreas at the histological level. Histological changes were directly related to a corresponding increase in AFB_1 in different groups. The important changes in the heptopancreas were a reduction in the number of R cells, B cells and F cells, loss of structure of cells and tubules, desquamation in the tubules, fibrosis, necrosis, cellular inflammation, haemocytic nodule formation and haemocytic infiltration. Other organs affected were the gills, lymphoid organ and antennal gland. Thus, the histological analysis clearly showed the disruption of the digestive functions of the hepatopancreas by AFB_1. The disruptions would upset the function of absorption and storage of nutrients due to the reduced number of R cells, production of digestive enzymes by F cells and secretion of enzymes by the B cell culminating in the disruption of the digestive, metabolic and detoxification functions of the hepatopancreas.

To conclude, the present study has important implications in practical feeding programmes of *P. monodon* culture. It demonstrates how low levels of AFB_1 (50 ppb) could affect the functions and architecture of the hepatopancreas without affecting the survival. Histologically, aflatoxin directly attacks the hepatopancreas, the main organ for detoxification of xenobiotics, and several categories of hepatocellular pathology are now regarded as reliable biomarkers of toxic injury and representative of a

biological endpoint of contaminant exposure. Consequently, the hepatopancreas has attracted the most attention as a target organ for biological effects' monitoring programmes since pathological alterations occur at a very early stage of exposure. We recommend the enforcement of strict regulation of 20 ppb AFB_1 in feeds and foods. This calls for the need for safe storage of feeds.

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References

AOAC (1990) Association of Official Analytical Chemists. In: *Official Methods of Analysis* (ed. by K. Helrich), 15th edn. 868pp. AOAC, Arlington, VA, USA.

Bell T.A. & Lightner D.V. (1988) *A Handbook of Normal Penaeid Shrimp Histology*. World Aquaculture Society, Baton Rouge, FL, USA, pp. 1–114.

Boonyaratpalin M., Supamattaya K., Verakunpiriya V. & Suprasert D. (2001) Effects of aflatoxin B_1 on growth performance, blood components, immune function and histopathological changes in black tiger shrimp (*Penaeus monodon* Fabricius). *Aquaculture Research* **32**, 388–398.

Chavez S.M.C., Palacios C.A.M. & Moreno I.O. (1994) Pathological effects of feeding young *Oreochromis niloticus* diets supplemented with different levels of aflatoxin B_1. *Aquaculture* **127**, 49–60.

Coulombe J.R.A. (1991) Aflatoxin. In: *Mycotoxin and Phytoalexins* (ed. by R.P. Sharma & D.K. Salunkhe), pp. 103–143. CRC Press Inc, Boca Raton, FL, USA.

Cruz D.L. & Tendencia E.A. (1989) Aflatoxin in commercial prawn feeds. *SEAFDEC Asian Aquaculture* **11**, 4.

Fabricius J.C. (1798) *Supplementum Entomologiae Systematicae*. Proftet Storch .*Hafniae* (= Copenhagen), pp. 1–572.

FDA (1989) *Action levels for aflatoxins in animal feeds*. FDA Compliance Policy Guide 7126.33. Office of Enforcement, Division of Compliance Policy, May 18, 1989, 3pp.

Harwig J. & Scott P.M. (1971) Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. *Applied Microbiology* **21**, 1011–1016.

Jantrarotai W. & Lovell R.T. (1990) Subchronic toxicity of dietary aflatoxin B_1 to channel catfish. *Journal of Aquatic Animal Health* **2**, 248–254.

Jantrarotai W., Lovell R.T. & Grizzle J.M. (1990) Acute toxicity of aflatoxin B_1 to channel catfish. *Journal of Aquatic Animal Health* **2**, 237–247.

Kalaimani N., Ali S.A., Shanmugasundaram K.R. & Sarathchandra G. (1998) Quality of shrimp feed with special reference to aflatoxin. In: *Advances-and-Priorities-in-Fisheries-Technology* (ed. by K.K. Balachandran, T.S.G. Iyer, P. Madhavan, J. Joseph, P.A. Perigreen, M.R. Raghunath & M.D. Varghese), pp. 284–288. Society of Fisheries Technologists India, Cochin, India.

Lavilla-Pitogo C.R., Bautista M.N. & Subosa P.F. (1994) *Histopathology of shrimp, Penaeus monodon, juveniles fed aflatoxin B_1 contaminated diets*. International-Symposium on Aquatic Animal Health, Program and- abstracts. Davis, CA, University of California, School of Veterinary Medicine, 105pp.

Liao I. (1977) A culture study on grass prawn, *Penaeus monodon*, in Taiwan – the patterns, the problems and the prospects. *Journal of the Fisheries Society of Taiwan* **5**, 11–29.

Lightner D.V., Redman R.M., Price R.L. & Wiseman M.O. (1982) Histopathology of aflatoxicosis in the marine shrimp *Penaeus stylirostris* and *P. vannamei*. *Journal of Invertebrate Pathology* **40**, 279–291.

Loizzi R.F. (1971) Interpretation of crayfish hepatopancreatic function based on fine structure analysis of epithelial cell lines and muscle network. *Z. Zell Forsch* **113**, 420–440.

Ostrowski-Meissner H.T., LeaMaster B.R., Duerr E.O. & Walsh W.A. (1995) Sensitivity of the Pacific white shrimp, *Penaeus vannamei* to aflatoxin B_1. *Aquaculture* **131**, 155–164.

Reiss J. (1972) Toxic effects of the mycotoxins aflatoxin B_1, patulin, and diacetoxyscirpenol on the crustacean *Cyclops fuscus*. *Journal of Association of Official Analytical Chemists* **55**, 895–896.

Sinnhuber R.O. & Wales J.H. (1978) The effects of mycotoxin in aquatic animals. In: *Mycotoxic Fungi, Mycotoxins, Mycotoxicoses. An Encyclopedic Handbook* (ed. by T.D. Wyllie & L.G. Morehouse), pp. 489–509. Marcel Dekker Inc, New York, NY, USA.

Spensley P.C. (1963) Aflatoxins, the active principle in turkey "X" disease. *Endeavour* **22**, 75–79.

Wiseman M.O., Price R.L., Lightner D.V. & Williams W.R. (1982) Toxicity of aflatoxin B_1 to penaeid shrimp. *Applied and Environment Microbiology* **44**, 1479–1481.