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Biochemical responses in penaeids caused by contaminants

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Abstract

The shrimp aquaculture industry has become increasingly aware of the need for developing sensitive and precise diagnostic tools (Biomarkers) with predictive capability for assessing the toxic effect of commonly encountered chemicals on shrimp culture. Potentially damaging compounds used in shrimp culture include disinfectants, therapeutics, feed additives, algicidals, pesticides, and fertilizers. These chemicals may cause biological damage at all life stages during shrimp production. Since many chemicals may be stressors to the organisms, lower production rates may occur, compromising the sustainability of the shrimp production. This manuscript gives a brief overview about toxic effects associated with the chemicals used directly or indirectly during shrimp production.

Keywords: Penaeid; Biomarker; Contamination

1. Introduction

In many countries, the consumption of shrimps has grown yearly but the natural stocks of these decapod crustaceans have been intensively depleted. Alternatively, shrimp culture has been adopted and consolidated as one of the largest profitable aquaculture activities all over the world.

Methods of shrimp cultivation adopted by the shrimp aquaculture companies vary from intensive farming to extensive rearing systems. The former requires auxiliary inputs and capital, while the second is to a great extent dependent on natural processes

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(Folke and Kautsky, 1989). In both systems, significant levels of chemical products are used including antibiotics, disinfectants, therapeutics, algaecides, pesticides, water and soil treatment and feed additives (Primavera, 1998). In some cases, this management is absolutely necessary to prevent massive losses of production, but it is important to be aware of the associated biological damage to the organisms that could compromise shrimp health as well as the health of the consumers.

Physiological/biochemical pathways through which environmental stressors produce pathological changes in crustaceans have not been examined to the same extent as in fish, but analogous pathways are thought to exist (Sindermann, 1996). These chemical stressors may cause damage at all life stages during the shrimp production. Comprehension of the mechanisms related to the sub-lethal effects caused by different chemicals upon shrimp metabolism would help to develop sensitive and precise diagnostic tools (Biomarkers) with a predictive capability in assessing the toxic effects, thus contributing to better pond management and sustainable aquaculture.

Depending on the chemical structure and the concentration of the contaminant, distinct biochemical responses can be observed. Several toxic effects in shrimp caused by contaminants have been described in the literature, including cytochrome *P*450 and monooxygenase induction, chitin synthesis disturbance, metallothionein induction and acetyl cholinesterase inhibition. Here, I will describe some of the possible mechanisms involved in the stressing effects caused by different chemicals that can be found in shrimp culture.

2. Cytochrome *P*450 and monooxygenase induction

The presence of many pollutants can be assessed through the analysis of enzymatic systems involved in the biotransformation reactions of xenobiotics. Probably, the best-studied biomarker in aquatic organisms is the induction of the multi-family cytochrome *P*450-dependent monoxygenases (CYP450). These phase I enzymes metabolise several endogenous substrates, including ecdysteroid hormones, fatty acids, prostaglandins, cytokines, biogenic amines, as well as different kinds of xenobiotics (Oberdöster et al., 1998b). According to Nelson (1995), at least 325 different CYP450 sequences have been identified belonging to 50 families and 82 subfamilies of more than 67 species.

Probably, the most extensively studied CYP forms in aquatic organisms belong to the CYP1A subfamily. These proteins are widely used as biomarkers of aquatic contamination, since they are highly induced by very low levels of toxic compounds, most notably polynuclear aromatic hydrocarbons (PAHs), planar polychlorinated biphenyls congeners (PCBs), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Stegeman, 1993; Rattner et al., 1993; Bucheli and Fent, 1995). However, most of these studies have been done with fish, but in crustaceans this induction is controversial. James and Boyle (1998) suggested that in crustaceans other CYP450 families could be inducible by these compounds, such as CYP2, CYP3 and CYP4. Most of the studies in crustaceans have been carried out on blue crab *Calinectes sapidus*, spiny lobster *Panulirus argus* and American lobster *Homarus americanus*, but scarce

information is available about the induction of *P*450 in shrimps, particularly that belonging to the genus *Penaeus* (James and Boyle, 1998).

An important aspect on the health of shrimps is the role of some CYP450 forms in the steroid biosynthetic pathway, which produces ecdysteroids and controls moulting, differentiation and behaviour (Tyler et al., 1998). Recently, Snyder (1998) observed that over the course of the lobster *H. americanus* moult cycle, CYP45 expression, a new cytochrome *P*450 family, mirrored the hemolymph titter of ecdysteroids, suggesting its potential involvement in moulting hormone dynamics. Considering this possibility, it can be hypothesized that some contaminants could react with CYP450 forms, disturbing ecdysteroid synthesis and promoting endocrine disruption in shrimp, similar to that which occurs in mollusks, as well as in mammals (for review see Tyler et al., 1998). Likewise, some chemicals, such as tributyltin and plant flavonoids, could mimic ecdysteroid hormones disturbing the endocrine system as well as affecting other ecdysteroid-dependent biochemical pathways (Oberdöster et al., 1998a; Snyder, 1998). Further investigation is required to confirm such a hypothesis and to clarify some aspects related with the regulation and the normal function of cytochrome *P*450 in penaeids.

3. Chitin synthesis inhibitors

Chitin is a structural polysaccharide polymer consisting of linked β1-4 *N*-acetylglucosamine residues found in the exocuticle and endocuticle of crustaceans and serving both as a shell component and a support for muscle attachment (Hadley, 1986). The synthesis of the crustacean cuticle is initiated and completed in the epithelial cells that lie under the cuticle. At the apical membrane of these cells, proteins, chitin and other glycoconjugated substances are secreted via exocytosis (for revision, see Horst et al., 1993). The chitin synthetic pathway involves a series of enzymatic steps required to transform glucose into the UDP-*N*-acetyl-D-glucosamine (GlcNAc) substrate for chitin synthetase. This membrane-bound enzyme adds molecules of *N*-acetyl-D-glucosamine to form the macromolecular polymer.

Some pesticides, as well as antibiotics, are known to inhibit chitin synthesis in insects. Diflubenzuron (DFB), a benzophenylurea compound used as an insect growth regulator, can block the incorporation of GlcNAc into the cuticle of the moulting crustacean (Weiss et al., 1992). Some authors have proposed different modes of action for DFB in brine shrimp *Artemia salina*, blue crab (*C. sapidus*) and fiddler crab *Uca pugilator*, including blocking transport of GlcNAc, direct inhibition of chitin synthetase, alteration in the levels of ecdysteroid hormones, modulation of CAMP-dependent protein kinase, but the precise mechanism that could affect the penaeid shrimp metabolism still remains to be clarified (Oberlander, 1976; Leighton et al., 1981; Weis et al., 1987; Ishi and Matsumura, 1992). It is also interesting to point out that the antibiotics polyoxin D and nikkomycin, both UDP-GlcNAc analogues, inhibit the chitin synthesis, respectively in larval *Artemia* and postmoult crabs (Horst et al., 1993). More studies are required to further investigate the possible effects of these antibiotics on the chitin synthesis pathway in penaeids.

4. Metallothionein induction

The induction of metallothioneins is a common defense strategy in all organisms to protect against heavy metal exposure (Rainbow, 1988). Metallothioneins are non-enzymatic cytosolic proteins with low molecular weight (6000–7000 Da), possessing an elevated cysteine content responsible for binding the metal ions via mercaptide bonds (Rainbow, 1988).

There is evidence that metallothioneins play a role in the normal physiology of decapods, involving copper (Brouwer et al., 1986) and zinc (Engel and Brouwer, 1989). Copper bound to metallothionein can be transferred to the respiratory pigment haemocyanin (Engel et al., 1985). Metallothionein regulates copper levels during the moulting cycle (Engel and Brouwer, 1987).

The binding of nonessential metals, such as cadmium and mercury, to metallothionein most likely represents a sequestration function associated with protection against metal toxicity (Roesijadi, 1992). The induction of cadmium–metallothionein in the hepatopancreas of *Penaeus vannamei* has been demonstrated and shows a good potential in monitoring programs to evaluate the heavy metal pollution on shrimp farms (Moksnes et al., 1995). Considerable similarity appears to exist between the amino acid sequences of crab and lobster metallothioneins, indicating homology among decapod crustaceans (Roesijadi, 1992). In this way, one could use the probes developed for crabs and/or lobster to evaluate the metallothionein expression in penaeids.

5. Acetylcholinesterase (AchE) inhibition

AchE is closely associated with cholinergic synapses and hydrolyses acetylcholine into choline and acetic acid. There are at least two clearly defined parts in its binding site for substrates: one which is anionic and binds the cationic head of the substrate (and of inhibitors), and an esteratic site in which hydrolysis takes place with the formation of an acyl-enzyme (Coulson, 1988). Compounds arising from two distinctly different chemical classes, the esters of phosphoric or phosphorothioic acid and those of carbamic acid, are well known as anticholinesterase insecticides, since they possess structures related to acetylcholine which inhibit the AchE (Ecobichon, 1996). The active site of AchE contains a serine hydroxyl group that binds to the organophosphorous ester, generating a phosphorylated unreactive enzyme which under normal conditions can be reactivated only at very low rate (Ecobichon, 1996). However, some organophosphorous ester insecticides irreversibly inhibit this enzyme, provoking serious and persistent damage to the nervous system, as well as the death of the organism.

It is not uncommon to observe intensive use of insecticides in agricultural areas adjacent to shrimp farming zones. This can lead to massive losses of shrimp production, but in the case where the farmer makes use of tools for the elucidation of the contamination status of the organisms, this loss can be avoided. In this respect, the use of AchE inhibition could be a very useful biomarker of organophosphorus insecticide.

Recently, Lignot et al. (1998) observed differential effects of fenitrothion (*O*,*O*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothionate), an organophosphorus insecticide, on the

AchE activity in two penaeids, shrimps *P. stylirostris* and *P. vannamei*. No significant changes in the AchE activity were observed in *P. vannamei* treated with sub-lethal concentrations of fenitrothion. Contrariwise, AchE activity was decreased in *P. stylirostris* treated with this insecticide. The authors concluded that AchE inhibition appears to be a good indicator of physiological disturbance induced by this insecticide for *P. stylirostris* but not for *P. vannamei*.

A complementary method to evaluate the degree of AchE inhibition in crustaceans, based on reactivation techniques and using the nucleophilic reagent pyridine 2-aldoxime methiodide, was developed by Escartin and Porte (1996). This method to diagnose exposure is particularly useful when reference animals are not available, and the authors propose its use as biomarker of organophosphorous exposure.

6. Recommendations

Since various chemicals used in shrimp aquaculture and contaminants from unpredictable sources may be stressors to the Penaeid, lower production rates can be observed concomitantly with an increased risk to the consumers, thus compromising the sustainability of the system and the profit of the farmers. Thus, it is necessary to intensify the studies at a molecular level in order to search for biomarkers that can be used as early warning signals for predicting more drastic impact on the production. For the development of new biomarkers, a sound research strategy could involve both producers and scientists, sharing their needs to direct and focused research efforts.

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