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Effects of dichlorvos aquaculture treatments on selected biomarkers of gilthead sea bream (*Sparus aurata* L.) fingerlings

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Abstract

The gilthead sea bream (*Sparus aurata*) is the most important marine cultured species in the Mediterranean. Dichlorvos is one of the main chemical agents used in bath treatments against ectoparasites of marine farmed fish. The main objective of this study was to investigate the effects of 24 h dichlorvos baths on selected biomarkers that are involved in important physiological functions or indicative of gilthead sea bream fingerlings growth. To attain this objective, the *in vivo* effects of dichlorvos on cholinesterases' activity (ChE), lipid peroxidation (TBARS), RNA/DNA ratio, glutathione *S*-transferases activity (GST) and heat shock proteins HSP70 were studied. The characterization of ChE in brain and muscle was previously determined to select the most sensitive tissue in this species. The exposure of gilthead sea bream fingerlings to dichlorvos caused an inhibition of ChE activity, an increase in lipid peroxidation (TBARS) and a decrease in the RNA/DNA ratio. In contrast, no significant changes in GST and HSP70 were found. The results indicate that ChE, TBARS and RNA/DNA ratio seem to be sensitive biomarkers to detect sub−lethal effects of dichlorvos aquaculture treatments in gilthead sea bream fingerlings.

Keywords: Gilthead sea bream; *Sparus aurata*; Dichlorvos; Acetylcholinesterase; Biomarkers; Oxidative stress; RNA/DNA; GST; HSP70; Aquaculture

1. Introduction

A range of chemicals, such as organophosphate (OP), carbamate (CB) and pyretroid pesticides are used as antiparasitics in European marine fish farming. Dichlorvos is one of the main chemicals used in bath treatment against ectoparasites, mainly in the salmonid aquaculture (Mladineo et al., 2006). However, its use has been extended to other marine farmed fish, including the gilthead sea bream (*Sparus aurata*). In bath treatments, dichlorvos is absorbed mainly through the gills and body surface. Even though it does not bioaccumulate in fish (Ellis, 1991) its toxicity for fish is moderate to high and

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may produce both lethal and sub-lethal effects (Galgani and Bocquené, 1990; Bocquené et al., 1995; Sievers et al., 1995; Varó et al., 2003).

An early detection of biochemical and physiological changes caused by chemical bath treatments in early stages of fish development is essential to assess the potential adverse effects of the chemicals used in these baths on fish health and to predict their impact on survival and growth during rearing conditions. The use of biomarkers as "early biological responses" to chemical stress in fish is very common in environmental studies. However, their use to evaluate the biological effect of chemicals agents (dichlorvos) used against ectoparasites infestation in commercial fish species is scarce. There are several studies on lethal and sub-lethal toxicity of dichlorvos to fish (Pavlov et al., 1992; Hai et al., 1997; Chuiko, 2000), but not many studies have focused on early life stages in gilthead sea bream, the most important marine cultured species in the Mediterranean.

The mechanism of action of OP pesticides is the inhibition of acetylcholinesterase (AChE), a member of the family of enzymes known as cholinesterases (ChE). AChE is the enzyme responsible for the degradation of the neurotransmitter acetylcholine in cholinergic synapses of both vertebrate and invertebrates. The inhibition of the enzyme by OP pesticides causes an accumulation of acetylcholine in the synapse and the continuous stimulation of the post-synaptic membrane, a process that may lead to death. In addition to AChE, OP pesticides also inhibit pseudocholinesterases like butyrylcholinesterase (BChE) and propionlycholinesterase (PrChE), which are close related enzymes that hydrolyse some xenobiotics and bind to others, including OP pesticides. AChE and BChE are often present in the same tissue as, for example, in the muscle of marine fish species (Ludin, 1962, 1968; Sturm et al., 2000; Varó et al., 2003). Since different enzymes may have distinct sensibility to anti-cholinesterase agents, it is recommendable to perform a characterization of the ChE present in the species and tissue to be used before employing the activity of these enzymes as an environmental biomarker in biomonitoring studies with wild species or as an effect criteria in toxicity assays (Bocquené et al., 1990; Garcia et al., 2000; Varó et al., 2003; Rendon-von Osten et al., 2005).

Several studies have used ChE activity to diagnose the exposure of fish to OP and CB pesticides and/or to assess their neurotoxic effects (Boone and Chambers, 1997; Bocquené et al., 1990; Sturm et al., 1999, 2000; Varó et al., 2003). In addition to interfere with the neurological functioning, OP pesticides have been showed to induce other adverse effects on fish such as oxidative stress and lipid peroxidation (LP) (Bagchi et al., 1996, Hai et al., 1997), dichlorvos being one of these agents (Hai et al., 1997).

Glutathione *S*-transferases (GST) play an important role in the defence against oxidative damage and peroxidative products of DNA and lipids (George, 1994). They are also of fundamental importance in the detoxification (phase II) of some endogenous substances and xenobiotics.

The RNA/DNA ratio gives a measure of the synthetic capacity of the cell and it has been extensively used in early life stages of fish to estimate growth, as well as physiological and nutritional conditions (Clemmesen, 1988; Buckley et al., 1999).

Other cell mechanisms to face stress conditions are the stress proteins (or "heat shock proteins", HSPs). They are part of the cell's strategy to protect itself from damage and are involved in normal biochemical processes, including spatial and folding arrangements of cellular proteins (Hartl, 1996). It is well established that stress proteins are induced in response to various kinds of environmental and physiological stresses.

The objective of the present study was to evaluate the *in vivo* effects of 24 h baths of dichlorvos, in conditions simulating those used in some aquaculture treatments, on selected biomarkers (ChE, LP, GST, RNA/DNA ratio and HSP70) of gilthead sea bream fingerlings. The biomarkers used are parameters involved in important physiological processes, such as nervous system function, detoxification, stress defenses and growth.

2. Materials and methods

2.1. ChE characterization

ChE characterization of gilthead sea bream was performed *in vitro*. Juvenile fish (83.1±9 g, 17.1± 0.4 cm) reared at the "Instituto de Acuicultura of Torre de la Sal (CSIC), Castellón (Spain)" were kept in 500 L fiberglass tanks filled with natural seawater (salinity: 38 g/L) in open circuit, with continuous aeration, at 22–28 °C, and natural photoperiod (40° 5′ N; 0° 10′ E). Fish were fed with commercial food pellets (Pro-Aqua®, Spain). For ChE characterization, fish were anaesthetised in ice-cold chilled water and sacrificed by decapitation, then, the whole brain and a piece of dorsal muscle were isolated, washed in ice-cold phosphate buffer 0.1 M (pH=7.2), homogenized in ice-cold phosphate buffer using a tissue disrupter (Ultraturrax, T-8, IKA, Germany) and centrifuged at 8000 ×*g* for 5 min, at 4 °C. The supernatant was stored at −20 °C for