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Modulatory effects of deltamethrin-exposure on the immune status, metabolism and oxidative stress in gilthead seabream (*Sparus aurata* L.)

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ABSTRACT

Deltamethrin, a sintetic pyrethroid, is the insecticide that has been replacing recently to others like organochlorines, organophosphates and carbamates which are less toxic for birds and mammals, although, unfortunately, all of them are highly toxic to various non-targeted aquatic organisms including fish. In the present study, the consequences of the exposition of gilthead seabream (*Sparus aurata* L.) specimens to sublethal bath dose of deltamethrin (0.1 ppb) on organo-somatic indexes, immunity, seric metabolic parameters, oxidative stress and liver histology were determined after 1, 3, 7 and 14 days of exposure. Deltamethrin alters gilthead seabream immune status, the hepato-somatic index and various seric metabolic parameters since the first exposure day while important progressive deleterious morphological changes in liver were also observed. However, no statistically significant deviation was detected in the expression of oxidative stress-related genes whilst the expression of cytochrome P450 gene was up-regulated in head-kidney and liver of exposed fish. Overall, the present results indicate severe immunotoxicological and metabolic effects of deltamethrin in gilthead seabream, the species with the highest rate of production in Mediterranean aquaculture. In general, the values obtained for the tested parameters during the trial seem to indicate that specimens try to adapt to this adverse situation although the continuous presence of the toxic impede the hypothetic recovery of homoeostasis. The use of deltamethrin in the proximities of seabream farms should be carefully considered.

1. Introduction

Traditional pesticides such as organochlorines, organophosphates and carbamates are recognized for their ecological hazards [1] and they may cause various diverse side effects in animals and humans, for example: changes in DNA structure [2], sperm malformations [3], generation of reactive oxygen species [4], increments in the level of free radicals [5], inhibition of specific enzymes [6], changes on antioxidant defense system [7], decrease of expression levels of growth-related genes [8], act as inducers of heat-shock protein in tissues and cells in different organisms [9] or provoked the formation of micronucleus in human lymphocytes [10]. Thus, elimination of their use or reduction in the adverse effects of pesticides is desirable for the environment.

In this sense, the pyrethroids, a new generation of compounds, have proved to be good substitutes of traditional pesticides and they are extensively used in agriculture and forestry, for controlling pests, insects and vectors of endemic diseases, protecting seeds during storage and fighting household insects [11]. Pyrethroids have a low persistence in the ambient, high bio-efficacy, biodegradability and lower toxicity to mammals and birds comparing to traditional insecticides [12] because they have a short life in most animals as they are readily metabolized [13]. Specifically, deltamethrin [(S)-α-ciano-3-fenoxibencil, (1R, 3R)-3-(2,2-dibromovinil)-2,2-dimetilciclopropancarboxilato] ($C_{22}H_{19}Br_2NO_3$) is one of the most important pyrethroids widely used as pesticide and insecticide because it has a wide range of application in both industrial and agricultural purposes. Deltamethrin is a synthetic analogue of the pyrethrins, derived from natural extracts from the *Chrysanthemum cinerariaefolium*, with a similar structure to these but modified in order to improve its stability in the environment [14]. Although primarily it was thought to be less toxic, numerous reports have demonstrated deltamethrin toxicity in laboratory and wildlife animal species [15–17]. Strikingly, fish are an exception for its clearance since they are

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reported to be deficient in enzymes involved in pyrethroid hydrolization [18], due to this peculiarity fish are extremely sensitive to the neurotoxic effects of these pesticides, in general, and of deltamethrin, in particular [19–24]; in other words, pyrethroids are up to 1000 times more toxic to fish than to mammals or birds [25]. According to the World Health Organization [26] the acute toxicity data (LC_{50}) for deltamethrin in fish after 96 h of exposure, with ranges between 0.4 and 2.0 μg L^{-1}, is classified as highly toxic. Furthermore, deltamethrin is also toxic for marine invertebrates [17] and zooplankton communities [27] so its effects on marine fauna are very negative and remain largely unknown.

Exposure of fish to deltamethrin produces different adverse effects: histopathological alterations in different organs [23,25,28,29], affects both energetic metabolism and ionic regulation [30], blocks the sodium channels of nerve filaments [31], affects the gamma-aminobutyric acid (GABA) receptors in the nerve filaments [32], causes different effects on the reproductive state [22], causes several symptoms of stress [33], inhibits acetylcholine esterase [34], affects innate immunity [35,36], causes increased lipid peroxidation [1] and decreases antioxidant superoxide dismutase (SOD) and catalase (CAT) activities [1,21]. Deltamethrin also provokes alterations on vital tissues of fish as gills (the primary route for the entry of pesticides) and liver (major site of storage, biotransformation and excretion of pesticides). For this reason, both organs were chosen as criteria for the sublethal action of deltamethrin in fish and resulted to be an extraordinarily sensitive tool to reveal harmful effects in fish health [37]. In aquaculture, deltamethrin is commonly used in rainbow trout and Atlantic salmon to treat sea lice (*Lepeophtheirus salmonis*, *Caligus elongatus*) [38,39] in many aquatic larvicidal programs [25]. Therefore, further characterization of the potential negative effects that this toxicant may exert on aquatic environments is mandatory.

Regarding immune status, in general, pesticides are pollutants that cause immunosuppressive effects in fish. In spite of the importance of this problem the available studies of how pyrethroids disrupt the functioning of the immune system of teleosts are very scarce and none focus on gilthead seabream (*Sparus aurata* L.), the main cultured fish species in the Mediterranean area [40]. Then, the aim of the present study was to investigate the consequences of the exposure of gilthead seabream specimens to a sublethal waterborne dose of deltamethrin (0.1 ppb) on main innate immune parameters, as well as on the spleen and liver organo-somatic indexes, seric metabolic parameters and liver histology after 1, 3, 7 and 14 days of exposure. Furthermore, the effects on the expression levels of several genes related to the oxidative stress or detoxification were analyzed by real-time PCR in head-kidney and liver, with the purpose of discriminating which of these parameters could be useful for evaluating the effects of pesticides in fish immune system.

2. Materials and methods

2.1. Animals

Forty-eight (104 ± 26 g weight and 18 ± 1.5 cm length) of the hermaphroditic protandrous seawater teleost gilthead seabream (*S. aurata* L.), obtained from Doramenor Acuicultura S.L. (Murcia, Spain), were kept in recirculating seawater aquaria (250 L) in the Marine Fish Facility at the University of Murcia in recirculation systems. The water was maintained at 20 ± 2 °C with a flow rate of 1500 l h^{-1} and 28‰ salinity. The photoperiod was of 12 h light:12 h dark and fish were fed with a commercial pellet diet (Skretting) at a rate of 2% body weight day^{-1}. Fish were allowed to acclimatise for 15 days before the start of the experimental trial. All experimental protocols were approved by the Bioethical Committee of the University of Murcia.

2.2. Experimental design

Fish were randomly assigned and divided into two identical tanks as unexposed (control group) or exposed to a sublethal dosage (0.1 ppb) of deltamethrin. To do this, deltamethrin (Sigma) was dissolved in acetone at 1 μg μl^{-1} and the precise amount added to the tank. The control group received the same volume of acetone alone. Six fish per group were sampled after 1, 3, 7 and 14 days of exposure.

2.3. Sample collection

Fish were dissected under sterile conditions and the whole fish, liver and spleen weighed. Liver fragments were sampled for histology. Head-kidney (HK) and liver fragments were stored in TRIzol Reagent (Invitrogen) at −80 °C for later isolation of RNA. Blood samples were obtained from the caudal vein of each specimen with a 27-gauge needle and 1 ml syringe. After clotting at 4 °C, each sample was centrifuged and the serum removed and frozen at −80 °C until use. Other HK fragments were cut into small fragments and transferred to 8 ml of sRPMI [RPMI-1640 culture medium (Gibco) supplemented with 0.35% sodium chloride (to adjust the medium's osmolarity to gilthead seabream plasma osmolarity of 353.33 mOs), 2% foetal calf serum (FCS, Gibco), 100 i.u. ml^{-1} penicillin (Flow) and 100 μg ml^{-1} streptomycin (Flow)] for leucocyte isolation [41]. Cell suspensions were obtained by forcing fragments of the organ through a nylon mesh (mesh size 100 μm), washed twice (400 × *g*, 10 min), counted and adjusted to 10^7 cells ml^{-1} in sRPMI. Cell viability was determined by the trypan blue exclusion test.

2.4. Determination of organo-somatic indexes and condition factor

Whole body, spleen and liver were weighted. The organo-somatic index (OSI) for spleen and liver was calculated with the following formula: OSI = (g tissue g $body^{-1}$) × 100. Condition factor (*K*) was calculated according to the following formula: K = (g body cm $length^{-3}$) × 100.

2.5. Determination of metabolic parameters in serum

The presence of aspartate aminotransferase (AST), creatine kinase (CK), uric acid (UA), glucose (GLU), calcium (CA^{+2}), phosphorus (PHOS), total protein (TP), albumin (ALB), globulin (GLOB), potassium (K^+) and sodium (Na^+) were determined in the serum of seabream gilthead specimens using samples of 100 μl of serum in an automated analyzer (VetScan, Abaxis Veterinary Diagnostics) and rotor (VetScan Avian–Reptilian Profile Plus) according to the manufacturer's instructions.

2.6. Light microscopy

Liver samples were fixed with 10% neutral buffered formalin (Panreac) at room temperature for 24 h. After serial dehydration steps in alcohol, samples were embedded in hydrophilic resin JB-4 according to routine procedures. Sections were cut at 5 μm (Microm), mounted and stained with haematoxylin and eosin (HE). Slides were analyzed by a light microscope (Leica 6000B) and images were acquired with a Leica DFC280 digital camera.

2.7. Immune parameters

2.7.1. Natural haemolytic complement activity

The activity of the alternative complement pathway was assayed using sheep red blood cells (SRBC, Biomedics) as targets