

# EX VIVO IMMUNE RESPONSES IN FRESHWATER FISH LEUCOCYTES EXPOSED TO ENVIRONMENTAL SEROTYPES OF LIPOPOLYSACCHARIDES

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## ABSTRACT

In aquatic ecosystems, detected endotoxins or lipopolysaccharide (LPS) contamination can be linked to cyanobacteria blooms or enterobacterial contaminations. Water contaminations by endotoxins is of particular interest as these microbiological molecules may be released in freshwater often in combination with other chemicals (pharmaceutics...). Their immunomodulatory properties rise interest towards their pro-inflammatory action on mammals and aquatic vertebrates. Despite the fact that some *E.coli* LPS serotypes such as O55:B5 are often used as immunostimulant in experimentations, no data are available concerning some other serotypes as O157:H7 even if they may significantly contaminate freshwater ecosystems, particularly in agricultural field contexts.

We aimed to compare here the immunomodulation patterns potentially induced by two *E.coli* LPS serotypes (O55:B5 and O157:H7) alone or in combination with diclofenac, an anti-inflammatory drugs which chronically contaminate European freshwaters. Using an *ex vivo* approach, we studied immune parameters (Oxidative activity, Phagocytosis and cytotoxicity) of roach (*Rutilus rutilus*) leucocytes populations (lymphocytes, monocytes/macrophages and granulocytes) isolated from blood, head-kidney and spleen in response to endotoxins exposure. Leucocytes were exposed from 6 to 12 hours to sub-lethal concentrations of LPS and/or diclofenac (1µg/mL LPS, 0,1µM diclofenac) determined from preliminary results.

Like diclofenac, endotoxins induced significant decrease in intracellular ROS production by leucocytes at time 0. After 12 hours of exposure to the *E.coli* O55:B5 LPS / diclofenac combination, the oxidative activity was stimulated in spleen leucocytes and, simultaneously, they showed a significant decrease in phagocytosis. Moreover, while the *E.coli* O157:H7 LPS alone showed tendencies to decrease phagocytic activity, the two endotoxin / diclofenac combinations significantly decreased it in head-kidney leucocytes after 6 hours.

All these results will be set versus an ongoing *in vivo* experiment which would allow us to study more inflammation aspects like cytokines and immune-related factors production.

Whereas environmental monitoring and studies of endotoxins presently concern only human health like in wastewater units, our results rise the questioning on environmental reality of endotoxins and their potential immunomodulations in fish.

**KEYWORDS:** Lipopolysaccharides, Fish, Oxidative stress, Phagocytosis

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