

**O-076.****Rainbow trout IgM+ B cells preferentially respond to thymus-independent antigens but are activated by CD40L**

A.G. Granja<sup>\*</sup>, P. Perdiguero<sup>\*</sup>, A. Martin-Martin, P. Díaz-Rosales, I. Soletto, C. Tafalla<sup>#</sup>.

Animal Health Research Center (CISA), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), 28130 Valdeolmos, Madrid, Spain

**Abstract**

In mammals, B cells can be activated by either thymus-dependent (TD) or thymus independent (TI) antigens. TI responses do not require T cell cooperation and are faster in generating antibodies. On the other hand, conventional B cells (B2 cells) are activated in response to TD antigens within the lymphoid follicles, triggering the formation of germinal centers (GCs). Within these sites, the close interaction with T follicular helper cells stimulate B2 cells to divide and differentiate into antibody-secreting cells (ASCs), reaching a terminal state of plasma cell (PC) or into memory B cells. Among the signals involved in T cell / B cell cooperation, the interaction between CD40 expressed on B cells and its ligand (CD40L) transitionally expressed on activated T cells, is of critical importance. In the absence of lymph nodes or GCs, the mechanisms through which teleost B cells mount extrafollicular IgM responses remains mostly unexplored. In this work, we demonstrate that rainbow trout IgM+ B cells, as B2 mammalian cells, respond to CD40L with effects on survival, proliferation and differentiation. Despite this effect, trout IgM+ B cells, when stimulated with different types of antigens, only reached a general activation state in response to thymus-independent 1 (TI-1) antigens such as TNP-LPS. Interestingly, this response against TNP-LPS was blocked by TLR inhibition, suggesting that TLR cross-linking is an essential signal for B cell activation. Finally, when different types of antigens were combined with CD40L to study their capacity to generate synergies, only TI-1 antigens significantly synergized with CD40L. Our results suggest that in lower vertebrates there is not a clear dichotomy between TD and TI responses whereas accumulation of stimulatory signals is the best approach to generate a full activation state. **KEY WORDS:** B cells, CD40L, TD response, extrafollicular response, TLR.

# Corresponding author.

E-mail address: tafalla@inia.es (C. Tafalla).

\* These authors have contributed equally to this work.

**O-077.****Effect of nanoencapsulated clove-oil anaesthesia in the physiological response and immune status of Nile tilapia**

A.E. López-Cánovas<sup>1,#</sup>, A. García-Ayala<sup>1</sup>, A. López-Gómez<sup>2</sup>, J.M.O. Fernandes<sup>3</sup>, J. Galindo-Villegas<sup>3</sup>.

<sup>1</sup> Department of Cell Biology and Histology, Faculty of Biology, Universidad de Murcia, Campus de Espinardo, E-30100, Murcia, Spain

<sup>2</sup> Department of Food Engineering and Agricultural Equipment, Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 48, E-30203, Cartagena, Spain

<sup>3</sup> Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway

**Abstract**

In aquaculture, to minimize the negative side-effects produced by the intrinsic and varied fish management practices, required throughout the farming process, the use of anesthetics is recommended. Paradoxically, adverse side effects resulting from the incorrect management of the same in species-specific domesticated fish have been extensively reported. Taking advantage of the aggregate morphology transition of  $\beta$ -cyclodextrins to form a unique lipophilic 3D complex, just recently we have provided the aquaculture industry with a new nanoencapsulated essential clove-oil (CEO+ $\beta$ CD) anesthetic formula. CEO+ $\beta$ CD preparation that if correctly applied displays a high efficiency on the physiological and

immunological behavior in aquacultured fish (Lopez-Canovas et al., 2019). In Nile tilapia farming, the characterization of the physiological effects resulting from using the most extended commercial anesthetics is reasonably available. However, to broaden the anesthetic choices in this species here we aim to characterize extensively the effects of a single 20 mg/L optimal dose in water of our CEO+ $\beta$ CD formula to achieve a 2-2 narcosis stage and compare it with the more traditional formulas, namely AQUI-S (Isoeugenol) and MS-222 (Buffered tricaine methanesulphonate). Five groups of nine naïve Nile tilapia of 450 g average weight each in duplicates would be reared in small 100 L tanks for treatment with anesthetics for 3 min. Once the physical effect of each anesthetic is determined, animals in each group will be transferred to individual rearing tanks. On days 1, 3 and 9 after treatment, three animals per tank will be killed by an overdose of the same anesthetic previously used for treatment. In each sampling, whole blood, skin mucus, gills, head kidney, and skeletal muscle would be aseptically collected to conduct biochemical, mass spectrophotometry, chromatography, histology, scanning-electron-microscopy, and RNA-seq transcriptomics and metagenomic analyses followed by qPCR quantification of target genes. Resulting from this study we anticipate obtaining an extensive physical profile in mucosal tissue subjected to the action of the present anesthetics, the molecular immune pathways related, and the bioaccumulation, if any would be revealed. Moreover, a guided protocol for domesticated Nile tilapia using our CEO+ $\beta$ CD formula would be provided that may contribute to ensure and minimize the animal welfare according to an ethical standard while the particular physical and inflammatory affection of anesthetics improved. All data would be provided and discussed along with the Congress.

**Keywords:** Anesthetics, CEO+ $\beta$ CD complex, Nile Tilapia, qPCR, RNA-seq, SEM

# Corresponding author.

E-mail address: jorge.galindo-villegas@nord.no (A.E. López-Cánovas).

**O-078.****Fish thermoregulation and the promotion of cellular antimicrobial defenses**

Daniel R. Barreda<sup>1,2,#</sup>, Michael E. Wong<sup>1</sup>, Farah Haddad<sup>2</sup>, Caitlin Thomson<sup>1</sup>, Débora Torrealba<sup>2</sup>, Ryan D. Heimroth<sup>3</sup>, Keith B. Tierney<sup>1</sup>.

<sup>1</sup> Department of Biological Sciences, University of Alberta, AB, Canada

<sup>2</sup> Department of Agricultural, Food & Nutritional Science, University of Alberta, AB, Canada

<sup>3</sup> Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico, USA

**Abstract**

Core body temperature impacts molecular and cellular immune responses. Herein we demonstrate that discrete thermoregulatory programs provide fish with a broader range of immunological benefits than previously anticipated. Complementing prior findings on the modulation of immune gene expression, high resolution quantitative monitoring of cell function showed predictable changes to leukocyte recruitment and pathogen killing capacity. Assessment of kinetics of leukocyte infiltration to a challenge site and changes to the distribution of cellular subsets showed changes in the efficiency of the teleost acute inflammatory response. *Ex vivo* characterization of cell function as well as *in vivo* evaluation of host-pathogen interactions pointed to enhancements in immune defenses and pathogen clearance. These have obvious positive implications for fish health and performance, setting the stage for valuable applications in the aquaculture industry.

**Keywords:** Teleost fish; cellular immunity; thermoregulation; white blood cell function; aquaculture health and performance

# Corresponding author.

E-mail address: d.barreda@ualberta.ca (D.R. Barreda).