



Apresentações Internas do CIIMAR

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Horário: 14:00 – 15:00

Local: Auditório CIIMAR, Rua dos Bragas 289

Coordenador: Dr. Rodrigo Ozório

Programa:

14:00 - 14:15 The detection of toxic cyanobacteria in drinking water supplies and in dietary supplements produced for human consumption

Matin Saker – Laboratorio de Ecotoxicologia Dr. “Augusto Nobre”

14:20 - 14:35 Nutritional needs of *Diplodus sargus* – A general overview

Rui Sá – Laboratório de Nutrição

14:40 - 14:55 Host/pathogen interactions in Gram-positive bacteriosis in farmed turbot (*Scophthalmus maximus*)

Sónia Alexandra da Rocha Dias Gomes - Laboratório de Imunobiologia

Resumos:

The detection of toxic cyanobacteria in drinking water supplies and in dietary supplements produced for human consumption

Matin Saker – Laboratorio de Ecotoxicologia Dr. “Augusto Nobre”

Cyanobacterial blooms dominated by *Microcystis aeruginosa* occur annually in several Portuguese water bodies. These blooms, which degrade water quality for recreational use, can also produce toxins that can affect the health of humans as well as domestic and wild animals. The microcystins are heptapeptide hepatotoxins with LD₅₀ values (ip mouse) in the range of 50 to 500 µg kg⁻¹. To date over 60 chemical forms have been identified. The toxicity of *M. aeruginosa* can vary at the subspecies level and it has been suggested that natural bloom populations of this species consist of a mixture of toxic and non-toxic genotypes that cannot be distinguished by conventional techniques such as microscopy. Clearly there exists a need to understand the factors that affect the toxicity of *M. aeruginosa* at the sub species (strain) level.

In this study we used a range of techniques to investigate the toxicity of strains isolated from a bloom of *M. aeruginosa* in the Tamega River. Matrix-assisted laser desorption/ionisation time-of-flight spectrometry (MALDI-TOF MS) was applied to investigate the presence of different microcystins. Of the 26 strains sourced from the cyanobacterial bloom, 9 were found to contain microcystins (35%). Twelve chemically distinct forms of microcystin were identified including

microcystin-LR, -AR, -RR, -Y R and -WR. Several other peptides including aeruginosins, anabeanopeptins and microginin were also identified. The application of ELISA analyses showed that there was a 3-fold variation in the microcystin content of the microcystin producing strains. The results indicate that bloom populations consist of a range of different “chemotypes”, and that the relative proportions of toxic and non-toxic strains can affect the overall toxicity of the bloom. Molecular probes, targeting the genes responsible for microcystin production were shown to be useful for distinguishing between toxigenic and non-toxigenic genotypes and showed good agreement with the results obtained from the other toxicological analyses.

We have also recently tested the application of molecular techniques to detecting contamination of health food supplements (produced from the non-toxic cyanobacterium *Aphanizomenon flos-aquae*) with toxigenic cyanobacteria. All of the products sourced from a range of commercial distributors in Europe and North America were shown to be contaminated with toxin-producing strains of *M. aeruginosa*. The molecular methods described in this presentation represent a useful technique that can potentially be used for the quality control of products made for human consumption that may be contaminated with toxic cyanobacteria.

Nutritional needs of *Diplodus sargus* – A general overview

Rui Sá – Laboratório de Nutrição

In order to counteract nowadays market saturation, one of the candidates to diversify the main species actually produced in Europe aquaculture is *Diplodus sargus*. Information on the nutritional needs of this species is very scarce or inexistent. For this, we proposed to determinate the nutritional needs of some of the main macronutrients: protein, lipids and carbohydrates for *Diplodus sargus*. Growth and digestibility trials were performed with sargus. Although a large variety of inclusion levels of protein, lipid and carbohydrate were tested, growth performance and feed utilization in every trial were substantially lower than that achieved with *Sparus aurata* or *Dicentrarchus labrax* given similar experimental diets. No differences in growth and feed utilization were detected for the different amounts and type of carbohydrate (native or gelatinized starch) used. Furthermore, no differences were found when fish were fed the various lipids levels. Yet, fish grew differently at different protein inclusion levels. *Diplodus sargus* juveniles appear to adapt well under a broad dietary protein levels. Diets with 42% protein led to a better feed utilization. Yet, 25% dietary protein did not compromise growth or feed utilization. Our results showed that further work still needed before large scale production of *Diplodus sargus* can take place.

Keywords: *Diplodus sargus*, diversification, aquafeeds, nutrient digestibility, marine aquaculture

Host/pathogen interactions in Gram-positive bacteriosis in farmed turbot (*Scophthalmus maximus*)

Sónia Alexandra da Rocha Dias Gomes - Laboratório de Imunobiologia

Aquaculture is widely recognised to have emerged as a significant source of fish products, being projected to increase further by its contribution to global fish supplies for human consumption.

Turbot (*Scophthalmus maximus*) aquaculture production in Portugal has benefited from the above conditions, as well as, by its high commercial value. In Portugal, turbot aquaculture production in the year 2003 accounted for 12.60 % of the total marine fish aquaculture production. The total value of marine fish aquaculture production in the same year, in Portugal, amounted to

16.054 million euros, to which turbot aquaculture contributed with 2.884 million euros. However, the development of aquaculture production throughout the world has had to deal with emerging diseases due to infectious agents appearing as a direct consequence of intensive conditions on fish farms and leading to high mortalities as well as to severe economic consequences. Most of the studies concerning bacterial infections in fish consist of Gram-negative bacteriosis. However, during the last decade, Gram-positive bacteria have been recognized as important fish pathogens. Two pathogenic Gram-positive bacteria were selected to characterise the immune response of turbot. One belongs to the **Streptococcaceae** family (*Streptococcus parauberis*), being an extracellular parasite, fast grower and induces mainly an acute infection. The other bacterium belongs to the **Mycobacteriaceae** family (*Mycobacterium marinum*). This one is an acid-fast bacilli, intracellular microorganism, slow grower and induces a chronic inflammatory response on the host. Although there are many *in vitro* immunological studies made in turbot, there is a generalised lack of *in vivo* experiments. In that case, the knowledge of turbot/Gram-positive bacteria interaction and fish immunity will result in a better understanding of the basic immunopathological interactions, contributing to the development of proper diagnostics and safer vaccine formulations to farmed turbot.

The main aim of this work is to study and characterise the turbot non-specific and specific immune response against these two groups of Gram-positive bacteria, using the peritoneal cavity as a model.

Preliminary assays have showed that the unstimulated peritoneal leucocyte population in turbot is similar to that encountered in rainbow trout and sea bass. Moreover, during the intraperitoneal injection of several flogistic agents, the inflammatory immune response has been analysed and till 7 days post-injection, there was a typical immune response, with an increase in the number of macrophages in the first 48 hours, in the groups injected with inactivated *S. parauberis* or *M. marinum*. The group injected with Freund's Incomplete Adjuvant showed an increase later on, followed by a moderate decrease, in opposite to that observed in the previous referred groups. So it seems feasible to suggest that inactivated bacteria and adjuvants act as inflammatory stimulants in turbot, as observed in other fish species.