ABSTRACT BOOK

CONGRESS

Sustainable Management of Aquaculture Bacterial Diseases: an Interdisciplinary Approach

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Monterey Bay Aquarium

Puerto Varas · 2024

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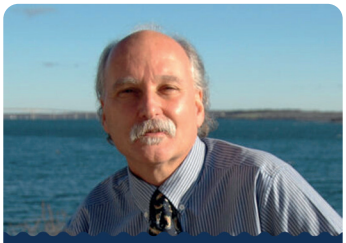

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Sustainable aquaculture
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General Information

The global aquaculture industry depends on the well-being of the ocean and the health of farmed seafood. The rapid expansion of aquaculture coupled with climate change is increasing the risk of disease in farmed seafood. Producers frequently use antibiotics as a cost-effective, efficient way to control disease. However, there are growing concerns that antibiotics may have negative impacts on the marine ecosystem and human health.

The aquaculture industry is beginning to develop strategies for managing antibiotic use, but these strategies must address many of the complex socio-economic, technical, and regulatory issues related to disease control.

A Multidisciplinary Approach to Sustainability – the first Antibiotic in Aquaculture Congress – will bring together experts from academia, the aquaculture industry, and non-governmental organizations to discuss ongoing efforts to promote responsible antibiotic use and explore innovative alternatives to antibiotic use.

Topics that are be covered at the Congress include:
- One Health
- Environmental Impacts
- Prevention Strategies
- Efficiency of treatment and management of use
- Policy and Regulation
- Food Safety
- Socioeconomics
- AMR
- Market and Certification

ABSTRACT

Session "Policy And Regulations"

DESAFÍOS PARA LA EVALUACIÓN DE PRODUCTOS VETERINARIOS DIRIGIDOS A LA ACUICULTURA

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The need to have safe and effective antimicrobials to combat animal diseases, both aquatic and terrestrial, is a challenge for both the pharmaceutical industry and the Official Veterinary Services of each country, who act as guarantors of veterinary public health and sanitation. In order to understand the origin of the antimicrobials available for hydrobiological species, it is necessary to know the authorization process carried out in our country by the Agricultural and Livestock Service (SAG) in conjunction with the National Fisheries and Aquaculture Service (SERNAPESCA). This process is based on an evaluation with a comprehensive approach, carried out by professionals in the veterinary, environmental and pharmaceutical areas, who analyze the background information presented, verifying that these products are safe for the species, the environment and people, as well as effective for the proposed purposes. Hydrobiological species, compared to terrestrial animals, have a smaller number of registered antimicrobials, this is mainly due to the low interest of the pharmaceutical industry in creating new developments, which increases the risk of generating resistance in microorganisms. The aim to improve the health of production systems and mitigate the risk of resistance is focused on prevention, based on good production practices and the use of alternative products to antimicrobials, such as pre/probiotics, microbiome, immunomodulators, phages and vaccines. Official Veterinary Services are called upon to evaluate and promote the development of these alternatives to improve the health of production systems and optimize the use of antimicrobials to mitigate AMR.

SURVEILLANCE OF ANTIMICROBIAL RESIDUES IN AQUACULTURE PRODUCTS: WHERE ARE WE TODAY?

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Antimicrobials in aquaculture in Chile are exclusively permitted for therapeutic and metaphylactic purposes, necessary to treat infectious diseases and ensure animal welfare. However, these drugs can generate residues in the final products, which concerns food safety. Since the 1980s, when the alarm was raised about the use of diethylstilbestrol (DES) in food animals, strict global regulations have been established, such as Maximum Residue Limits (MRLs), and the use of certain substances, as well as prophylactic use of antimicrobials, has been banned in many countries.
In Chile, the monitoring of drug residues in aquaculture is conducted through pre-harvest sampling in farming centers and monthly analyses in processing plants, carried out by SERNAPESCA with the support of authorized laboratories that must be accredited under the ISO 17025-2017 standard. Under this framework, our laboratory role as an official verification laboratory for the sanitary authority's program.
Currently, the challenges in residue detection involve the use of advanced technologies that allow the implementation of confirmatory methods such as mass spectrometry, which enables precise detection of residues in complex matrices. These measures aim to ensure that drug residues in food do not exceed permitted limits and minimize public health risks. In this context, all available information, such as the Rapid Alert System for Food and Feed (RASFF) and the Food and Feed Alert Network (RIAL), among others, show a low percentage of detections or non-compliant products from our country, demonstrating the excellent food safety and quality status of our product, resulting from a robust monitoring system.

CONTROLS ON ANTIBIOTIC USE IN SHRIMP AQUACULTURE – ARE THEY SUFFICIENT AND EFFECTIVE?

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As global consumption of shrimp continues to grow, this need is being met primarily through intensive shrimp farming operations mainly using whiteleg shrimp (Litopenaeus vannamei) with the main producing countries being China, Ecuador, India, Indonesia and Vietnam. For a variety of reasons, on occasions, antibiotics are used in production and for a short cycle species (3 months) this proves problematical with regards to sufficient withdrawal periods to make the product food safe.

Controls on antibiotic use are mainly through regulatory/government run programs and third-party accredited certification. Despite these two control mechanisms in place, results from testing of finished product at the consumer ready level shows that there still are detections of antibiotics from some regions and in some cases, there are unapproved or illegal therapeutants.

Aquaculture certification has developed over the last 20 years to give assurances on a range of topics, one of which is Food Safety, which has therapeutant use as one of its key areas of concern. There are varying levels of stringency in the standards around controls on antibiotic and therapeutant use, ranging from prohibited use to finished-product testing. In some regions there are also government run programs to control the use of antibiotics in shrimp production, which tends to focus on the hatchery level, involving also the residue testing of shrimp.

ECOLOGICAL RISK ASSESSMENT OF VETERINARY MEDICINAL PRODUCTS USED IN EUROPEAN AQUACULTURE

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Aquaculture production relies on the use of a wide range of Veterinary Medicinal Products (VMPs) with different physico-chemical properties and toxicological modes of action. To ensure that these VMPs do not pose unwanted ecotoxicological effects on ecosystems, a regulatory risk assessment is established prior to the registration and commercialization of these compounds. In Europe, the regulatory Ecological Risk Assessment (ERA) is performed following the guidelines issued by the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products (VICH). This framework establishes physico-chemical and ecotoxicological data requirements for each of the evaluated compounds, and comprises a tiered approach supported by several guidance documents for the calculation of environmental exposure concentrations and risk quotients. This presentation will provide an overview of the list of compounds registered for use in European aquaculture and will describe the minimum data requirements needed for their evaluation as well as the principles and tools available for performing a regulatory ERA. Furthermore, this work will provide some recommendations to advance the ERA of VMPs used in aquaculture and highlight the potential extrapolation of these tools and principles to other major aquaculture producing regions.
Acknowledgments: I thank the Talented Researcher Support Programme - PlanGenT (CIDEGENT/2020/043) of the Generalitat Valenciana.

COMPROMISO: YELCHO, UN COMPROMISO DE LA INDUSTRIA SALMONERA A UNA NUEVA FORMA DE RELACIONARSE CON EL SECTOR DE SALUD ANIMAL

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Project Yelcho is an unprecedented collaboration between the salmon-producing powerhouses of Chile: Aquachile, Australis, Blumar, Camanchaca, Cermaq, Cultivos Yadran, Marine Farm, Mowi, Multi X, Salmones Austral and Ventisqueros. Together, this group of companies produce over 90% of the Atlantic Salmon of Chile. Project Yelcho unites salmon producers with industry associations and regulatory authorities, with the goal of significantly reducing antibiotic use and bringing about effective solutions for the prevention and control of Salmon Rickettsial Syndrome (SRS).

Initiated in 2023, Yelcho Project is inspired by Chile's COVID-19 strategy, and it is a commitment from the Chilean salmon farming industry to a new way of working with pharmaceutical companies that fosters innovation. This project aims to work closely with pharmaceutical companies to develop solutions for common bacterial diseases, including SRS. Since the companies started collaborating in the year 2023 under the Yelcho name, they have been working closely with pharmaceutical companies to identify prospective areas for collaboration.

Other result of this project took place in January 2024, Yelcho signed a formal collaboration agreement with Chile's Agricultural and Livestock Service (SAG) and the National Fisheries and Aquaculture Service (Sernapesca) that aims to further facilitate the advancement of innovation and greater technical collaboration between authorities and the private sector. This is the first public-private collaboration to catalyse an innovation of its kind in the Chilean salmon farming industry, which makes the involvement of SAG and Sernapesca in Yelcho Project of huge significance.

SURVEILLANCE, ALERT AND RESPONSE SYSTEM (SVAR) FOR THE USE OF ANTIMICROBIALS IN CHILEAN SALMON FARMING

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The "Surveillance, Alert, and Response System (SVAR) for the Use of Antimicrobials in Chilean Salmon Farming" project aims to reduce antimicrobial use (AMU) by 25% over four years in the Los Lagos and Aysén regions, where Piscirickettsia salmonis poses a significant challenge. This project will enhance science-based governance of AMU through the implementation of the SVAR system. The project is structured in two phases: the first phase focuses on identifying factors influencing AMU, resulting in a catalog of practices, cost-effectiveness evaluations, and a proposal for regulatory updates with incentives. Metrics will be developed to classify salmon farming centers by AMU levels, integrating these into the SVAR design.

The second phase involves field evaluation of selected measures to reduce AMU, with results disseminated to middle - and low - income countries in Latin America. The SVAR system will enable the identification and categorization of centers with high, medium, or low AMU, integrating diverse information sources, and strengthening regulations to identify measures for reducing AMU.

Six work packages cover the review of variables and practices, economic analyses, regulatory requirements, infrastructure and platform design, SVAR evaluation, and identification of barriers and facilitators for system scaling and dissemination.

Session "Antimicrobial Resistance"

THE MENACE OF ANTIMICROBIAL RESISTANCE IN CHILEAN SALMON FARMING: THE RESISTOME UNDER A ONE HEALTH PERSPECTIVE

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Antimicrobial resistance (AMR), which is the ability of microorganisms to adapt and survive under diverse antimicrobial selection pressures is a growing public health concern worldwide, and it is now regarded as a critical One Health issue. One Health is an interdisciplinary concept considering the interdependent human and animal health in association with the ecosystem, where they live. Evidence exists that antimicrobials used in salmonid farms are disposed and accumulated in aquatic environments, leading to the selection of bacteria carrying different antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs), which are considered to serve as a vehicle for the dissemination of ARGs, mostly including plasmids and integrons. Amphenicols, β-lactams, tetracyclines, fluoroquinolones and sulfonamides have been classified by the World Health Organization (WHO) as critically important antimicrobials for animal health, thus studying related ARGs are becoming crucial for understanding the dissemination and transmission of AMR and ARGs in different domains of One Health. The incidence and characterization of ARGs encoding for resistance to phenicols (floR and fexA), tetracyclines (tet), sulfonamides (sul), and quinolones (qnr), have been mostly detected in Chilean freshwater farms, but information on their occurrence in seawater farms is very scarce. Furthermore, co-selection and persistence of specific ARGs may occur even in absence of direct selective pressure. To address AMR through a multifaceted One Health approach is an urgent need, considering that all domains contribute to the emergence, evolution, and spread of ARGs, which is a significant risk factor for human and animal health associated with Chilean salmonid farming industry.

WILD-TYPE AND NON-WILD-TYPE VS RESISTANCE AND SUSCEPTIBLE— THAT IS THE QUESTION

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Research on mitigating antimicrobial resistance in aquaculture emphasizes the necessity of using antimicrobials responsibly and judiciously, particularly those most effective in managing and reducing mortality associated with fish species. To achieve this, standardized methods for antimicrobial susceptibility testing and effective treatment protocols are essential for fish pathogens. The Clinical and Laboratory Standards Institute (CLSI) has published internationally recognized protocols, including VET-03 and VET-04, for assessing bacterial susceptibility in aquatic animals.
Epidemiological cut-off values are determined solely based on in vitro susceptibility data, such as minimum inhibitory concentration, without supporting clinical parameters. These cut-off values serve as thresholds for susceptibility measurements for fully susceptible bacterial isolates. Isolates with susceptibility indistinguishable from completely susceptible members are classified as wild-type, whereas those with reduced susceptibility are termed non-wild-type.
The terms 'resistant' or 'susceptible' cannot be applied to any species without a defined clinical breakpoint, relying solely on epidemiological cut-off values, as noted by Smith (2020). Thus, classifying a bacterial isolate as resistant indicates that the microorganism has developed resistance mechanisms to antimicrobials, potentially leading to treatment failure.
In aquaculture, confusion often arises regarding these concepts, resulting in misinterpretations of laboratory susceptibility test results. Therefore, any extrapolation to field conditions must be approached with caution, especially in fish production. This presentation aims to address these issues and includes case analyses of key bacterial pathogens, such as Piscirickettsia salmonis or Tenacibaculum dicentrarchi, that impact Chilean salmon farming.

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LOW COST AND EFFECTIVE ANTIMICROBIAL RESISTANCE DETECTION USING NGS

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Antimicrobial resistance (AMR) poses a significant threat to global human health, increasing the demand for fast and reliable detection methods of AMR. Next-generation sequencing (NGS) offers a comprehensive approach to identify resistance genes with high accuracy, yet traditional applications have often been limited by cost and accessibility constraints. This work explores a streamlined, cost-effective workflow for AMR detection using NGS, emphasizing innovations in DNA extraction and library database preparation. We compare the rate of gene identification of purified bacterial cultures and metagenome-assembled genomes (MAGs) from a series of bacteria from environments associated with salmon production, including matrices of Salmo salar, seawater, freshwater, marine sediment, and purified bacterial samples from processing plants, against the gold standard Illumina DNA Prep. Our results demonstrated comparable gene detection rates, but with a reduced cost per gene identified up to 20x. We demonstrate how NGS can be made accessible for routine AMR screening, providing a powerful tool for laboratories worldwide to combat AMR effectively showing how these tools can provide AMR information throughout the entire production process.

Session "Prevention Strategies"

PISCIRICKETTSIOSIS in vivo INFECTION MODELS: FROM BASICS TO OUTCOME PREDICTION

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Piscirickettsiosis is one of the most important sanitary challenges for the salmon farming industry in Chile and the major driver for the use of antibiotics. In vivo testing is a critical stage for research in several fields, including the evaluation of new products and antibiotic therapies. The robustness of disease trial models in salmon farming depend not only on following wet laboratory good practices, but also on an optimal quality of biological material, especially the inoculum used to infect fish. In this work, we show how epidemiological data are useful to predict and define Piscirickettsia salmonis strain candidates, whose suitability for bioassays was tested. Taking advantage on the ADL strain collection, which includes around 700 P. salmonis well-characterized specimens, and TEKBios's accumulated experience on fish trials, we deeply investigated the disease manifestation and reproducibility for different P. salmonis strains, representative of the most relevant genogroups, using intraperitoneal and cohabitation infection models. This approach allowed us to select the best and most up-to-date strains according to the objectives of each assay. We also demonstrated that, despite identical minimal inhibitory concentration values for some antibiotics, the assay outcome depends largely on the virulence displayed by the bacterial strain utilized. Finally, considering the statistics gathered in multiple assays, we ranked the most important variables and built a preliminary machine learning model for the performance prediction of piscirickettsiosis in vivo tests. We conclude that only a few variables, mainly microbiological, are needed to obtain a satisfactory accuracy level.

GENETIC IMPROVEMENT PROGRAM FOR SRS RESISTANCE: A CONTRIBUTION TOWARD SUSTAINABLE SALMON FARMING

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Aquagen's program of genetic improvement focuses on increasing profitability and sustainability of the Salmon farming industry by the selection of breeders that show heritable traits with economic impact such as P. salmonis resistance. Genomic selection models are trained through experimental challenges with an infection by cohabitation model, by which is possible to select breeder candidates with a similar genetic profile as the survivors of the challenges, and to generate eggs either for productive purposes (client) and/or for the formation of new families. The objective of this research is to evaluate the effect of the genetic improvement program in promoting the resistance to Salmonid Rickettsial Syndrome (SRS) outbreaks in field conditions. To achieve this, data from two sea sites that endured a SRS outbreak were collected. The evaluated phenotype was binary survival (alive or dead), where sampling for genotyping was performed. One of the centers showed genetic correlations between 0 and 0.33 with the experimental challenges. This site was treated with antibiotics. In the case of the non-treated center, the genetic correlations were among 0.76-0.97 with the experimental challenges performed in the parental generation. These results would support the improvement effect of the Aquagen program over the production of Atlantic salmon eggs with higher resistance to P. salmonis, thus reducing the application of antibiotics.

ANTIBIOTIC USE IN SALMON AQUACULTURE CAN BE SUCCESSFULLY DECREASED BY EMPLOYING PROPER STRATEGIES AND PRACTICES

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Chile is the second-largest producer of famed Atlantic salmon in the world. Like most intensive animal food productions, salmon farming is not exempt from the use of antibiotics to treat diseases. In Chile, more than 90% of antibiotic treatments are used to address an endemic bacterial disease caused by Piscirickettsia salmonis. Although a decreasing trend in antibiotic use has been observed over the years, the main concern regarding sustainability in the Chilean salmon production is the use antimicrobial treatments. To reduce antibiotic usage and improve animal welfare in salmon farming, efficient and effective strategies are required. The Pincoy Project is a collaborative initiative that encourages strategies on a wide range of production practices, from genetic factors and functional and high-performing diets to smolt selection and vaccination, all aimed at reducing antibiotic use in the Chilean salmon industry. To evaluate the effectiveness of these practices and strategies through statistical approaches, comprehensive data sets are required. The project has been collecting and analyzing data at cage and farm site level over the years, providing insight of variables that are related to a reduction of the antibiotic use and mortality. Factors such as fish genetic background, functional feeds, vaccine and production strategies, site and environmental conditions significantly explain antibiotic use and associated mortality rates. Additionally, the antibiotic use and mortality data from the industry are compared. The appropriate strategies and practices encouraged by the Pincoy Project can effectively reduce the use of antibiotics in Atlantic salmon production.

INFECTIOUS DISEASES PREVENTION USING NUTRITIONAL TOOLS

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Infectious diseases in the salmon industry generate a huge impact on both health and the economy, particularly salmonid rickettsial septicemia (SRS) caused by Piscirickettsia salmonis, a disease that causes the largest use of antibiotics in the industry. In recent years, Mowi has been working on nutritional strategies with a preventive and strategic focus by using additives/functional ingredients that allow us to face health challenges, this approach seeks to enhance fish physical barriers, such as gills and skin. The study objective was to assess how this nutritional strategy helps to prevent and control diseases. Regarding SRS, these tools have enabled to delay the first outbreak ie: pens receiving 2 or more pulses of additives or functional diets have means of ~40 days delayed in comparison with control pens (without additive). As a result, the time of first antibiotic intervention has also been delayed with a mean of ~50 days. The results show higher survival rates when strategic use is carried out, particularly against SRS, Amoebic gill disease (AGD) and Tenacibaculum infections. The use of this preventive nutritional tool has made it possible to avoid the negative effect of diseases and delay the first outbreaks of SRS, contributing to the prevention and control of other infectious diseases, such as AGD.

TOWARDS COST-EFFECTIVE REDUCTIONS OF ANTIBIOTIC USE IN SALMON AQUACULTURE

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In recent years, many in the Chilean salmon industry have committed to reducing their use of antimicrobial products. While national data suggests substantial progress, achieving these targets is likely to be both challenging and expensive as there exist few alternatives to antibiotics for treating common infections, such as Piscirickettsiosis. In this research, we explore how the design of antibiotic-use restrictions is likely to impact the productivity of fish farms exposed to a communicable disease. Leveraging insights from both economics and epidemiology, we develop a stylized model of a farm as a forward-looking, profit-maximizing entity where the biomass at harvest is determined by a compartmental model of disease dynamics. Farm managers control disease by applying treatment with temporary effectiveness. Further, we assume that pathogens are transmissible between individuals on the farm and between farms in a region, such that the disease management choices of one farm impact its neighbors. We solve the model as a finite-horizon, deterministic optimal control problem and simulate a selection of restrictions that are parameterized to achieve a targeted reduction level.
Our model suggests that the optimal treatment strategy is a function of both on-farm disease dynamics and ambient disease levels. However, in many scenarios, a reduction in ambient disease pressure does little to reduce optimal antibiotic-use. We also illustrate that, consistent with a theory, a cap on the total quantity of antibiotics used in a production cycle is the most cost-effective policy for achieving an intended target, when compared with a variety of other standard policies.

Session "Sustainability And Enviroment"

ANTIBIOTICS USE IN AQUACULTURE AND ANTIMICROBIAL RESISTANCE: PARTICIPATORY EXPERTS OVERVIEW AND RECOMMENDATIONS

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56 worldwide experts gathered for the Antimicrobial Assessment on Global Aquaculture Production (AGAP) series of workshops to address 1) Evaluating the current state of knowledge on antimicrobial use and identifying existing gaps, 2) Formulating strategies to identify ecologically relevant impact indicators and establish thresholds for assessment, 3) Identifying pivotal socio-economic factors and effective governance mechanisms essential for implementing monitoring practices in aquaculture and extending them across sectors, and countries for aquaculture sustainability, 4) Developing pathways to enhance our comprehension between antibiotic use in aquaculture and antimicrobial resistance, and 5) Exploring potential antibiotic monitoring tools that can be universally adapted and implemented across region and sectors. The main outcomes were a roadmap for establishing investigation priorities on the relevant topics regarding antibiotic use in aquaculture, socio-economic drivers for using antibiotics and behaviors that need more robust and transparent regulatory frameworks to guide farmers, training on antimicrobial use, access to veterinarians and extension services agents for education. Overall, the workshop evidenced the power of collaboration in addressing complex global challenges, to achieve sustainable aquaculture. Despite diligent efforts, some constraints may have inadvertently narrowed the possibility of having more experts and left some pertinent topics unaddressed, but they are needed in the discussion.

SOCIO-ECONOMIC SUSTAINABILITY: WHAT REMAINS TO BE DONE ABOUT ANTIBIOTIC USE IN SALMON AQUACULTURE?

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This paper reviews the literature on the economic sustainability of antibiotic use in animal production, particularly emphasizing the gaps in salmon farming in Chile. The work starts by examining the sustainable management of antibiotics in other industries, to identify potential gaps in the salmon farming industry through comparison. Then, it reviews economic techniques and tools present in the literature that could be used in Chilean aquaculture to improve the economic sustainability of the industry. Finally, the work concludes with the gaps identified based on the literature and recommendations to make the use of antibiotics more sustainable.

Work has not been published.
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RESPONSE OF MARINE PLANKTONIC MICROBIAL METABOLISM TO FLORFENICOL USED IN SALMON FARMING: A MICROCOSM APPROACH

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Florfenicol (FFC) is the most used antibiotic in Chilean salmon farming to control fish diseases in the marine phase. Its use poses a potential risk to the biodiversity of natural microorganisms and biogeochemical processes in the aquatic environment. This study evaluates the responses of marine planktonic microbial activity to FFC additions (1 µg/L and 150 µg/L), evaluated in a microcosm experiment using microbial communities from Coliumo Bay (central Chile) and Achao (Chilean northern Patagonia), the latter is an area influenced by salmon farming. We estimated respiration rates, extracellular enzymatic activity (EEA), carbon substrate utilization, and changes in nutrients and biomass. The results showed different responses with respect to microbial community origin and FFC additions. At both sites, a significant decrease in specific respiration and EEA was observed at different FFC concentrations. Respiration rate decreased up to 40%, while EEA decreased between 22% and 50% compared to the control (without FFC). These results are consistent with the significant decrease in ammonium concentration observed in the FFC treatments for both areas. The utilization of carbon substrates was variable, but showed a decreasing trend in Coliumo, while it increased in Achao, in both treatments. Our results suggest that FFC can cause a disruption in the metabolism of the marine microbial community, limiting the capacity to process different carbon sources. The decrease in the capacity to degrade organic matter when exposed to FFC suggests a possible decrease in the capacity of the ecosystem to handle the organic load generated by salmon farming.

Acknowledgements
This research was funded by the Interdisciplinary Center for Aquaculture Research, INCAR (FONDAP-INCAR 1523A0007) and by the Monterrey Bay Aquarium (MBA).

METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES IN MARINE SEDIMENT ASSOCIATED WITH SALMON FARMS UNDER ANTIMICROBIAL TREATMENTS IN LOS LAGOS REGION, CHILE

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To evaluate the effect of oral florfenicol administration on the microbiome of marine sediments around salmon farms, samples were collected from three fish farms in Los Lagos Region. In salmon farms 1 and 2, florfenicol was administered for 33 and 20 days, respectively, while the control farm did not receive antimicrobial treatment.

Marine sediment DNA was extracted, and the V3-V4 region of the 16S rRNA gene was amplified using PCR. The resulting DNA sequences were analyzed using a bioinformatics pipeline. Each Amplicon Sequence Variant (ASV) was assigned a bacterial taxonomy using a Naïve Bayesian classifier. Statistical analyses were conducted to determine the relative abundance of ASVs and their relative abundance at phylum level.

Significant differences between the control and farm 1 were observed in the phyla Bacteroidota, Bdellovibrionota, Crenarchaeota, Deferrisomatota, Desulfobacterota, Fibrobacterota, Firmicutes, and Fusobacteriota. Meanwhile, between the control and farm 2, significant differences were found in the phyla Bdellovibrionota, Calditrichota, Crenarchaeota, Deferrisomatota, Desulfobacterota, Fusobacteriota, Nanoarchaeota and Nitrospirota.

Alpha diversity, measured by the Shannon Index, revealed significant differences between groups with antimicrobial treatment and the control group at phylum, class, order, and family levels. The predominance of certain bacterial groups adapted to antimicrobial pressure has been documented in numerous studies, including those focused on the gut microbiota of farmed salmon. However, this is the first study to consider the microbiota of marine sediments. The direct and indirect effects of microbiota changes around farms—ranging from treatment efficacy to impacts on local communities—must be addressed using an ecosystem-based approach.

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BENEFITS OF OXIGEN SUPLLY IN FISH HEALTH Salmo salar AND ITS CONTRIBUTION TO THE EFFICACY OF ANTIBIOTICS TREATMENTS

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The permanent use of Oxymar equipment under challenging environmental conditions allows for better fish health and improved efficacy of antibiotic treatments (AT). The present study focuses on the effect of permanent oxygen supply as a support for the reduction of infectious diseases in general, and in the reduction of the incidence of pathologies such as salmonid rickettsial septicemia (SRS) and Tenacibaculosis, in particular. The study is based on comparing salmon sites that do not have Oxzo systems with those that do. In addition, it seeks to clarify whether the permanent use of oxygen improves the efficacy of AT treatments. The results of this study show that infectious diseases decrease from 3.55% to 3.04% (-14.36%) and, specifically, SRS decreases from 1.86% to 1.53% (-17.74%) and Tenacibaculum decreases from 1.09% to 0.75% (-31.19%), between sites without Oxzo and those with Oxzo, respectively. The efficacy of AT treatments is better and more consistent in centers with Oxzo systems, measured from the beginning of treatment until two weeks after the end of treatment (4 weeks in total), analyzing SRS mortalities in both groups. The study demonstrates that oxygen could be a powerful tool for the reduction of infectious diseases, and a great contribution to achieve an improvement in the efficacy of AT, which can be used as a complement in the sanitary strategies in salmon farming centers.

Session "Antimicrobial Treatments Optimization"

In vitro RELEASE OF MICROENCAPSULATED OXYTETRACYCLINE IN FORMULATIONS FOR ORAL ADMINISTRATION IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Oxytetracycline (OTC) is one of the most widely used antimicrobials in freshwater aquaculture, particularly for treating bacterial infections in salmonids. However, its effectiveness is often compromised due to its low bioavailability when administered orally. This limitation arises from the formation of non-absorbable chelates with polyvalent ions, leading to reduced absorption and increased environmental contamination through excretion via both feces and urine. To address these challenges, new formulations, such as microencapsulated formulations, are being explored to improve the controlled release and absorption of OTC. This study evaluated the in vitro release profile of microencapsulated OTC in two formulations designed for salmonids: zinc-alginate and a co-encapsulation with aromatic-aromatic interactions. Three different core-to-wall ratios—0.5:1, 1:1, and 2:1—were tested to assess their impact on release kinetics. The formulations were exposed to simulated gastric (pH 2.0) and intestinal (pH 8.0) conditions, with samples collected at regular intervals over a 2-hour period. OTC release was quantified using direct absorbance measurements at 380 nm via spectrophotometry. The results demonstrated that the zinc-alginate formulation with a 2:1 core-to-wall ratio provided the most controlled and sustained release in the intestinal phase, which is the primary site of absorption in salmonids. This suggests that the zinc-alginate formulation, particularly with the 2:1 core-to-wall ratio, is most appropriate for in vivo testing to enhance bioavailability, reduce environmental contamination, and improve therapeutic outcomes in aquaculture feeds.

Acknowledgments to the National Agency for Research and Development (ANID) for the funding provided through the FONDEF ID project N° 22I10071.

EVALUATION OF THE EFFECT OF THREE FLORFENICOL DOSES AGAINST SRS IN Atlantic salmon CHALLENGED BY IP-INJECTION WHEN ADMINISTERED AT AN EARLY STAGE OF INFECTION

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In this study the effect of various doses of florfenicol (FFC) (5, 7.5, and 10 mg/kg/day) was assessed to test the efficacy against SRS when treatment is initiated at an early stage of the infection. Since salmonids infected with Piscirickettsia salmonis typically lose appetite as the disease progresses, an early treatment was administered 5 days post-challenge (DPC5), when the fish still had a strong specific feeding rate (SFR) and a better likelihood of receiving the appropriate therapeutic dose prescribed. The average weight of the fish on the day of challenge was 255.1 ± 39.7g. The fish were acclimated for 13 days prior to the challenge, which were conducted in seawater at a temperature of 14 ± 1°C. On the day of challenge experimental fish were injected intraperitoneally (IP) with 0.2 ml of the challenge inoculum (9.07 x107 cfu/ml) of P. salmonis Genogroup LF. Fish mortality, behaviour, clinical signs, feed intake and SFR were registered during the challenge study, which lasted for 35 days.
An important finding in this study was that all the antibiotic doses tested halted the disease progression in the fish challenged with P. salmonis and prevented mortality in all treated groups. In the control group, mortality reached 32,2% and clinical signs of SRS were distinctive. From these results it is evident that an early treatment is crucial to ensure effectiveness of a FFC treatment against this disease.

EVALUATION OF THE EFFICACY OF TIMELY FLORFENICOL TREATMENTS AGAINST SRS (Piscirickettsia salmonis) IN ATLANTIC SALMON BY AN IP CHALLENGE MODEL

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As most intensive animal food productions, salmon farming is not exempt from the use of antibiotics to treat diseases. In Chile, more than 90% of antibiotic treatments are used to treat an endemic bacterial disease caused by Piscirickettsia salmonis. To optimize the use of antibiotics and improve animal welfare in salmon farming, efficient and effective therapies are required. Major shifts in P. salmonis genogroups along with significant differences in virulence and antibiotic susceptibility have been documented from farm outbreaks in recent years. To assess the efficacy of florfenicol therapies against a recent isolate of P. salmonis (LF-89) in Atlantic salmon, an intraperitoneal (IP) challenge model with timely oral therapies was employed. Two florfenicol doses, 10 and 20 mg/kg were evaluated during a 10 days treatment. Feed intake and specific feeding rate (SFR) in control fish were significantly reduced before mortality appeared. The treated groups kept targeted SFR during the treatments. Survival and mortality curve analysis showed no differences within tank replicates, but significant differences between treated and control fish. Under the conditions of the performed study, the probability for a mortality event in fish treated with a dose of 10 mg/kg and 20 mg/kg was, respectively, 76.0% and 95.4% lower compared with control fish. There were significant differences in mortality between florfenicol dose of 10 mg/kg and 20 mg/kg with a recent isolate of P. salmonis.

PHARMACOKINETIC PROFILE OF MICROENCAPSULATED OXYTETRACYCLINE USING AROMATIC-AROMATIC INTERACTIONS AND ZINC-ALGINATE MICROPARTICLES FOR POTENTIAL ORAL USE IN SALMONIDS

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Oxytetracycline (OTC) is used in Chilean salmon farming to treat various bacterial pathogens, being preferably administered orally. However, it has been proven that the oral bioavailability of OTC in salmonids is low due to its chemical structure, which contains multiple ionizable functional groups and a large number of conjugated double bonds, facilitating the formation of non-absorbable chelates in the presence of metal ions. To enhance OTC absorption, microparticles with aromatic-aromatic interaction and zinc-alginate were developed through spray drying. An in vivo study was conducted in rainbow trout (Oncorhynchus mykiss) to compare the bioavailability of the developed microparticles against a commercial OTC formulation in plasma. Nine sampling points were taken, and the quantification of OTC was carried out by UPLC-MS/MS. The results showed greater absorption of the microencapsulated formulations compared to the commercial OTC formulation. Additionally, the Cmax of the microencapsulated formulation with aromatic-aromatic interaction was reached 10 hours earlier than the commercial formulation, being 76% higher. On the other hand, the formulation developed with zinc-alginate reached the Cmax 12 hours later than the commercial formulation, although its bioavailability was 31% higher compared to the commercial formulation used as a reference.
In conclusion, OTC microencapsulation improves drug absorption, which could potentially allow for reduced doses in salmon farming in the future, thereby decreasing environmental contamination from these residues.

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Session "Therapeutic Alternatives To The Use Of Antimicrobials"

ON-SITE VALIDATION OF A PROBIOTIC TO INCREMENT FISH RESILIENCE AGAINST SRS

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A novel probiotic with postbiotic activity enhances the immune response of salmon against intracellular bacteria like P. salmonis, the causative agent of Salmon Rickettsial Syndrome (SRS), often treated with antibiotics.
Since 2021, the probiotic's effectiveness in controlling and reducing SRS outbreaks has been validated across multiple Chilean salmon farming sites with complicated sanitary histories and consecutive SRS outbreaks, using the probiotic for nearly 90 days. Various parameters were analyzed to assess the probiotic's effectiveness, including cytokine expression as indicators of the priming of fish on the Th1 component of immune system, that is involved in the response against intracellular pathogens. Additionally, histological analyses of key organs affected by P. salmonis were carried out as well as productivity monitoring.
Results showed that after 20 days of feeding with a diet supplied with the probiotic (test group), cytokines IFNg and IL12 increased by 32x and 8x, respectively, in comparison to the control group (fed with a standard diet without probiotic), indicating a strong stimulation of Th1 immune response. During SRS outbreaks, combining medicated feed with antibiotics and the probiotic led to a more rapid mortality decline, therapeutic success, and controlled mortality rates, suggesting the probiotic enhanced the immune response of fish, preventing therapeutic failure and reducing antibiotic reliance.
In conclusion, the probiotic strengthens fish resilience against SRS, improves survival, and extends the interval between SRS outbreaks.

AQ BIOCARE PLUS®: A NATURAL ESSENTIAL OILS-BASED SOLUTION FOR ENHANCING FISH HEALTH AND WELFARE IN SALMON FARMING

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AQ Biocare Plus® is a naturally derived essential oils formulation with antimicrobial, antioxidant and anticancer properties. Designed to enhance fish health through bath treatments, extensive in vitro and in vivo studies in Atlantic salmon have been done and demonstrated its antibacterial efficacy against key pathogens, including Flavobacterium, Aeromonas hydrophila, Aeromonas salmonicida, and Tenacibaculum. In vitro studies validated through absorbance and MIC assays that AQ Biocare Plus® shows strong antimicrobial activity lasting 24 to 48 hours during immersion treatments.

Beyond its antimicrobial properties, AQ Biocare Plus® enhances innate immune responses, increases skin mucus production, and bolsters disease resistance. It significantly strengthens natural immunity by improving cutaneous, mucosal, and serum immune parameters while upregulating immune-related genes. At the molecular level, it enhances inflammatory, immune, antiviral, and antibacterial responses while mitigating oxidative stress by reducing and scavenging free radicals. Lower levels of inflammatory markers (IL1b, TNFa, IL10) and tissue remodeling indicators (Col1A1, MMP2) suggest reduced skin damage and a decreased need for repair. The formulation also demonstrates superior control over reactive oxygen species (ROS), resulting in reduced oxidative stress, with lower activity of SOD3, GPX, and CAT, alongside high lysozyme levels, indicating enhanced bacterial protection.

Histologically, AQ Biocare Plus® increases mucous cell density in the skin, strengthening the fish's natural barrier. Welfare analyses, including serotonin, cortisol, and osmolality measurements, highlight its positive impact on fish well-being.

These comprehensive benefits position AQ Biocare Plus® as an innovative and sustainable alternative in salmon farming, effectively protecting fish health, enhancing productivity and improving animal welfare.

POSITIVE EFFECT OF MARINE SYMBIOTICS SUPPLEMENTATION ON FISH INTESTINAL MICROBIOTA DYSBIOSIS INDUCED BY ANTIBIOTIC TREATMENT

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The aquaculture industry is continuously interested in a deeper understanding of the impacts of farm practices on targeted species with the aim to improve and adjust its sustainable development. It is well known that intestinal microbiota has strong interactions with numerous physiological processes and its composition fluctuates depending on various parameters, including the exposure to antibiotics, which can lead to rapid emergence of resistant bacteria threating global human health.

A 40-days feeding trials was performed in a fresh water system on Oncorhynchus mykiss (20 g initial body weigth, n=150.000 fish/tank, 3 replicates) receiving a 15-days antibiotic treatment with florfenicol (FLOR, 20 mg of active principle/kg) followed by a 30-days feed supplementation of a marine probiotics consortium encapsulated in algae (marine symbiotics). A control group fed with a conventional food underwent the same antibiotic treatment. Evolution of microbiote composition (metabarcoding) before and after the FLOR treatment and at the end of the 30-days supplementation period was analyzed. The dysbiosis induced by FLOR treatment in gut microbial community will be presented (on going analysis). Dietary inclusion impact of marine symbiotics after FLOR treatment will be shown on the intestinal microbiota recovery.

In a previous study, we demonstrated that Oxytetracyclin (OTC) treatment induced a dysbiosis and the predominance of a high amount of γ-proteobacteria (pathogens) in the control group. Marine symbiotics supplementation before and after OTC treatment showed a reduction of negative impact of OTC and a stronger recovery, limiting the installation of opportunistic pathogens in the intestinal microbiota.

ASTRIX® NEW ORAL IMMUNOSTIMULANT AS AN ALTERNATIVE TO THE USE OF ANTIBIOTICS

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The infectious disease Salmonid rickettsial septicemia, known as SRS presents a great health challenge to the aquaculture industry. This disease, caused by the bacterium Piscirickettsia salmonis in the seawater phase, is the main pathology affecting salmonid farming and explains the large use of antimicrobials, being responsible for 93.21%, equivalent to 339 TONS.

To solve this problem, FAV has developed a prebiotic immunostimulant formulation of CD4 helper lymphocytes for aquatic organisms, that includes a concentrated protein fraction microencapsulated with immunomodulatory biopolymers that allow for its protection in the gastric environment, and thus, the targeted protein concentrate delivery in the distal intestine of the fish, to improve immunity against the pathogen, via mucosal stimulation.

With ASTRIX® as an oral additive, we seek to enhance the immune response of vaccinated fish, as a booster on healthy salmonids that are in the seawater culture phase.

NANOSTRUCTURED PROTEINS FOR DISEASE CONTROL IN AQUACULTURE: DEVELOPING SUSTAINABLE ALTERNATIVES FOR THE INDUSTRY

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The sustainability of aquaculture is under threat from infectious diseases, which are a major concern. It is estimated that around 10% of all cultured aquatic animals worldwide are lost due to these diseases. This underscores the urgent need for technological development and innovation in this field to reduce outbreaks caused by pathogens and to reduce the use of antibiotics. Our research on nanostructured proteins for disease control in aquaculture is a step towards meeting this need. These recombinant proteins are mechanically stable, non-toxic biomaterial produced in recombinant cells. Nanostructured proteins mainly comprise recombinant heterologous protein, which maintains their function in a high percentage. The other protein fraction works as a protective scaffold for the functional protein. Our results show that it is possible to obtain nanostructured proteins of key proteins of the salmonid's immune system (immunomodulators) and from highly antigenic pathogens proteins (chimerical vaccines). These nanostructured proteins present a nanometric size, diverse morphology and resist harsh physicochemical conditions in vitro (pH and temperature) while maintaining their functionality. In in vitro experiments, we observed the immune system's modulation and immune cells' ability, such as macrophages, to phagocytize them. On the other hand, in vivo challenges with bacterial pathogens, we observed that nanostructured proteins confer protection and increase fish survival. Moreover, these nanostructured proteins are absorbed by intestine cells and can reach and accumulate in immune-relevant organs such as the head kidney and spleen. Notably, these results open the door to exploring this biomaterial as an alternative prophylactic method in aquaculture.
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C3COS®: BOOSTING SUSTAINABILITY AND HEALTH IN SALMON AQUACULTURE VIA GUT HEALTH MANAGEMENT, IMMUNITY IMPROVING, AND OXIDATIVE STRESS CONTROL

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C3COS® is a residue-free, natural formulation broad spectrum, designed to enhance multiple aspects of salmon aquaculture, offering significant benefits in the health of the fish that extend from biological function to overall production efficiency. This formulation supports the balance of intestinal microbiota, reduces the feed conversion ratio (FCR), and maximizes nutrient absorption, which collectively lead to improved growth performance and shorter production cycles. By optimizing these physiological processes, C3COS® contributes to a more sustainable and economically viable aquaculture system.

Empirical studies have demonstrated the bacteriostatic properties of C3COS®, with inhibition of bacterial growth observed at concentrations as low as 1.000 ppm. Notably, in trials involving Tenacibaculum dicentrarchi, fish administered C3COS® exhibited a reduction in mortality exceeding 90% compared to those treated with antibiotic, highlighting its potential to mitigate bacterial resistance and enhance fish health.

On a histological level, C3COS® significantly increases the density of mucous cells in the skin, thereby fortifying the fish's natural barrier against environmental stressors and pathogens, also increases intestinal villus height, enhancing the nutrient absorption efficiency. At the molecular scale, the formulation augments inflammatory, immune, antiviral, and antibacterial responses, contributing to a robust physiological defense mechanism. Additionally, C3COS® effectively mitigates oxidative stress by reducing free radical presence, which promotes the longevity and overall health of the fish.

These comprehensive benefits position C3COS® as an innovative and sustainable intervention in salmon farming, not only enhancing productivity but also improving animal welfare and the quality of the final aquaculture product.

NANOBITE® PASSIVE IMMUNOTHERAPY FOR SALMONIDS BASED ON ALPACA NANOBODIES

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Antibiotics have been widely used in salmon aquaculture as an important tool in managing bacterial diseases. The use of antibiotics improves fish welfare, but reliance on antibiotics use is fraught with significant disadvantages that pose serious risks to both aquaculture industry and public health. A primary concern is the development of antibiotic resistance, long-term ecological damage and diversity loss.
Regulatory frameworks and industry standards are focused on balancing the benefits of antibiotic use with the imperative to mitigate their risks. Many countries are implementing stricter regulations on antibiotic use, promoting a shift to alternative health management strategies. Lessening the reliance on antibiotics, the aquaculture industry can enhance its sustainability protecting ecosystem integrity.
We hunt, select and produce recombinant VHH domains of heavy chain only antibodies (HChA) from Alpacas immune system also known as Nanobodies. Nanobodies are small, very stable and easy/cheap to produce antibodies domains with sub-nanomolar affinity. Nanobodies has proved neutralization capability against viruses, so Nanobodies offers a reliable solution to complement/substitute the use of antibiotics.
Here we show pioneer technology, NANOBITE®, based on the use of Nanobodies as food supplements as a tool for a passive immunization protocol through salmon feeding. We demonstrate that Nanobodies are traceable into the blood serum of O. Mykiss feeds Nanobodies supplemented pellet. Moreover, moreover the Nanobodies enriched serum of the group feed with NANOBITE® where active efficiency recognizes target proteins. Our results put on the table the use of Nanobodies as novel technology to inmuno-activate salmonids diet and help them to fight against pathogens in the most sustainable possible manner.
Acknowledgement CORFO Crea y Valida 22CVC-206539 & IMAGINE WAVES

Session "Pitch Sessions"

DEVELOPMENT AND EVALUATION OF A NOVEL QUINONE ANTIBIOTIC FOR MANAGING BACTERIAL DISEASES IN AQUACULTURE

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Aquaculture is one of the most relevant economic activities in Chile. Salmonids occupy the first place in aquaculture production, where Atlantic salmon is the most produced. These salmon farms are threatened by various pathologies, where bacterial diseases such as salmonid rickettsial syndrome (SRS), caused by Piscirickettsia salmonis, are one of the most relevant. This pathology has historically been treated with antibiotics such as florfenicol or oxytetracycline, which is a scarce therapeutic arsenal available to treat bacterial pathologies. This promotes a low possibility of rotating antibiotics, and this, coupled with the misuse of these drugs, allows for the emergence of non-susceptible isolates. Accordingly, in this work we sought to synthesize a new quinone antibiotic not related to any structural family used in human medicine or other animal species in order to offer a new alternative for the treatment of bacterial pathologies. The compound was synthesized by traditional green chemistry methods from commercial precursors. The compound was tested on Aeromonas salmonicida subsp. salmonicida ATCC 33658 and Piscirickettsia salmonis ATCC VR-1361, where a minimum inhibitory concentration (MIC) value of 64 and 128 ug/mL was obtained, respectively. The synthetic compound is currently being tested on clinical isolates of both species. These results suggest that quinonic compounds have a high activity against both pathogens, with the potential to be used as a new pharmacological resource to control bacterial pathogens in salmon farms.

EVALUATION OF THE IN VITRO ANTIMICROBIAL EFFECT OF CHITOSAN SULPHATED (CH-S), ON THE VIABILITY OF Piscirickettsia salmonis

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Research objective: To evaluate the in vitro antimicrobial effect of chitosan sulphated (Ch-S) on the viability of Piscirickettsia salmonis. Materials and methods: Ch-S was obtained from low molecular weight commercial chitosan (LMW-Ch Sigma®), after functionalization with H2SO4 for enhancing its antibiotic activity. One strain of P. salmonis genogroup LF-89 was obtained and propagated in Austral medium at the Biovetec-Favet-laboratory, University of Chile, and was genetically characterized by sequencing the 16S ribosomal gene, obtaining an alignment coverage of 99% and being located in a clade with frequent and consistent distances with respect to all P. salmonis GenBank sequences. The method used was microdilution in 96-well plates, where each well contained 155µL of Austral broth, 20µL of bacterial inoculum (standardized to 0.5/McFarland scale) and 25µL of different concentrations of reference control antibiotics (tetracycline and florfenicol 0.5, 8, 64 and 128 µg/mL) or Ch-S (0, 5, 10, 15 and 20 mg/mL). The assay was performed in triplicate for each antimicrobial, and the plates were incubated at 18°C with constant shaking for 14 days. Bacterial viability was assessed using the LIVE/DEAD kit. Results and conclusions: A higher percentage of dead bacteria (red fluorescence) was observed in plates treated with reference antibiotics compared to those treated with Ch-S, indicating lower antibacterial activity of Ch-S compared to reference antibiotics. However, the results suggest the need to perform new experiments to evaluate the possible synergistic effect between Ch-S and reference antibiotics, which could contribute to preventing the risk of antimicrobial resistance.

EVALUATION OF AN ENDEMIC PLANT EXTRACT FROM CHILE FOR THE PREVENTION OF BACTERIAL INFECTIONS IN SALMONIDS

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Aquaculture in Chile is an economic activity of great importance, with most of its production taking place in the south of the country, where Atlantic salmon is the species of largest production. Although salmon is farmed in areas where conditions are optimally controlled so that it can develop as efficiently as possible, there are factors that are difficult to prevent, such as infectious diseases. The use of synthetic antibiotics, although usually a very effective therapy, often brings with it undesired environmental impacts, production difficulties, limitations on the export of the final product and the potential generation of resistance in target and non-target bacteria. These problems generate the need for alternative therapies to combat infections affecting salmonids. In this context, an extract of an endemic plant to Chile was prepared using ultrasound extraction and low environmental impact solvents. Dry extracts were obtained with good yields where all the solvents could be removed. The extracts obtained has a high content of polyphenols determined by the Folin-Ciocalteu technique. The extract was tested on Aeromonas salmonicida subsp. salmonicida ATCC 33658, where a minimum inhibitory concentration (MIC) value of 256 ug/mL was obtained. The extract is currently being tested on clinical isolates of the same species. These results suggest that the extract possesses biocidal activity, with the potential of being used as a water conditioner to reduce the bacterial load of this or other bacterial pathogens in salmon farm.

RETENTION OF OXYTETRACYCLINE IN BIOCOMPATIBLE POLYESTERS FOR USE IN AQUACULTURE

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In the present work, a new technology is introduced to solve the current methods of administration of antibiotics in aquaculture, which have caused environmental problems such as bacterial resistance, contamination of water bodies, and potential risks to human health, according to the WHO. This study is focused on those antibiotics used to treat the Piscirickettsiosis disease (SRS), focusing specifically on oxytetracycline (OXY), whose administration dose ranges between 75-100 mg/kg-1 P.V in salmonids.
In this way, a control method in the dosage of this antimicrobial is the retention of it in biocompatible polyesters due to its properties and characteristics for a controlled release; this is possible because the polyesters have different crosslinking degrees and, derived from castor oil and polyethylene glycol 1000 g/mol-1 called PEE 1000. Thus, it allows for avoiding the permanent availability of the drug in the environment and regulating the amount administered to the salmonids.
The retention capacity of OXY was studied at pH 7, with concentrations of 2.3E-03, 0.4, and 1% m/v of antibiotic. The results show that the retention percentage is lower at low concentrations; nevertheless, the percentage is around 85%. Likewise, the retention at various polymer masses (23, 50, 150, and 500 mg) at a concentration of 1% m/v was analyzed to determine the appropriate amount of polyesters. OXY was retained in 99% of all cases when the mass was varied, indicating that this technology allows a high amount of drug to be retained in a small amount of polymer, obtaining a drug load of 216%.

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AI-DRIVEN DESIGN OF PHYTOGENIC ADDITIVES FOR SALMON HEALTH

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The use of antibiotics in aquaculture, specifically salmon farming, raises significant consumer safety concerns. There is an urgent need for alternative solutions to mitigate these risks. Plant-derived feed additives have emerged as promising antibiotic substitutes due to their diverse biological functions and lower likelihood of inducing resistance. In this study, an innovative methodology for discovery, designing and optimization of phytogenic additives has been developed using artificial intelligence (AI). This approach allows for the precise identification and development of plant-based compounds targeted at specific pathologies. As a case study, we focused on Piscirickettsia salmonis, a major pathogen in the Chilean salmon farming. Our AI-driven design process led to the discovery of effective phytogenic additives, which demonstrated promising results by significantly reducing cell mortality in in vitro assays.
Experimental results showed that the phytogenic additive reduced cell mortality by 50% relative to the untreated control, with the control samples exhibiting 60% mortality by day 10. Additionally, gene expression analysis indicated that treatment with the phytogenic additive modulated the expression of key inflammatory markers, such as IL-1β, IL-8, nfkβ, and IL-12. This study highlights the potential of using AI to develop phytogenic additives as a sustainable and effective alternative to antibiotics in aquaculture.

STUDY OF THE RETENTION AND CONTROLLED RELEASE OF FLORFENICOL (FF) FROM POLYMERIC MATRIX

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The latent concern of the World Health Organization is that prolonged exposure to antimicrobial agents encourages the development of bacterial resistance. In the field of aquaculture, the significant increase in production entails the management of the pathogenic bacteria Piscirickettsia salmonis, responsible for Salmon Rickettsial Septicemia (SRS), which is the main target of antibiotics used in this industry to avoid production losses. Among these antibiotics, florfenicol has been widely used, with 92.60% used for preventing and controlling SRS, which could generate microbial resistance in the future.

In this context, the retention and subsequent controlled release of florfenicol is studied using biocompatible cross-linked polyesters, synthesized from castor oil derivatives and different molecular masses of polyethylene glycol (PEG), as an alternative to the regular administration of this drug, to reduce FF exposure. For this purpose, three polyesters with different degrees of cross-linking were evaluated; the retentions were carried out from a mixture of phosphate buffer and acetonitrile (6:4) adjusted to pH=8 for 24 hours with constant stirring at 130 rpm. The results indicated that the three polyesters could retain 99% of the drug. Consequently, the releases were carried out using the dialysis method in the solution prescribed for the retention, stirring at 130 rpm for 79.12 hours. Subsequently, the amount of florfenicol released was determined by absorbance measurements at 266 nm. From the release curve, approximately 15% of florfenicol was released during the first 24 hours, and around 26% remained at the end. Therefore, the system can control the release of florfenicol over a wide time range.

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INNOVATIVE DISEASE MANAGEMENT: COMPARATIVE EFFICACY OF FLORFENICOL AND C3COS AS A POWERFUL DUO AGAINST Piscirickettsia salmonis IN ATLANTIC SALMON

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C3COS® is an innovative formulation provides a sustainable alternative to antibiotics in aquaculture, enhances antimicrobial efficiency and reduces the risk of pathogenic resistance, supporting fish health and performance. C3COS® is a natural combination, composed of organic acids and plant extracts that improve animal well-being, ensures the quality of the final product and taking care of the environment. This study compared the efficacy of Florfenicol at 20 mg/kg for 10 days with the combination of C3COS + Florfenicol at 10 mg/kg for 10 days of treatment in Salmo salar infected with Piscirickettsia salmonis under controlled conditions. The fish were inoculated intraperitoneally with P. salmonis, and mortality, immunogenic markers, bacterial load, and general health indicators were evaluated. During the study, S. salar maintained a feed intake above 1.5% of its body weight and showed no significant changes in biometric parameters. The disease manifested between days 20 and 26 post-inoculation in the positive control group, with a cumulative mortality of 40% at 37 days. Both treatments, Florfenicol and C3COS + Florfenicol reduced mortality to 6.7%, matching the negative control. Both treatments achieved a significant reduction in mortality and bacterial load compared to the positive control. The combination of C3COS + Florfenicol at 10 mg/kg demonstrated comparable efficacy to the full dose of Florfenicol (20 mg/kg), suggesting a compensatory effect on immune response or an enhancement of Florfenicol. C3COS is proposed as a sustainable treatment strategy for the salmon industry, potentially reducing Florfenicol use by 50% in the treatment against P. salmonis.

BIBLIOGRAPHIC EVALUATION OF PLANT EXTRACTS AS AN ALTERNATIVE TO THE USE OF ANTIBIOTICS IN SALMON FARMING

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Salmon farming in Chile, a key industry for the country's economy, faces significant challenges. The intensive use of antibiotics has resulted in resistance to these drugs and negative effects on the ecosystem. This situation demands the search for efficient but also sustainable alternatives. Plant species possess a wide range of bioactive, biodegradable and environmentally friendly metabolites. Thus, this study proposes to evaluate the antibacterial potential of plant extracts against Piscirickettsia salmonis, Tenacibaculum spp., Renibacterium salmoninarum, Aeromonas salmonicida, Flavobacterium sp. and Vibrio sp.
This document presents the first stage of the study, which consisted of a bibliographic review on the presence of plants with antibacterial activity in Chile. The search for articles was carried out using databases such as Science Direct, Springer link and Google Scholar, considering as a selection criterion, species whose minimum inhibitory concentration was classified as highly active or significantly active against Gram-negative and/or Gram-positive bacteria.
50 species were found that possess these characteristics. The study of these plants and their bioactive compounds represents a promising alternative to the use of antibiotics in Chilean salmon farming. The information generated in the research that begins with this data could contribute to reducing the risks to human health and the environment associated with the use of drugs in salmon farming.

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DEVELOPMENT OF A BACTERIOCIN-BASED FEED ADDITIVE FOR THE CONTROL OF AHPND IN SHRIMP AQUACULTURE

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Acute Hepatopancreatic Necrosis Disease (AHPND), caused by Vibrio parahaemolyticus, presents a significant challenge to global shrimp aquaculture, leading to severe mortality and production losses. Organicin Scientific has developed a novel bacteriocin-based feed additive as a non-antibiotic solution to mitigate AHPND in whiteleg shrimp. Two independent trials were conducted to evaluate the efficacy of this feed additive. A trial at the University of Arizona demonstrated that shrimp fed with low and high doses of bacteriocins achieved survival rates of 93% and 100%, respectively, compared to 35% in positive control groups subjected to AHPND. Similarly, a larger-scale trial conducted at ShrimpVet showed a 38% improvement in survival at the lowest dose post-challenge, alongside enhanced immune responses and weight gain. These results highlight the potential of bacteriocin-based feed additives to significantly reduce shrimp mortality, improve zootechnical performance, and offer an effective, non-antibiotic strategy for controlling AHPND in shrimp farming systems. Further research is ongoing to refine dosage and explore additional health benefits.

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