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Immunomodulatory and Gut-Protective Effects of a New Probiotic Feed Intended for Salmonids

Introduction

The intensive fish-farming relies heavily on efficient feeding and high fish densities, which in turn heightens the susceptibility to diseases. Factors such as repeated handling, bad water quality, or inadequate nutrition contribute to stress, compromising the fish's immune system and increasing disease susceptibility. The main risks are microbial diseases, which are responsible for serious losses in aquaculture. Prevention measures have been developed against some of the diseases, such as vaccination, but antibiotic medication remains the most common way to combat bacterial infections. These medications have adverse effects on both meat quality and the environment. As an alternative, probiotics are successfully used in aquaculture, as in other animal species. The majority of successfully used probiotic preparations contain lactic acid bacteria (LAB). When using autochthonous strains of probiotic microorganisms, there is a higher probability of their good adaptation to the conditions of the relevant species or its environment as compared to non-autochthonous strains. Therefore, our research is focused on the development of probiotic feeds based on autochthonous LAB strains capable of increasing the resistance of fish to stress and infectious diseases.

Methods

Based on the selection criteria, which included antagonistic activity against bacterial pathogens of salmonids, a high survival rate in the conditions of the digestive tract of fish and water, two strains with the best properties were selected - *Lactiplantibacillus plantarum* R2 Biocenol™ and *Limosilactobacillus fermentum* R3 Biocenol™. Both strains were obtained from the intestines of healthy trout and met the antibiotic sensitivity criteria according to EFSA. The influence of R2 strain on the immune response of healthy and infected (*Aeromonas salmonicida* and *Yersinia ruckeri*) primary trout intestinal cell cultures was tested. For aquatic application, two novel forms have been designed by incorporating these strains into fish feed with preservatives. Subsequent experiments involved assessing continuous and cyclical application in non-infectious trials on trout and administration to salmon with induced intestinal inflammation. Then, probiotic feed was given to trout infected with *A. salmonicida* and in the final stage it was fed to trout in large scale farm experiment.

Results

The results indicated that both strains positively impacted the immune response and gut microbiota, as evidenced by reduced enteritis in salmon and increased TGF- β gene expression in trout. Furthermore, cyclic administration stimulated the gene expression of other immune-related molecules in the gut, such as CD8, IgM, IL-8, and TLR-9, without elevating proinflammatory cytokines like IL-1 and TNF- α . Beneficial LAB predominated in the intestinal microbiota and the absorption area of gut was increased.

Discussion

Based on the obtained results, we can conclude that continuous application can be used for stabilization of gut microbiota in favor of LAB without overstimulation of intestinal immunity. However, cyclical application provides the opportunity to modulate the immune response in critical periods associated with stress, such as transport, change of feed, bad weather conditions, etc. In addition, the newly developed application forms proved to be suitable, as it ensures the long-term survival of the probiotic strains, are cheap with a simple preparation technology and accepted by the fish.

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