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Red seaweed: Promising, sustainable feed additive combats ISA virus

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Inclusion in the diets provided good growth and antiviral protection

When included in diets for salmon, the red seaweed *Pyropia columbina* was shown to improve growth and antiviral activity.

The outbreak of infectious salmon anemia (ISA) in Chile resulted in the loss of some U.S. $5 million for the salmon production sector from 2007 to 2009, as well as a 75 percent reduction in the production of Atlantic salmon (*Salmo salar)*. In response, the Chilean government and the salmon industry applied a series of measures to control and eradicate the disease. However, ISA and the potential emergence of new diseases continue to be problems for salmon production in Chile and other producing countries.

Infectious salmon anemia is caused by a virus of the *Orthomyxoviridae* family. In its clinical stage, the disease affects cultivated Atlantic salmon in their seawater stage. However, the disease has not been described in wild Atlantic salmon, even though the aquatic environment naturally hosts high concentrations of pathogens, including the ISA virus. This indicates that natural factors allow wild salmon to remain in good health.

Seaweed traits

The effects of diet on animals' immune systems are well known, in particular in relation to disease resistance in fish. Both macronutrients and micronutrients can affect responses to pathogens such as viruses. Functional diets that include bioactive ingredients have been shown to increase disease resistance while also increasing growth and feeding, making them a powerful tool to prevent diseases. Among the dietary options is the use of seaweed.

One of the most important characteristics of marine seaweed is its capacity to absorb and concentrate mineral salts, vitamins, oligo-elements and amino acids, which they use to meet metabolic needs. Because of this, marine seaweed is considered a major reservoir of natural molecules. Given this capacity to concentrate nutrients, seaweed is an important food source, although its nutritional composition varies by species, geographic location and harvesting season.

Red seaweed or rhodophyta (*Phylum rhodophyta*) is considered a much more important source of biologically active metabolites than other types of seaweed. Its bioactive content includes sulfated polysaccharides, Vitamin C, amino acids, peptides, fatty acids and proteins. Among the bioactive proteins are two important groups: lectines and phycobiliproteins. The latter are found only in cyanobacteria and red algae.

Given the above, red seaweed is a potential source of antiviral components for fish. The unique nutritional properties of red seaweed include high levels of protein, and the seaweed can be cultured in a sustainable and environmentally friendly manner.

ISA study design

The authors performed a study to assess a strategy of using natural ingredients that form part of the first trophic level as part of the diet of Atlantic salmon in the marine phase in a strategy to combat the effects of the ISA virus. The impacts of the diet were assessed by an ex vivo protocol following the in vivo application of the diet to *Salmo salar*.

The Chilean red seaweed species selected for the experiment were *Pyropia columbina*, which has a high protein content throughout the year (25.20 percent crude protein in winter) and *Gracilaria chilensis*, which has the second-highest protein content (24.31 percent in winter) among Chile's red seaweeds. Both species are accessible in Chile through natural banks and by cultivation. *G. chilensis* is cultivated commercially in Regions I, III, IV, VII and X, while *Pyropia columbina* is cultivated in Region X.

The red seaweed used in this study was harvested in the area of Ancud, Chiloe Island, in southern Chile in the winter of 2012. Immediately after harvesting, the algae were taken to the city of Calbuco, Chile, to be freeze dried.

Lyophilized concentrates of both species were prepared and added to a commercial diet. Diets were prepared with 0.1, 1.0 and 10.0 percent *Gracilaria chilensis* and P*yropia columbina* separately, and 0.1 and 1.0 percent of a mix of both species at a 1:1 ratio. The experimental diets were extruded into 3.0-mm pellets.

The diets were fed twice a day to satiation for eight weeks to eight triplicate groups of 18 Atlantic salmon at the BioMar Chile outdoor tank facilities on the island of Chiloe in southern Chile. The initial average body weight of the fish was 149.05 ± 32.00 g.

Data on production parameters and blood samples were collected at two, four and eight weeks. Serum and its constituents were challenged with ISA virus in the presence of salmon kidney cells under controlled laboratory conditions. Antiviral activity against ISAV was evaluated through a plaque reduction assay.

The moisture content of red seaweed is calculated before the seaweed is freeze-dried.

Results

Nutritional characterization

The amino acid profiles showed interesting values for taurine in the freeze-dried samples from fish fed both *Pyropia columbina* (0.99 percent) and *Gracilaria chilensis* (1.06 percent) compared to levels in

fishmeal (0.69 percent) and rapeseed meal (0.06 percent), the latter two produced in Chile. This was a very promising result, considering the other plant sources for fish feed are deficient in taurine, an important amino acid involved in anti-inflammatory responses in fish, as well as a powerful antioxidant that protects fish against oxidative tissue damage.

The levels of arachidonic acid (ARA), an omega-6 fatty acid, in fish fed *Pyropia columbina* (10.88 percent) and *Gracilaria chilensis* (9.63 percent) were about five times as high as the 1.91 percent level in fishmeal produced in Chile. This was very promising, given that the presence of this fatty acid in fish results in higher rates of growth and survival.

ARA and ARA/eicosapentaenoic acid levels increase significantly in Atlantic salmon during smoltification. Fish with higher ARA content in gill phospholipids adapt better to the conditions and challenges of saltwater. Considering that fish have a limited capacity to synthesize ARA, the amino acid needs to be provided in their diets.

Fish performance

The growing performance of experimental fish was not negatively affected by the inclusion of red seaweeds in the experimental diets. In fact, a considerable improvement was observed in fish performance in some cases, such as the diet with 10 percent *Gracilaria chilensis* (1.50 ± 0.12), which resulted in a higher specific growth rate than that of the control diet (0.92 ± 0.01), as shown in Fig. 1.

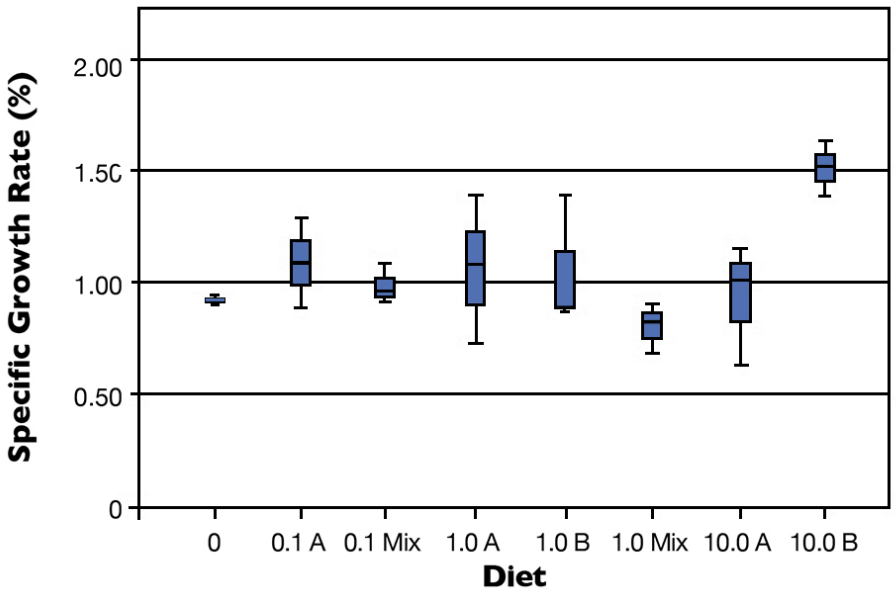

Fig. 1: Effects of diet on the specific growth rate of salmon.

A = *Pyropia columbina*

B = *Gracilaria chilensis*

0 = control diet

BioMar Chile maintains outdoor tank facilities on the island of Chiloe in southern Chile.

Anti-ISAv activity

After completing the challenge protocol to ISAv with cell monolayers in the presence of salmon sera from fish fed diets with red seaweed, lysis plaques generated by the virus were obtained by incubation and counted. Control samples were obtained from fish fed diets without red seaweed. No antiviral effects were detected in the control samples.

In contrast, the sera of fish fed the diets with red seaweed had significantly higher levels of antiviral activity against ISAv – 0.013 ($P < 0.05$) – as compared to the sera of positive control fish. The serum of fish fed diets with 0.1 percent seaweed mix (55.73 ± 19.63 percent) and 10.00 percent of *Gracilaria chilensis* (48.52 ± 5.05 percent) showed the greatest increase in antiviral activity compared to the control diet (4.12 ± 9.45 percent) (Figure 2).

Fig. 2: Effects of diet on anti-ISAV activity in salmon.
A = *Pyropia columbina*
B = *Gracilaria chilensis*
0 = control diet

The test results showed that red seaweeds increased the capacity of *Salmo salar* to resist the ISAv pathogen by 55.73 ± 19.63 percent.

Perspectives

Compared to serum from the control fish group, serum from fish fed diets supplemented with red seaweed showed a significant increase in anti-ISAv activity. This could explain why symptoms of infectious salmon anemia have been identified in farm-raised salmon and not in wild-caught salmon.

The inclusion of the Chilean red seaweed species *Pyropia columbina* and *Gracilaria chilensis* in the diets of *Salmo salar* provided two main benefits: good growth and antiviral protection. Both seaweed species can be farmed, so their use could be an excellent strategy to help ensure the sustainability of Chilean salmon farming.

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