- About
  - Who We Are
  - Our History
  - Our Team
  - Sustainable Development Goals
  - Careers
- Membership
  - Overview
  - Our Members
  - Corporate Membership
- Resources
- Certification
  - Best Aquaculture Practices
  - Best Seafood Practices
- GOAL Events
- Advocate Magazine
- Aquademia Podcast
- Blog
- Contact

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# Breaking the 20 percent soy barrier in fish feed

Responsible Seafood Advocate logo

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## Improved trout line boasts good growth, no distal enteritis

After 14 years of selective breeding, a line of rainbow trout grows rapidly on a high-soy diet and also does not develop distal enteritis. The trout phenotype can be a tool to further explore mechanisms of fish growth and health.

The 20 percent soy barrier in fish feed content has been an impediment to the development of more sustainable feeds for many farmed fish species for the last two decades. Many published papers describe the results of short-term feeding trials that show soybean meal can exceed 20 percent in feeds for salmon, cobia, trout, sea bass, sea bream and yellowtail. However, when such diets are fed for longer periods in commercial production, the results are often reduced fish growth, lower feed efficiency, increased mortality rates and distal enteritis.

Several lines of research on factors in soybean meal responsible for low fish performance have been explored, starting with assessing the effects of antinutrients in soy proteins, such as trypsin inhibitors, lectins, saponins and non-soluble carbohydrates. Phytic acid, the storage form of phosphorus in seeds, has also been blamed for reducing the performance of fish fed high-soy diets. Although phytic acid is not an antinutrient per se, it interacts with calcium ions to form insoluble complexes that reduce zinc digestibility, causing a conditional deficiency in salmonids.

Other research has focused on identifying nutrients that are lacking in soy protein ingredients compared to fishmeal, such as cholesterol, taurine, phosphorus or trace minerals, and fortifying high-soy diets to increase their levels. Finally, fishmeal contains androgenic steroids, unlike soy, which contains estrogen-like substances. Androgenic steroids in fishmeal increase growth rates in salmonids.

## Unsuccessful research lines

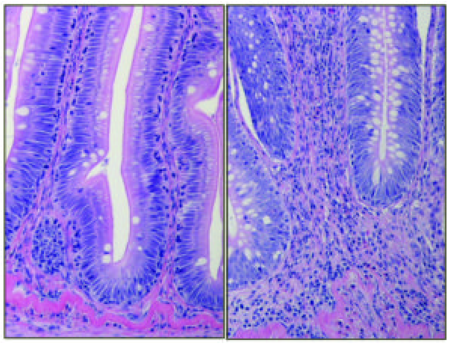

Fig. 1: Grade 2 (left) and grade 4 (right) histological slides of distal intestine. In the grade 4 photo, note the increased inflammation, as well as expansion of lamina propria and an increased number of goblet cells in the epithelium.

Fishmeal and soybean meal are complex products containing hundreds of compounds that are biologically important. In fish feed formulations, the usual approach is to ensure that all essential nutrients are present at levels above the minimum dietary requirements of fish. In practice, this means supplementing high-soy feeds with DL-methionine.

When feeds are formulated in this manner, the performance of fish improves, but not to control diet levels. Similarly, formulating high-soy feeds with supplemental amino acids to approximate the amino acid pattern of fishmeal improves performance, but still not to control feed levels.

Soybean meal can also be pretreated with heat to inactivate anti-nutritional factors. Pretreatment improves the nutritional value of soybean meal, but not enough to overcome reduced fish growth.

These research lines have been explored for many years in fish nutritional research without achieving breakthrough results. They are no longer considered the research avenues of preference likely to achieve them.

## Trout-breeding program

In 2001, a selective-breeding program for rainbow trout was started at the Hagerman Fish Culture Experiment Station in Idaho, USA, by the Agricultural Research Service and the University of Idaho. Focusing mainly on enhancing growth and dietary utilization, the program fed rainbow trout a plant protein-based diet.

After 14 years of selective breeding, the selected line of trout produced grows rapidly when fed an all-plant-protein, high-soy diet. However, another notable outcome of the program was a second trait linked with soy-based plant protein diets – the selected line does not develop distal enteritis. These results led the research team, with financial support from the soybean checkoff program through the Soy Aquaculture Alliance, to apply an alternative approach to studying soy-induced distal enteritis.

The selected line of trout exhibits a specific phenotype that can be used as a unique research tool to explore the mechanisms of distal enteritis development and other aspects of growth and health when combined with high-throughput sequencing and advanced bioinformatics.

For the first time, a fishmeal diet would not be considered the standard control diet. By comparing the genetic and physiological responses of the selected trout with those of non-selected trout when fed the all-plant-protein, high-soy diet, the mechanisms associated with the response of trout to high-soy feeds could be explored in an entirely new manner.

## Distal enteritis

Many carnivorous fish species exhibit distal enteritis when fed high-soy feeds. The condition is best described as an inflammatory process typically located in the first section of the distal intestine that alters the cell structure and morphology of intestinal villi. The incidence and degree of enteritis increases with the soybean meal level in feed and duration of feeding.

In rainbow trout, distal enteritis is not always evident after 12 weeks of feeding, but can become full blown after 24 weeks. The severity of distal enteritis is visually judged by scoring histological slides using a scoring system based on evaluation of mucosal folds, goblet cells, supranuclear vacuoles, eosinophilic granulocytes, subepithelial mucosa and the lamina propria. An additional criterion for scoring the severity of distal enteritis is total inflammatory cells.

Fig. 1 shows histological slides of distal intestine. The left image shows a grade 2 sample, while the other reflects grade 4. In the grade 4 photo, note the increased inflammation, as well as expansion of lamina propria, both between and below the crypts. There are also a loss of supranuclear vacuoles in epithelial cells and an increased number of goblet cells in the epithelium.

## High-throughput sequencing

The development of high-throughput sequencing technology has changed the ability of researchers to investigate genomic differences, changes in global gene expression and the roles of the microbiome in humans, livestock, companion animals and now fish. The roles of the intestinal microbiota of fish in digestion and disease resistance have only recently come under investigation. Results suggest that gut microbiota are involved in key processes, including nutrient metabolism, innate immune responses, epithelial proliferation and overall fish growth and health.

Next-generation sequencing has proven to be a very powerful tool to identify all microorganisms in a sample based on unique genetic sequences in each bacterial class, family, genera or species. Initial findings by the authors evaluating the intestinal microbiota of selected and non-selected fish raised on either plant- or fishmeal-based diets revealed distinct changes in microbial populations linked specifically to diet, strain of fish and intestinal region.

## Responses to high-soy diets

Research has been under way for several years at the Aquaculture Research Institute in Hagerman, Idaho, USA, to characterize the responses of selected and non-selected trout to a high-soy diet. Initial results confirmed that the selected strain of rainbow trout indeed adapted to the high-soy diet and was distinctly different from non-selected fish, thus validating the use of this genetic line as a model organism.

Selected and non-selected trout strains differed in their capacity to grow on the high-soy diet, and this trait appears to be linked, in part, to differences in efficiency of digestion. Differences between strains in plasma amino acid levels following a single feeding were found in the hepatic portal veins of the fish. Differences were also found in plasma amino acid patterns in samples taken from caudal veins, which suggested differences among strains in amino acid metabolism in peripheral tissues and in post-hepatic processing.

The selected strain did not exhibit distal enteritis after 28 weeks of consuming the high-soy diet, in contrast to a high incidence of enteritis in the non-selected trout strain. Surprisingly, histological examination of distal intestine tissue samples revealed a biofilm containing cocci in samples from non-selected fish, whereas none were found in tissue samples from the selected strain. This finding suggested a link between the fish gut microbial community, particularly the microbiome associated with intestinal mucosa, and distal enteritis.

## Perspectives

Ongoing research is currently focused on integrating data from strain single-nucleotide polymorphism identification, transcriptomic and proteomic analysis of individual tissues, and microbial population changes with physiological responses to determine the roles of diet, microbiome, host and their interactions associated with the increased efficiency of selected fish in utilization of all-plant, soy-based aquaculture feed.

The next step will be to identify genetic and physiological markers in selected fish fed high-soy diets associated with resistance to development of distal enteritis, rapid weight gain and improved protein efficiency. These biomarkers will be used to improve the performance of commercial rainbow trout strains. The outcome of this research will lead to a new understanding of how selected fish adapt to high-soy diets.

This information will facilitate accelerated selection in other farmed species and identify specific microbial species in the gut microbiota that are associated with high tolerance of soy and improved fish performance, potentially leading to candidate probiotics specifically designed for high-soy feeds.

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