# Science & Solutions

# A Close Look at Gut Health



<u>Aquacu ture</u>

Why It Matters in Aquaculture



Tools to Boost Immune Fitness

## Editorial

## Gut Health in Aquaculture

Several decades ago, the gut was only considered important for digestive functions. When interactions between the gut, its indigenous microbes, and the host were investigated, it became apparent that the role goes beyond mere energy uptake. Many studies of humans and animals elucidate the role of the gut and its microbiota in health and disease.

We now know that a healthy gut is essential to the well-being and health of aquatic animals. There is continuous interaction between the gut and its microbiota. The microbial populations build a barrier against incoming pathogens, produce enzymes and synthesize vitamins, modulate the immune system, promote development of the gut epithelium and the mucosal tolerance. In the zebrafish model, more than 200 genes were found to be regulated by gut microbiota, demonstrating the relevance of gut health for the overall well-being of an aquatic species. In this issue of **Science & Solutions** we introduce the first article in a series on gut health in aquaculture.

There are numerous ways to improve and maintain gut health, namely probiotics, prebiotics, and phytogenic substances. Probiotics, or beneficial bacteria, create a favorable microbial community in the gut. Prebiotics specifically stimulate growth of indigenous beneficial bacteria. Immune modulators strengthen the animal's immune response. Adding novel mixtures of essential oils or organic acids into the feed provides additional ways to improve gut health. The second article explores the link between these tools, gut health and immunity in aquaculture species.

Wishing you an enjoyable read.

Barbara WEBER Scientist





## Contents



## 2

## **Gut Health in Aquaculture**

First in a series of articles to discuss the growing importance of gut health for the industry.

By Rui Gonçalves and Gonçalo Santos



## **Tools to Boost Immune Fitness**

Numerous situations induce stress in aquaculture species. Prebiotics and probiotics can support immune competence and performance.

By Jutta Zwielehner

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All photos herein are the property of BIOMIN Holding GmbH or used with license. Printed on eco-friendly paper: Austrian Ecolabel (Österreichisches Umweltzeichen) Gut health [GAt hEl0] n. 1. improve efficiency 2. prevent disorders 3. re-establish gut integrity Related terms: immunity, per

# Gut Health in Aqua

by Rui Gonçalves, Technical Manager and Gonçalo Santos, R&D Manager

This first in a series of articles introduces three key factors and the main performance indicators of gut health, underscoring its growing importance in aquaculture.



he gastrointestinal tract is an extraordinary organ: it can be regarded as an inner skin. This barrier function separating an external environment from the organism's cells is one of the key features of the gastrointestinal tract. It also plays an important role in nutrient absorption and immunity.

## **Gaining ground**

In aquaculture, fish and shrimp live in close connection with the surrounding environment. Through the ingestion of water, aquatic farmed animals face pathogens and environmental stress constantly in the gut. Therefore gut health is extremely important in aquatic farmed species. Gut health is a concept that has been around for many years, however it has recently gained more attention in the field of aquaculture.

## Defining gut health

The term 'gut health' is part of a complex animal health definition that relies on a diverse set of gut performance indicators. In aquaculture the diversity of farmed species make this even a more complex topic. The key feature of the gastrointestinal tract comprises its ability to digest feed and make it suitable for absorption and growth under healthy conditions, e.g. in the absence of disease, leading to improved animal performance. At BIOMIN we define gut performance management according to three objectives: to improve the efficiency of the gut; to prevent gastrointestinal disorders and related side effects; and to re-establish gut integrity after a dysfunction.

## **Three factors**

Understanding gut health requires the elucidation of the complex interactions between different components that will allow the gut to perform under normal physiological functions and to maintain homeostasis, thereby supporting its ability to withstand infections and non-infectious stressors. Such complex interactions can

## Gut health has recently gained more attention



be grouped in three pillars: animal physiology, nutrition and environment. These three main factors interact, influencing the gastrointestinal microbiota community, and consequently, gut performance (*Figure 1*).

### Normal operation

An animal's physiological status can be characterized by its metabolism, which is set according to gastrointestinal anatomy/physiology variations according to each species, feeding habits, diet, nutrition and physiological condition. It is further influenced by health challenges and defence mechanisms. Defence mechanisms will have particularities inherent to each species and some pathogens will also be species-specific.

## Nutrition and alternative feeds

The second pillar focuses on nutritional aspects, where raw material quality and origin sources in particular can affect gastrointestinal health. Nutrient digestibility, anti-nutritional factors, mycotoxin contamination and lipid oxidation can all influence the microbiota community. Nutrition is especially important nowadays due to the pressure to replace fishmeal and fish oil with alternative ingredients (*Figure 2*).

#### **Environmental influences**

The final pillar, environment, takes into account both the richness of both the physical environments in their wide diversity as well as the richness of pathogens and antigenic stimuli. All those surrounding factors influence the animal's homeostasis. Aquatic animals are constantly ingesting the surrounding water, thus pathogens and antigenic stimuli present in water, are also ingested and will challenge or adapt the microbiota community. At the same time, they are exposed to variations of salinity, temperature, oxygen, pH and rearing stress. These environmental stressors will influence the host homeostasis by directly affecting the microbiota community and indirectly influencing the energy budget spent to recover homeostasis.

## **Community support**

Microbial metabolic activities ensure the uptake of energy and the absorption of nutrients, promote gastric development, and stimulate epithelial cell proliferation and differentiation, while maintaining mucosal tolerance and providing protective functions against pathogens. As a result, there is great scientific interest in modulation of the intestinal microbiota in favor of beneficial bacteria through the use of novel feed additives such as probiotics, prebiotics, phytogenics and organic acids – sometimes in combination.

## in the field of aquaculture.



Figure 2. Steady decline in fish meal and fish oil content of aqua feeds

Source: Adapted from FAO Fisheries and Aquaculture Technical Paper No. 564. FAO, 2011. 87 pp.

## **Hidden threats**

Aquatic farmed animals constantly face pathogens and environmental stress. These factors sometimes manifest themselves as disease. Often their impact remains at a sub-clinical level – redirecting valuable energy and nutrients away from growth performance and toward animal defences – with consequences that are difficult to detect but do have health and economic impacts. Through natural feed additives we can support animal physiology, harmonizing intestinal microbiota, immunity and resistance to disease and improve animal performance.

## Conclusion

The intestinal microbiota plays an important role in the health and well-being of aquatic animals. The anatomy and physiology of aquatic animals' gastrointestinal tracts will have important determinants for the establishment of the quantitative and qualitative aspects of its microbiota. The three pillars of gut health will allow us modulate some aspects to achieve an effective digestive process, a stable gut microbial population, correct structure and function of the gut barrier, and effective immune system function. The use of feed additives and bioremediation tools can in some cases influence the three factors to effectively support gut health and improve animal performance.



## More to come

In the upcoming issues of **Science & Solutions** dedicated to aquaculture we will address the topic of gut performance in more detail, how nutrition can influence gut health, and the importance of local gut immunity on the overall and health status of the aquatic species. Stay tuned...

# Tools to Boost Immune Fitness

by Jutta Zwielehner, Product Manager Microbials

Fish and shrimp encounter stress in situations such as transport, grading, sea water transfer, handling and during disease seasons. These events depress immune response, impairing health and performance. Prebiotics and probiotics can support immune competence and performance.

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## Stress impacts immunity

Aquacultured species often respond to stress of any sort with feed refusal. Fasting, particularly over a prolonged period (7 days or more), lowers the expression of critical innate immune factors in a manner consistent with lower immune fitness. Transportation stress has been demonstrated to suppress the immune response of fish fry and fingerlings for several days following the transport. Weakened immune response means greater susceptibility to pathogens, lower performance and higher mortality rates.

### Immunity vs. performance?

Some farmers hesitate to use immune boosting products out of a concern that doing so could sacrifice feed efficiency since stimulation uses additional energy that would otherwise be used for growth. While initial stimulation does consume energy, it increases the organism's readiness for tolerance, thus saving energy on the long run. This tolerance results in a fitter animal with a stronger immune competence.

## Prebiotics for immune support

For years *Saccharomyces cerevisiae*, or brewer's yeast, has been used as a prebiotic to improve livestock productivity. Prebiotics stimulate the activity of beneficial bacteria in an organism's gastrointestinal tract, supporting overall gut health. A wide body of evidence demonstrates that *S. cerevisiae* and its components such as nucleic acids (DNA and RNA), chitins (a long-chain polymer of a N-acetylglucosamine), beta-glucans (a type of polysaccharide) and mannan-oligosaccharides (MOS, non-fermentable saccharide polymers) support the immune systems of fish and shrimp (*Figure 1*).

The effectiveness of yeast-based prebiotics can be enhanced by breaking down whole yeast cells into component parts through a process known as autolysis which makes these substances more bioavailable in the animal.

Autolysis improves the immune-boosting effects of yeast.



Figure 1. Summary sketch on the functional ingredients of the yeast Saccharomyces cerevisiae and their associated effects on the immune system of aquaculture animals.



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Figure 2. Cell wall components of yeast activate the respiratory burst activity of primary carp head kidney macrophages in low dose.

**Regular** application of prebiotics and probiotics can support immune competence improvement in farmed species.

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#### Training immune competence

Immune receptors on the surfaces of immune cells and epithelial cells recognize a variety of different surface molecules of pathogens but also yeast components: chitin, B-glucans, MOS, nucleotides (intracellular receptors).

The first step in immune stimulation is binding between receptor and the microbe associated molecular pattern, or MAMP. The now-active immune cell is alert and ready to fight invading pathogens.

From here, the immune system has two options. An MAMP belonging to a dangerous bug invites war against intruders, causing inflammation. An MAMP belonging to a harmless or beneficial bug induces tolerance, and peaceful coexistence results. Which way the immune cell decides depends on the presence of certain factors. Interleukin- 2 (IL-2), for example, causes a prolongation of the life of the immune cell, which triggers war. TNF- $\alpha$ , in contrast, induces tolerance. Frequent contact between immune receptors with harmless MAMPs increases the organisms' readiness for tolerance and trains the immune system for critical situations.

Experimental evidence demonstrates in vitro that the different brewer's yeast cell wall components produce a strong immune activation. Figure 2 shows the respiratory burst activity (release of nitric oxide by macrophages, a type of white blood cell, when fighting pathogens and

other threats) to be on par with that elicited by endotoxin (part of the outer membrane of the cell wall of Gram-negative bacteria such as E. coli).

#### Probiotics and disease challenge

Probiotics can boost aquacultured species' immune competence, thereby improving performance and reducing mortality. Trout weighing 60g to 120g received a multi-strain probiotic product (AquaStar® Growout) were challenged with Yersinia ruckeri, the causative agent of red mouth disease. Probiotic treatment at 2g/kg feed (top coated) improved growth performance and reduced mortalities associated with the pathogenic challenge by 30% (Figure 3).

## Combined tools for shrimp

The development of specific pathogen free (SPF) stocks have allowed some level of control of disease in shrimp aquaculture. Closed or recirculating facilities are often considered the next best method to control the introduction of pathogens. However, a lack of natural resistance of the shrimp stocks results in a highly susceptible environment. Strong biosecurity protocols and disinfection methods are necessary in combination with immune modulators to face this challenging situation. A farm in Indonesia had previously reported heavy



Source: BIOMIN, 2008



Figure 3. Trout performance and mortality during a 13-day feeding period after Y. ruckeri challenge.

Source: University of Veterinary Sciences Vienna, Austria 2015

losses from white spot syndrome virus conducted a pilot scale study to gauge the effectiveness of a combined prebiotic and multi-strain pond probiotic application (Levabon® Aquagrow E; 3g/kg feed morning, 2g/kg feed afternoon and AquaStar® Pond; 100g/pond every 3 days) in a closed system.

Table 1 illustrates the trial results. All four ponds received the pond probiotic AquaStar® Pond, though this alone was not sufficient to reduce WSSV disease incidence. WSSV-related mortalities occurred in the control ponds from day 20. In the treatment pond first symptoms were observed only from day 60. In all three control ponds the crop was lost.

In treatment pond 4 the limited availability of peddle wheels led to low dissolved oxygen levels at night, leading to



Table 1. Pilot study results in a shrimp farm with a confirmed history of WSSV.

	Size	Flush out	Final harvest	FCR
Pond 1 (control)	0.4 ha	Day 26	0	-
Pond 2 (control)	0.38 ha	Day 31	0	-
Pond 3 (control)	0.2 ha	Day 40	0	-
Pond 4 (Levabon <sup>®</sup> AquaGrow E and AquaStar <sup>®</sup> Pond)	0.22 ha	-	3800 kg	1.33

Source: BIOMIN, 2015

early harvest of some shrimp at day 72 (312 kg, size 74) and day 81 (540 kg, size 63). Mortalities appeared from day 89. The remaining shrimp were harvested at day 91 (2983 kg, size 48).

## When to modulate immunity

In general, regular application of prebiotics and probiotics can support immune competence improvement in farmed species.

Higher or additional dosages can be applied in the days leading up to stress trigger events. For marine fish, it is recommended to introduce a prebiotic in the starter feed, for example 4 kg/t, and halve the dosage in growout and finishing feed.

## Conclusion

Stress impairs aquaculture species' immune response, health and performance. Prebiotics and probiotics can support improved immune competence and performance in fish and shrimp. In some cases, their combined application is required to achieve optimal effectiveness. Regular immune modulation offers a clear way to improve immune competence. 🥏





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