Hiding in plain sight: Gut health

by Karin Pittman [1,2], Sjur Øyen S[1], Mette Sørensen[3], Kjetil Korsnes [3,4], Dalia Dahle[3], Nimalan Nadanasabesan [3], Mearge Okubamichael[2], Grigory Merkin G[2], Ole Jacob Myre[2]
Dept of Biosciences, Univ of Bergen, Thormøhlensgate 53, 5006 Bergen, Norway
Quantidoc AS, Thormøhlensgate 41, 5006 Bergen Norway
Nord University, P.O. Box 1490, 8049 Bodø, Norway
BioVivo Technologies AS, Storbjørnen 17, 8029 Bodø, Norway

Fish wear their immune system on the outside and the answer to many industry conundrums is likely hiding in plain sight. This statement refers to the mucosal immune system, or rather all the slimy barriers in skin, gills and intestines (the outside of the inside), (See Figures 1A and 1B).

Why does this matter? We can think of this protection like a detective's tools to find out where a production challenge lies: the skin is the shield and able to tolerate some handling and damage, the gills are the sentinel guarding 50 percent of the surface area of the fish, while the guts are the foundation, reliant on incoming nutrition upon which almost all else in the fish is built.

The structure of skin, gill and gut mucosal barriers at the cell-level is almost identical. These tissues can regenerate, and they can learn: this innate mucosal (mucus) immune system is more important for fish than the typical acquired immune system is for mammals. Fish are hatched with this protective system, optimised quite free-of-charge by nature through about 500 million years of evolution. It knows a thing or two about protecting fish health, survival and tolerance.

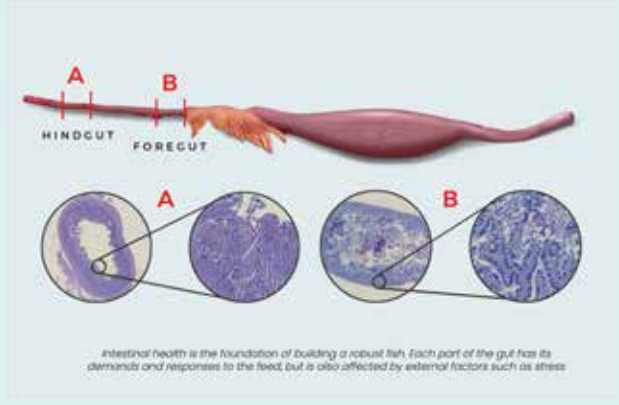
If we can read these immune responses, so what? Feeds give different signals to the guts and the skin, and sometimes to the gills. Everyone knows about the anti-nutrients that come from terrestrial plantstuffs, and everyone knows that fishmeal is generally more expensive than plant ingredients.

Fish gut microbiomes are clearly different from mammalian gut microbiomes and fish genes even more diverse than mammalian genes. This makes functional interpretation a challenge, but we have to start somewhere. To summarise the effects of genes, fatty acids, proteins, lipids and treatments we can look at the tissue which contains the cells we are interested in, the mucus or goblet cells.

This is a much higher level of biological organisation than genes and it is much less variable. The fish intestine reacts to the entirety of a recipe, not just the fashionable silver bullet ingredient (See Table 1).

Figure 1A: The skin's protective mucosal immune system is right on the surface of the fish and on the internal surface of the gastrointestinal tract (blue dots = mucous cells; pink bars = scales; blue tissue = epithelium; stained with Periodic Acid Schiff and Alcian Blue)

Figure 1B: Intestinal health is the foundation of building a robust fish. Each part of the gut has its demands and responses to the feed, but gut and skin are also affected by external factors such as handling and stress



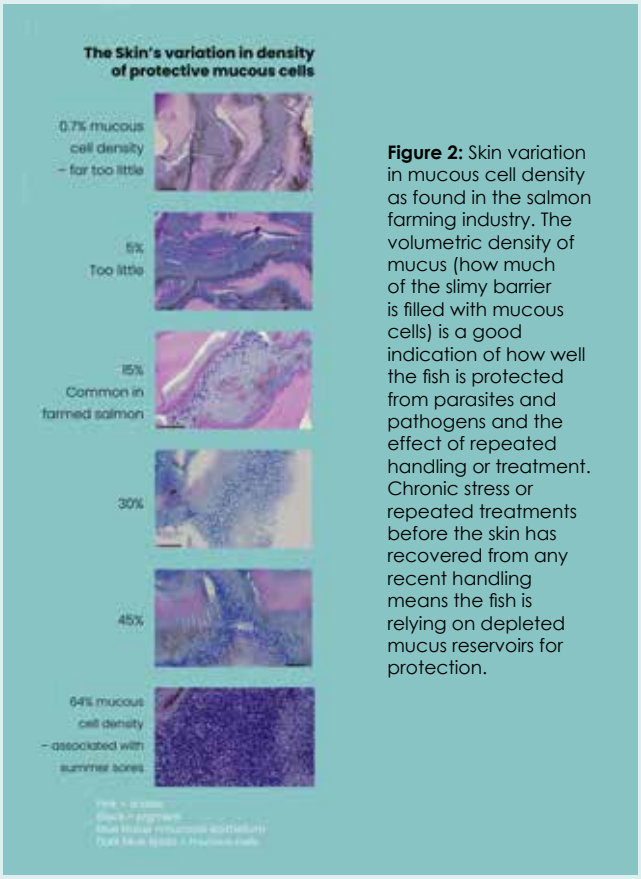


Figure 2: Skin variation in mucous cell density as found in the salmon farming industry. The volumetric density of mucus (how much of the slimy barrier is filled with mucous cells) is a good indication of how well the fish is protected from parasites and pathogens and the effect of repeated handling or treatment. Chronic stress or repeated treatments before the skin has recovered from any recent handling means the fish is relying on depleted mucus reservoirs for protection.

Veribarr: Verification of barriers

The trick is analysing in a way that can be used in comparative models across species and diets and that is statistically robust. This we have done and there are a number of scientific publications documenting this (eg Pittman et al 2013, Torrecillas et al 2015, Dang et al 2019) as well as industrial project results with clients.

Quantidoc has, over the last nine years, developed Veribarr™ to quantifiably assess the slimy barriers in skin, gills and guts in 10 species from nine countries in over 60 large-scale and lab scale trials. The results show that treatments can significantly deplete the immune reservoirs or, conversely, enhance them. A healthy gut can regain balance (homeostasis) shortly after a challenge.

Industrial relevance

There is no life in water without a mucous membrane and no mucous membrane without mucous cells. A tissue's mucous cells (goblet cells) are very dynamic not just in size (5–400 square microns, site specific) but also in the amount of the tissue which fills up with goblet cells.

Skin can literally dry out as a result of repeated handling or delousing (below a mean of 2% volumetric density of slime-producing cells in the skin; Pittman, Jensen, Sissener, Trengereid et al in prep), retain their normal industrial density at between 15-25 percent or be severely irritated and extremely slimy prior to "summer sores" (over 60% density; see Figure 2).

Fish gill mucosa reacts to water quality and therapeutic treatments (Soleng et al 2019). Fish mucus is proactive (See Table 1), so making sure tomorrow's supply can handle the challenges of industrial food production is an integral part of production planning.

Guts are equally dynamic. Gut dysfunction typically arises slowly, ending in chronic inflammation or other debilitating conditions such as little or no mucous cells, similar to human Inflammatory Bowel Disease. Fish diets can, as known, induce enteritis and a number of other chronic conditions which are

measurable by Veribarr™ .

An early application of the technology to trout production was the investigation of "fat gulping" and watery belly flap which was a usual occurrence at one farm. Over the course of seven months we looked at the pyloric caeca, the foregut, the hindgut and the anterior and posterior stomach.

Already by the second sampling, on the basis of changing mucous cell sizes and densities, we recommended a change in diet months before normal and at greater expense. The farmer did so, and the result was good.

"Based on input from Quantidoc AS, we chose to change the diet and monitor the intestinal health of the fish with their methodology over a longer period. This has helped to make this year's production one of the best, with very few problems related to fish growth or quality", notes Svein Fløl0, COO Hofseth Aqua AS.

Benchmarking

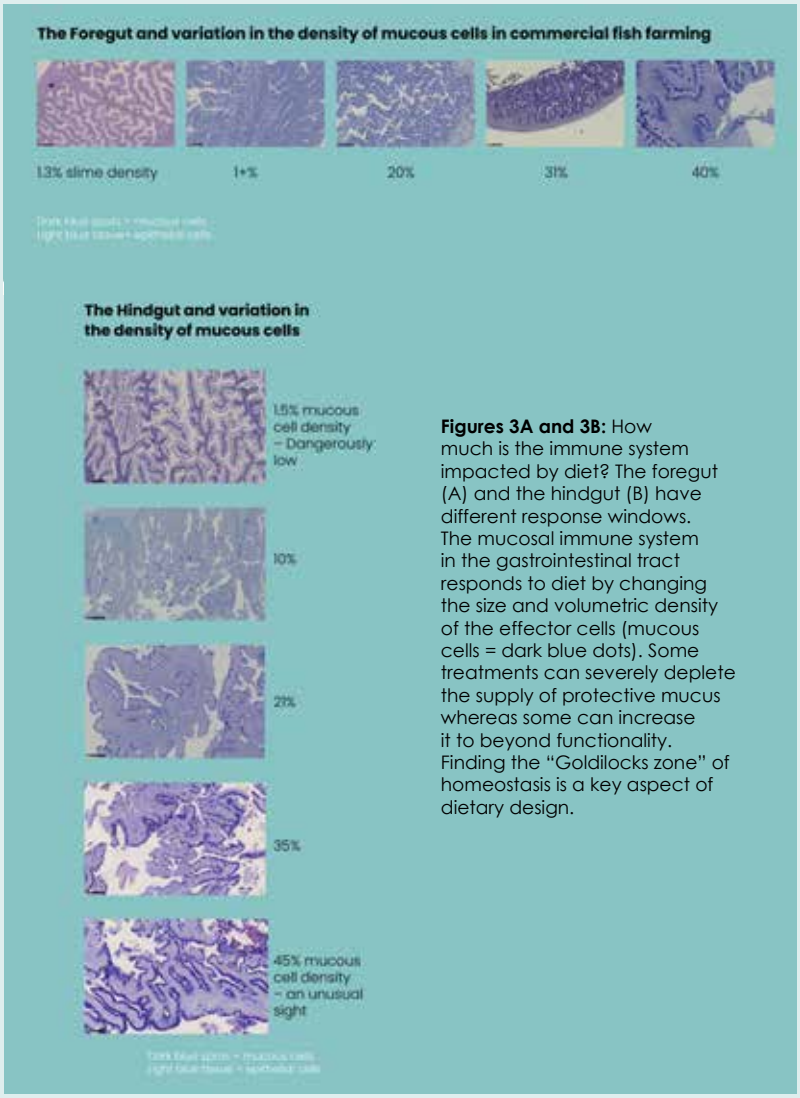
There are clearly differences in diets, and differences in their short-term and long-term effects. Often the "Control" is different between trials while the duration of a trial is between two and six weeks.

A small difference in gut response found after six weeks can translate to a large difference over the production cycle. Or not. One factor is that researchers are usually not told the actual dietary composition for trade reasons (understandable) but another factor is that this lack of basic knowledge confounds the hunt for key ingredients in reasonable, healthy diets and healthy treatments. We need benchmarks at the very least (See Figures 2 and 3).

We are currently finalising analysis of five diets with known recipes given in quadruplicate to 200g salmon in tanks. This careful work is in cooperation with Nord University and BioVivo, funded by MABIT. These diets include one based on fishmeal and fish oil, and several mixing different elements of plant- and fish-based components.

Analyses include antimicrobial peptides, gene expression, fatty acid profiles, growth rates, morphology and gut mucosa including the lamina propria. We appreciate the hard work that goes into learning histology and identifying structural differences. When this is joined with objective measurements the outcome is extremely valuable for all (See Table 2).

We have already determined that the diet of fishmeal and fish oil did not give the best growth and was in the middle of the mucosal responses of all the diets. This means there are many pathways to a satisfactory diet. As we proceed in the analyses, we expect subtle consistent differences in response variables, while the statistically robust barrier data are



Figures 3A and 3B: How much is the immune system impacted by diet? The foregut (A) and the hindgut (B) have different response windows. The mucosal immune system in the gastrointestinal tract responds to diet by changing the size and volumetric density of the effector cells (mucous cells = dark blue dots). Some treatments can severely deplete the supply of protective mucus whereas some can increase it to beyond functionality. Finding the "Goldilocks zone" of homeostasis is a key aspect of dietary design.

Table 1: Slime is pro-active with a wide variety of substances that protect fish from externalities (from Rakers et al. 2010 and 2013)

Substance	Antibacterial	Antifungal	Antiviral	Antiparasitic
H2A peptider	✓	✓		
H1 oncorhyncin2	✓	✓		
H6 oncorhyncin3	✓	✓		
Pleurocidin	✓	✓		
Sal-2	✓	✓		
Complement factors	Antigen-antibody	Antigen-antibody	Antigen-antibody	Antigen-antibody
Hydrolytic enzymes (proteases etc)	Degrade	Degrade	Degrade	Degrade
IgM, IgT	Basic antibodies	Basic antibodies	Basic antibodies	Basic antibodies
Lectins	Pathogen recognition	Pathogen recognition	Pathogen recognition	Pathogen recognition
Mucus extract			✓	✓
Interferon			✓	

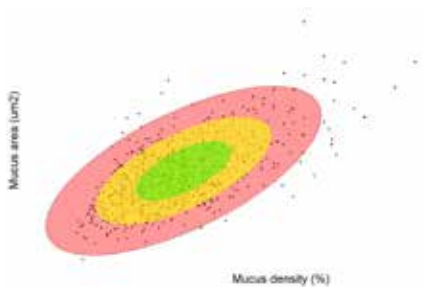
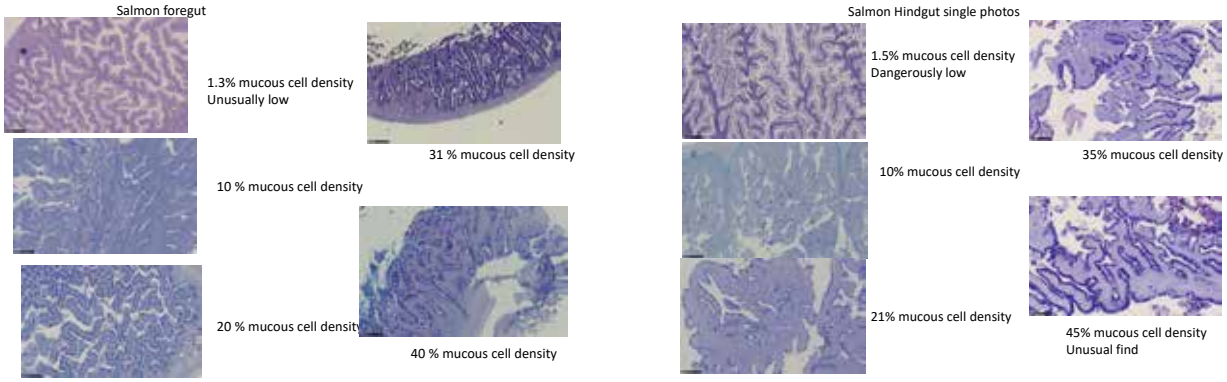


Figure 4: A proposed traffic light system for gut tissue based on the mucous cell sizes and mucous cell densities in hindgut biopsies of farmed Atlantic salmon. Mucous cell area is average mucous cell size at the cell equator, Mucous cell density is volumetric density of mucous cells in the mucosal epithelium. Green = homeostasis or Goldilock's zone; Yellow = dysregulating or recovering; Red = dysregulated. Anonymized data from Quantidoc AS



essential to the understanding the host health. The answer is hiding in plain sight. (See Figure 4).

Photos and figures from "The Robust Fish" (Akselsen et al 2019) except where otherwise indicated. We are very grateful for project support from MABIT AF0082, Norway.

References:

Akselsen B., Pittman KA and Myre OJ 2019. The little book about the Robust Fish and the protection of 0.07 mm. Quantidoc Bergen Norway. 40 pp, ISBN 978-82-691791-0-1

Dang M, Pittman K, Bach L, Sonne C, Hansson SV, Søndergaard J, Stride M and Nowak B. 2019.

Mucous cell responses to contaminants and parasites in shorthorn sculpins (Myoxocephalus scorpius) from a former lead-zinc mine in West Greenland. Science of the Total Environment 678:207-216

Dang M, Pittman K, Bach L, Sonne C, Hansson S, Søndergaard J, Stride M and Nowak B.

Mucosal mapping and traditional histological mucous cell analyses reveal different aspects of mucous cell responses in the gills and skin of shorthorn sculpins (Myoxocephalus scorpius). In submission.

Pittman K, Pittman A, Karlson S, Cieplinksa T, Sourd P, Redmond K, Ravnøy B and Sweetman E. 2013. Body site matters: an evaluation and application of a novel histological methodology on the quantification of mucous cells in the skin of Atlantic salmon, Salmo salar L.. Journal of Fish Diseases 36:115-127

Torrecillas S, Montero D, Cabballero MJ, Pittman KA, Custodio M, Campo A, Sweetman J and Izquierdo M. (2015) Dietary mannan oligosaccharides: counteracting the effects of soybean meal oil inclusion on European sea bass (Dicentrarchus labrax) gut health and skin mucosa mucus production? Frontiers in Immunology 6:397. doi: 10.3389/fimmu.2015.00397

www.quantidoc.com