Probiotics to Boost Immune Fitness and Gut Health

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Despite growing trends in probiotic use, their application in aquafeeds has been constrained by the aggressive processes used during feed manufacture, which kill or maim heat-sensitive bacteria. Advances in post-pellet application and other technologies have overcome this constraint, enabling probiotics to deliver their benefits to the global aquaculture industry.

Probiotics offer feed millers the opportunity to produce value added functional feeds. Once the feed is consumed, a successful probiotic will colonize the intestinal tract and exert a number of benefits, often relating to enhanced immunity and disease resistance.

The intestine is one of the main portals of entry for invading pathogens. In order to successfully infect the host, a pathogen must navigate and survive multiple obstacles and attacks, executed by the host's immune system.

IN BRIEF

- Heat-sensitive probiotics can be successfully added to feeds thanks to advances in post-pelleting and other technologies.
- Probiotics deliver a number of immune and disease-resistance benefits.
- An increase in goblet cell and IEL numbers was recorded in probiotic-fed fish. Goblet cells and IELs are important components of the immune system.
- Probiotics support immunity in the whole animal, as well as locally in the gut.

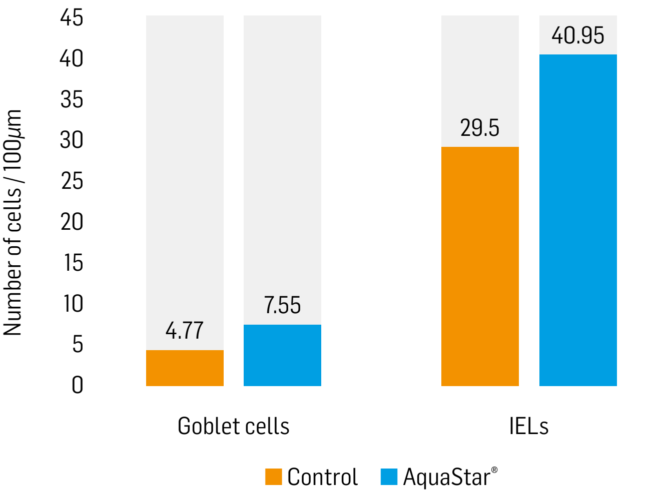
Innate immune response

As with mammals, the immune system of fish can be separated into innate (non-specific) and adaptive (specific) responses. Compared to mammals, fish are more dependent on the innate immune response for two main reasons. First, the innate immune system has developed to be non-specific and is therefore capable of mounting an immune response against a wide range of pathogens. Second, due to the ectothermic nature of fish, adaptive immunity can take considerable time. For example, antibody production in salmonids can take up to six weeks, compared to just hours or days for the innate immune system.

Reinforcing the first line of defense

The mucus layer produced by goblet cells provides the immediate line of defense. The mucus functions to trap and remove pathogens by providing both a physical and chemical barrier, since it contains a number of antimicrobial compounds. This mucus layer can be modified by the commensal microbiota as well as probiotic bacteria. For example, after feeding tilapia with a commercial probiotic (AquaStar®) for five weeks, there were approximately 60% more goblet cells in the intestine (*Figure 1*). These additional goblet cells could contribute to a greater production of mucus, thus providing a more impenetrable barrier, potentially retarding pathogens and preventing their attachment to the underlying epithelia.

Figure 1.
The abundance of goblet cells and intra-epithelial leucocytes (per 100μm) in the intestine of fish fed with and without dietary probiotics.



Source: BIOMIN

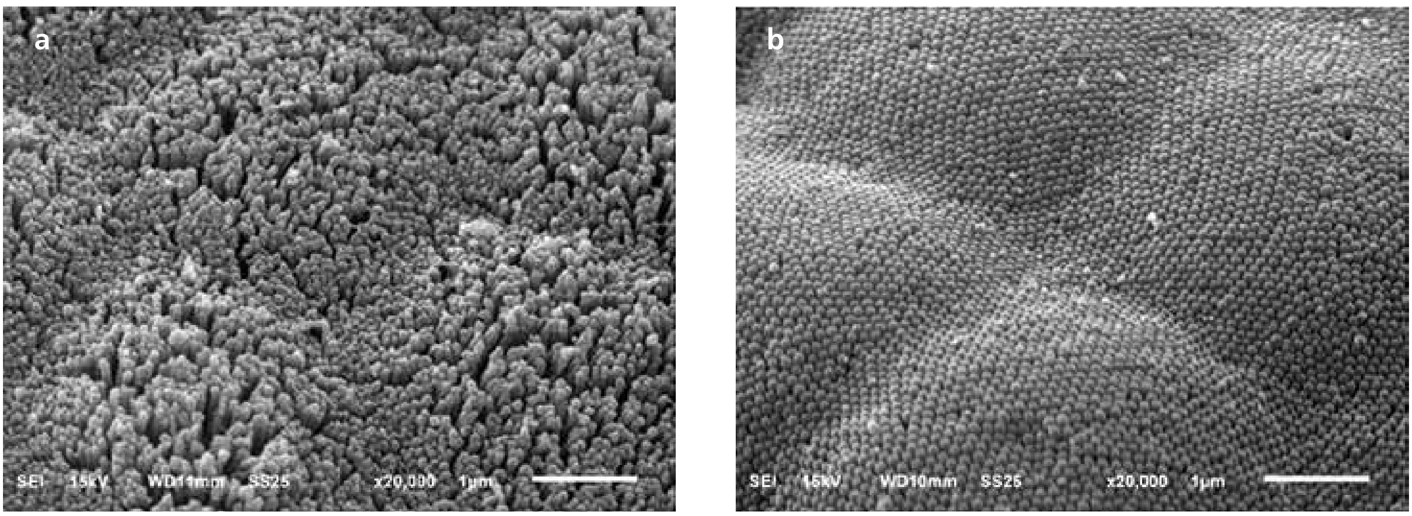
Strengthening the barrier: microvilli density

Beneath the mucus layer lies the epithelia, primarily consisting of enterocytes. These cells are lined with microvilli. Using electron microscopy, it was shown that feeding a probiotic significantly increased microvilli density in the gut (*Figure 2*). The benefit of this increase is two-fold. First, more numerous microvilli will increase the surface area so the host can acquire more nutrients from the feed. Second, any gaps between microvilli present an opportunity for pathogens to translocate the epithelia and infect the fish. Thus a higher microvilli density, resulting from probiotic inclusion in the diet, contributes to the gut barrier being more efficient and better able to block pathogens.

Larger leukocyte infantry

Assuming a pathogen was able to breach the epithelia, an army of white blood cells, collectively known as intra-

Figure 2.
Electron micrographs showing microvilli from fish fed (a) a control diet and (b) a probiotic supplemented diet. Gaps between microvilli as seen in micrograph a, provide an entry point for opportunistic pathogens. In micrograph b, the microvilli provide an impenetrable barrier, blocking pathogen entry.



Source: BIOMIN

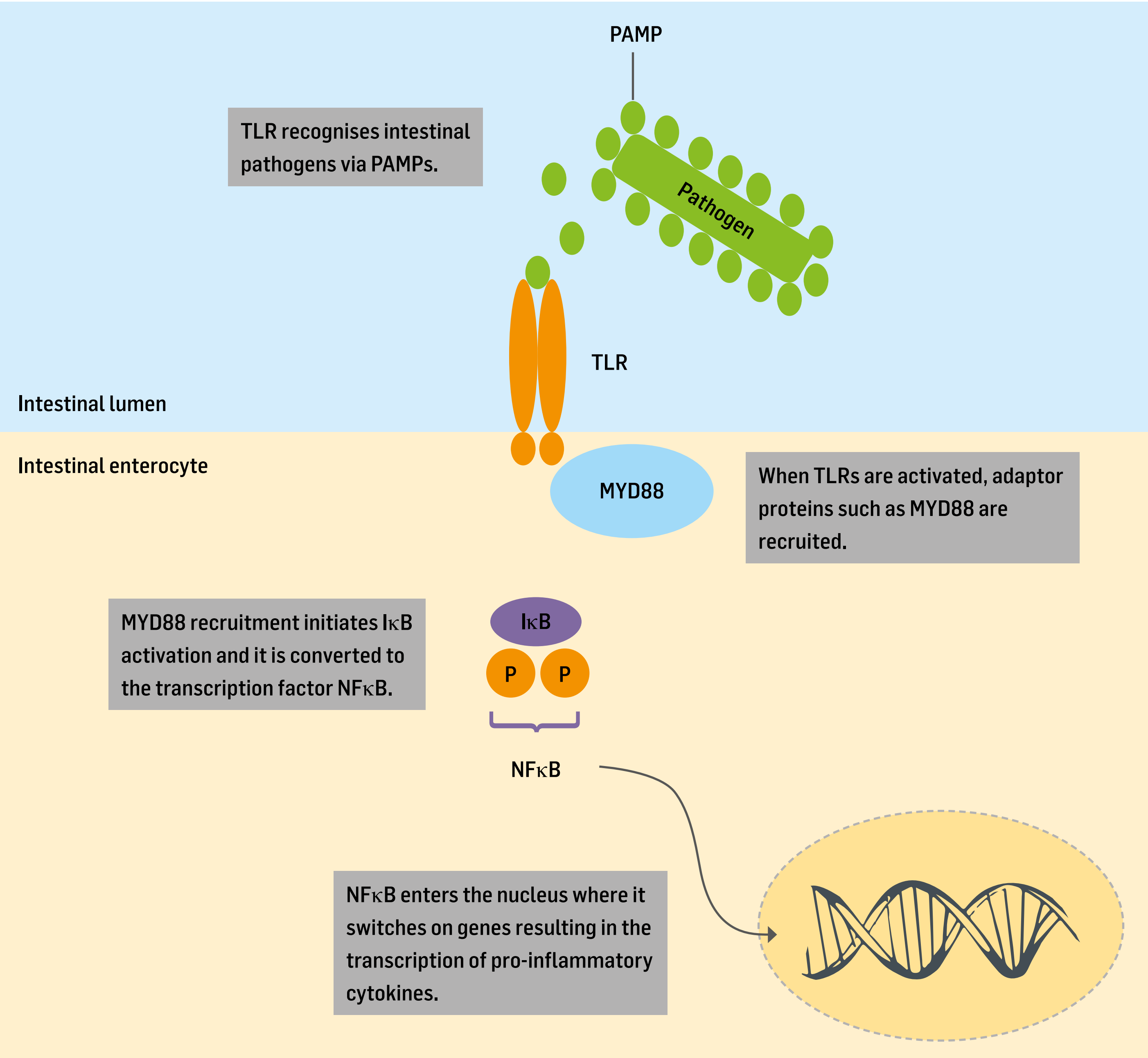
epithelial leucocytes (IELs), would be waiting to attack. Research at Plymouth University consistently demonstrated that tilapia feeds supplemented with probiotics resulted in significantly larger populations of IELs. This increase was between 22 and 38% depending on the probiotic dosage, and the duration of feeding (*Figure 1*).

Better immune readiness

All pathogens express pathogen associated molecular patterns (PAMPs) on their cell surface. These are recognized by their respective receptor molecules such as toll-like receptors (TLRs) which notify the host on the pathogen type (i.e. bacterial, viral, fungal; *Figure 3*). Intestinal gene expression analyses show that probiotics can up-regulate the expression of TLR2 by approximately five-fold in tilapia. TLR2 is important for recognizing Gram-positive bacteria. This is particularly important because tilapia (along with many other warm water species) are susceptible to a number of Gram-positive infections, most notably *Streptococcus*.

Once activated, TLRs initiate a number of molecular

Figure 3.
Modulation of intestinal immunity through TLR signalling. Pathogens (and probiotics) bind to TLR. Upon activation, adaptor proteins such as myeloid differentiation primary response protein 88 (MYD88) are recruited. When this happens, IκB (inhibitor of nuclear factor kappa B (NFκB)) is phosphorylated (P) and degraded by the cell. This allows NFκB to pass from the cytoplasm into the nucleus of the cell where it initiates the cytokine transcription.



Source: Adapted from Cerf-Bensussan and Gaboriau-Routhiau, 2010