Nutrient Uptake

Gut morphology

a key to efficient nutrition

Figure 2: Rainbow trout gut transect showing the more complex villi structures in the Bio-Mos fed fish Top: Bio-Mos Bottom: control

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Gut function and its efficiency is one of the keys to successful commercial production of fish species.

The digestive tract of fish can be described basically as a muscular tube lined by a mucous membrane of columnar epithelial cells that exhibit regional variations in structure and function (Ringo et al, 2007). The major function of the gastrointestinal (GI) tract is to process the ingested feed material and to digest and degrade this into a form that can be easily absorbed and assimilated by the animal and thereby supply dietary nutrients to the body tissues. Digestion by its very nature is a complex process involving enzyme and fluid secretions, motility, absorption and ultimately evacuation.

A healthy digestive system is crucial for optimal animal performance. The large surface area of the GI tract is necessary for the optimization of nutrient absorption and to allow the efficient passive transfer of nutrients to the blood. However the gut is protected by only a single layer of epithelial cells and damage to these structures can therefore impact nutrient uptake and affect growth and performance.

The histological study of the intestine is important in establishing the status of structural integrity, it acts as a tool that helps improve our understanding of dietary influences that can either positively improve structure or factors such as infectious diseases or anti-nutritional components that may ultimately cause physical damage.

The GI trace has been long recognized as one of the major routes of infection in fish (see Figure 1) and an array of protection systems exist within it to limit

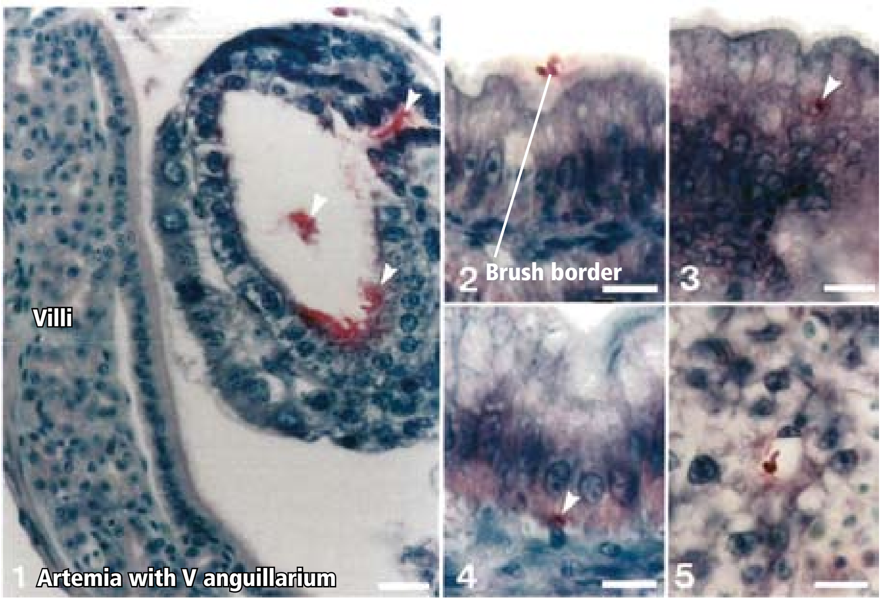


Figure 1: The mode of infection and spread of *Vibrio anguillarum* through oral challenge in turbot *Scopthalmus maximus* larvae through oral challenge through live feed. (Grisez et al, 1996). The route of larval infection is through the intestinal epithelium where bacterium are released into the lamina propria and transported via the blood to the internal organs of the larvae

Table 1: Results of agglutination tests using Bio-Mos

Bacterial strain	Aggregation to Bio-Mos
Aeromonas veroni #9071	Positive
A. veroni field isolate	Positive
A. hydrophilia #35654	Positive
A. hydrophilia #35654	Positive
A. caviae #15908	Positive
A. caviae field isolate	Negative
Edwardsiella tards isolate 1	Positive
Edwardsiella tarda fiels isolate	Negative
V. fluvialis field isolate	Positive
V. parahemolyticus CSUH	Positive
V. (Listonella) anguillarum	Positive
V. vulnificus field isolate	Positive
Photobacterium(Pasteurella) damselae ssp Piscidia #17911	Positive

the risk of damage to the GI tract. Mucosa is critical in digestion, absorption and metabolic processes and acts as a barrier to pathogenic infections preventing both viable and non-viable bacteria and their bacterial products from migrating from the intestinal lumen through the epithileal mucosa to infect otherwise sterile tissues.

Mucosa plays a role in the electrolyte balance, immune response and endocrine functions. Mucins and glycoproteins associated with the intestinal brush border serve as important barriers protecting the absorptive surface from feedstuffs, bacteria colonization and toxins. In addition endogenous acids, digestive enzymes and bile reduce bacterial growth while digestive flow and peristaltic movements transport the digesta through the tract limiting bacterial development.

Farmed fish are vulnerable to ubiquitous opportunistic bacterial pathogens that can take advantage of fish stocks when stressed. Stress and environmental conditions are closely interlinked and one of the consequences of stress can be the loss of intestinal integrity causing enhanced epithelial permeability that may lead to enhanced uptake of macromolecules, bacterial products and antigens across the epithelium leading to reduced performance and susceptibility to disease.

Therefore the interactions between intestinal microflora, gut morphology, the immune system and nutrient uptake will have a major influence on the animals health and performance. Enteritis and poor gut morphology can lead to inefficient feed conversion and the repair of damaged enterocytes is an energy consuming activity which in turn directs valuable resources from growth to the more immediate urgency of tissue repair and maintenance.

Role of mannan oligosaccharides (MOS)

Mannan oligosaccharides have been shown to affect gut health by pathogen adsorption and immune modulation, however the different fermentation conditions, the different strains of yeast used and the different processing methods used in their manufacture can alter their function. (Newman, 2007).

Bio-Mos® (Alltech Inc., USA) is a MOS derived from the outer cell of a specific strain of yeast *Saccharomyces cerevisiae* 1062 using a proprietary process developed by Alltech Inc. Its use in terrestrial animals has been well documented in over 500 trials and numerous peer review papers and it is only recently that is effectiveness in aquaculture has been established.

One of the key benefits of Bio-Mos is its ability to bind or agglutinate bacteria thereby preventing colonization of the gut and subsequent infection. Table I shows the agglutination of Bio-Mos to a number of strains of bacteria known to cause disease in fish and shrimp.

Trial results obtained with Bio-Mos

Microbiology

Zhou and Li (2004) reported that the intestinal microbial populations in Jian carp, were significantly affected by the dietary inclusion of 0.24 percent of Bio-Mos. The presence of Escherichia coli in the intestinal digesta were significantly decreased while significant increases were observed in bifidobacterium and lactobacillus. Recent work by Dimtroglou et al. (2007) demonstrated that Bio-Mos significantly reduced the bacterial load in the gut of both rainbow trout and sea bream by reducing the total aerobically cultivated bacteria. In the case of sea bream the percentage contribution of the various aerobically cultivated bacterial families to the total count was not significantly altered between the Bio-Mos fed fish and the controls. However in the case of rainbow trout the Bio-Mos fed fish had reduced numbers of Micrococcus spp., Staphylococcus spp., Aeromonas/Vibrio spp and other unidentified Gram + bacteria and increased Acinetobacteria spp., Pseudomonads spp. and Enterobacteria spp. Therefore Bio-Mos promoted the colonization of beneficial bacteria associated with the natural gut flora of the rainbow trout when healthy. The differences observed between the two species were reported to be due to a) the fact that marine fish effectively 'drink' significant amounts of water, to maintain osmotic regulation, via their gastrointestinal tract and b) the culture conditions - the rainbow trout were reared in an open flow system while the sea bream were reared in a closed circuit system.

Gut morphology

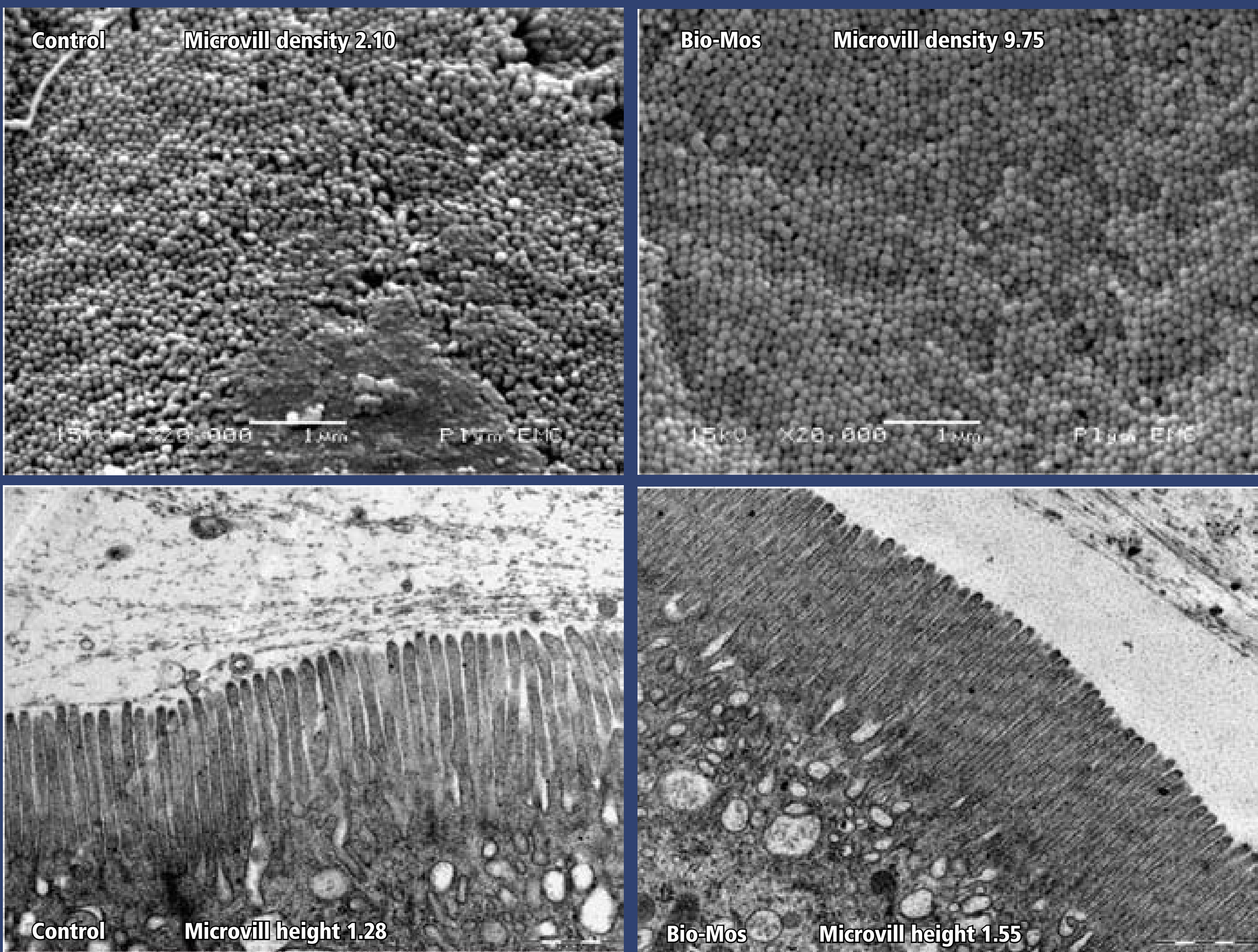
The effect of Bio-Mos on the gastrointestinal morphology of several species: rainbow trout, salmon, sole and sea bream has been reported recently (Dimitroglou et al, 2007). They were examined using optical and electronic microscopy:

- Light microscopy for evaluating the effect of Bio-Mos on the villi structure by calculating the perimeter ratio (ratio of gut perimeter to the perimeter of the gut lumen).
- Scanning electron microscope to evaluate the effect of Bio-Mos on the microvilli structure by looking at microvilli density and damage.
- Transmission electron microscope to investigate the effect of Bio-Mos on the length of the microvilli on the lumen surface of the enterocytes.

In adult rainbow trout and sole Bio-Mos had a significant effect on the external and internal perimeter ratio both in the anterior and in the posterior gut regions, indicating a more complicated architectural gut structure with longer villi, and hence a large surface area for nutrient absorption. (Figure 2).

The inclusion of Bio-Mos improved the microvilli density only in the posterior gut region of the fresh water fish, rainbow trout. In marine fish species, salmon, sole and sea bream, Bio-Mos produced a more pronounced integral effect by increasing the microvilli density in both the anterior and posterior gut regions. The microvilli length was also significantly increased in all the adult fish in both the anterior and posterior gut regions. In juvenile rainbow trout the

Figure 3: Microvilli structures in the GI tract of salmon fed Bio-Mos and their control groups without Bio-Mos in their diets



effect of Bio-Mos on the microvilli length was restricted to the anterior part of the gut.

Figure 3 shows the effect of Bio-Mos on the microvilli structures of salmon, where the microvilli density of the Bio-Mos fed groups was 4.62 times greater than that of the control groups of salmon and the microvilli length was increased by 21.1 percent in the Bio-Mos fed groups when compared to the control groups. The microvilli density photographs also indicate that there is considerably less damaged areas in the Bio-Mos fed groups.

It is clear from these results that Bio-Mos can have a significant effect on gut morphology. The changes observed: increased perimeter ratio, increased villi and microvilli density and length, indicate that the absorptive surface of the gut has been improved and hence a better absorptive capability appears to be possible. Increased microvilli density without signs of damage or irritation will also eventually lead to an increase of the absorptive capability of the enterocytes.

In the case of sole the Bio-Mos was added to the feed in order to improve the condition of vaccinated and non vaccinated fish. In both cases Bio-Mos improved the villi structure and increased the microvilli density. A disease outbreak in these fish and the overall mortalities were reduced in the Bio-Mos fed groups when compared to the control groups. (Dimitroglou et al, 2006).

Growth, performance and immune parameters

In addition to the improvements seen in gut morphology and function, Bio-Mos has been shown to improve growth, performance and immune parameters in a number of fish and crustacean species. Zhou and Li (2004), in addition to the changes in gut microflora mentioned previously, showed higher weight gains, improved feed conversion ratio (FCR) and immune parameters in Jian carp when Bio-Mos was included in the diet.

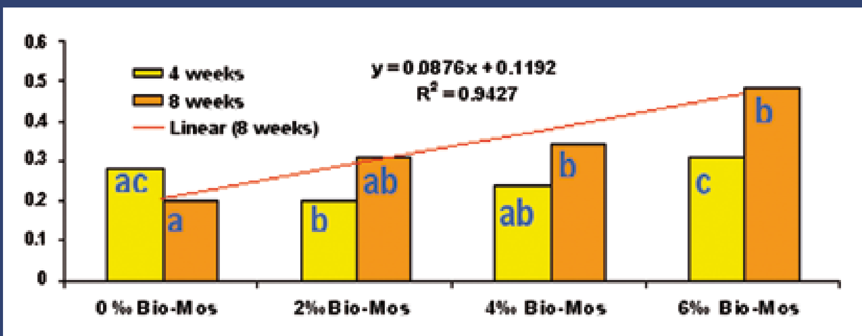
Similar effects of Bio-Mos on improved growth, lower FCR's, mortalities and improved immune parameters have been reported in carp (Staykov et al, 2005, Culjak et al 2006), rainbow trout (Staykov, 2007) and catfish (Bogut et al, 2006). Daniels (2005) reported decreased mortalities and therefore improved survivals in lobster larvae fed Bio-Mos to stage IV.

These improvements in performance have also been observed for marine fish species. In sea bass juveniles Torrecillas et al (2007a & b) has reported that the dietary incorporation of Bio-Mos significantly increases growth, by approximately 10 percent, and produces a better specific growth rate at low densities. When fish are stocked at higher densities the FCR is enhanced. The immune function was improved with the inclusion of Bio-Mos. The immune parameters, phagocytic activity of leucocytes and the bacterial activity of the sera in the Bio-Mos fed groups showed a statistically significant improved dose response when compared to the control group. (Figure 4)

Disease resistance to bacterial infection, both by cohabitative challenge and by direct inoculation in the gut, were enhanced when Bio-Mos was incorporated in the diets. In cohabitation trials the presence of *Vibrio alginolyticus* on the head kidney of sea bass was 33 percent for the control group and 8 percent and 0 percent respectively for the 0.2 percent and 0.4 percent Bio-Mos fed groups.

The incorporation of Bio-Mos also resulted in the improvement of

Figure 4: The immune parameters, phagocytic activity of leucocytes and the bacterial activity of the sera, show a significant dose related response in European sea bass fed Bio-Mos when compared to the control group



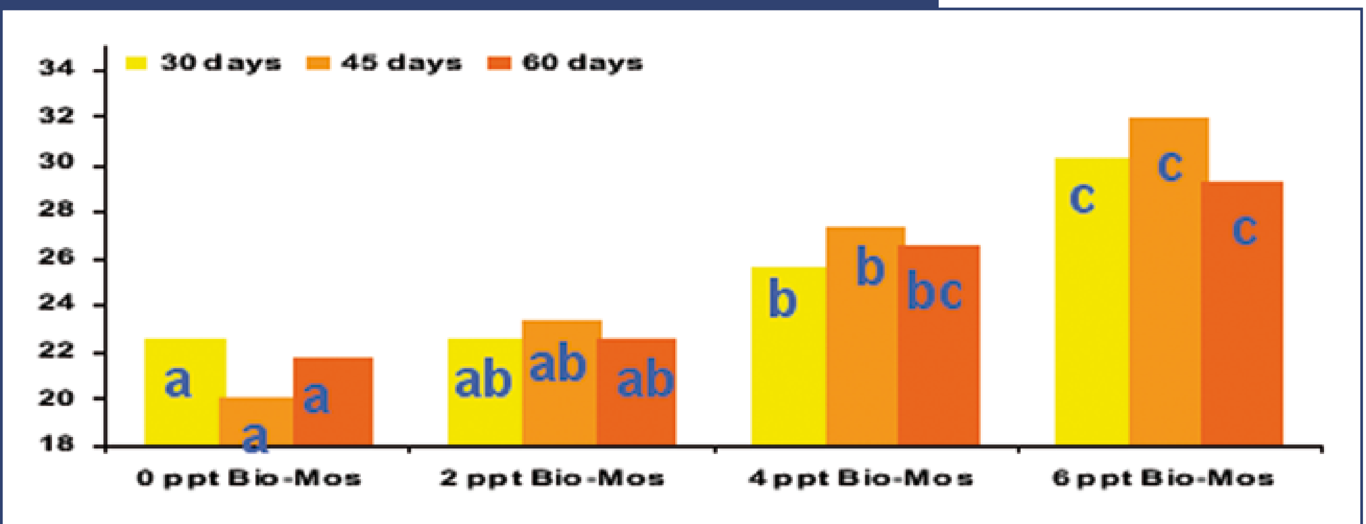
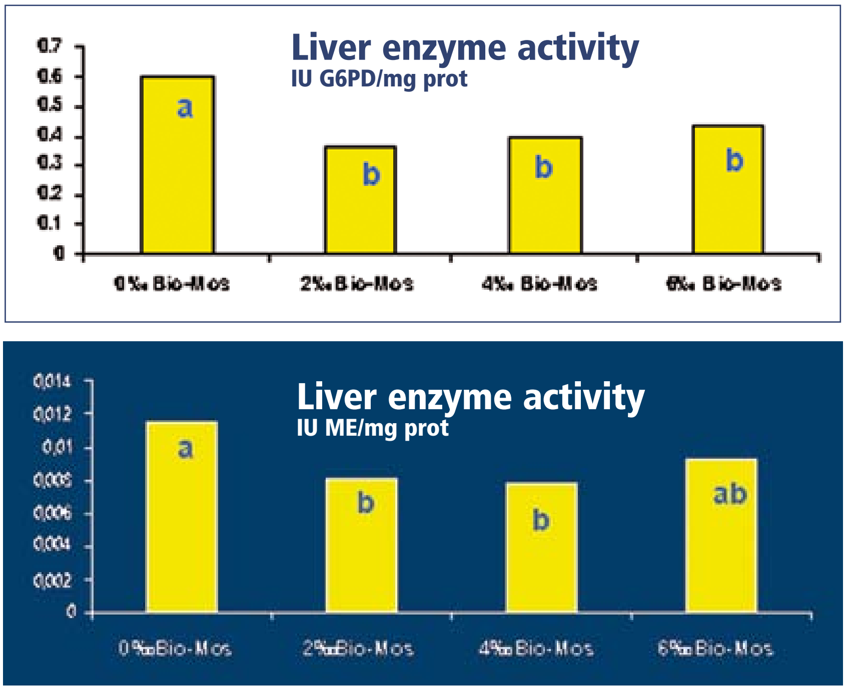


Figure 5: European sea bass fed Bio-Mos had a significant reduction in lipogenic enzyme activity



the hepatocyte morphology with more regularly shaped hepatocytes and less hepatocytes with displaced nuclei to the cellular periphery. The activity of lipogenic enzymes in the liver were significantly reduced at the three levels of Bio-Mos inclusion. (Figure 5)

The reduced liver fat deposition and the improved hepatic composition may well be an indicator of better utilization of dietary nutrients and this in association with improved gut morphology may suggest reasons for better growth and performance.

Conclusion

Bio-Mos has been shown to be an effective tool for feed producers and fish farmers increasing performance and health status of a number of important commercial species.

Acting in a prophylactic manner it provides multiple benefits when incorporated in to aqua feed diets for a number of species. Bio-Mos not only improves the GI morphology and therefore its function through an increased absorptive surface and better absorptive capability but also interacts with the immune system in a modulatory manner and alters enzyme activity promoting the better utilization of dietary nutrients therefore improving performance characteristics and immune function.

The combination of all these benefits results in better performance, livability and disease resistance and therefore gives a more cost effective production of interest to the commercial producer

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