What level of nucleotides should be incorporated into the diet?

There has been little research on the dose-response relationship between nucleotides and their beneficial effects. It is recommended that feed formulators start with a dietary level of between 2 and 5% of hydrolyzed additives based on single cell protein or yeast. However, nucleotide levels as low as 0.03% have been shown to result in significant improvements in performance although this may depend on nucleotide source, species and culture environment.

What is the future for nucleotide use in aquaculture?

The use of nucleotide supplementation of animal feeds is a relatively recent field of study. In terrestrial animals, the benefits are becoming increasingly clear and the existing work on nucleotide supplementation of feeds in aquaculture demonstrates similar results. The fact that their application has shown to be beneficial in a wide range of species indicates that the response is generic and not species specific. Thus, it is likely that the application of nucleotide-enriched diets will cover an increasing number of species.

To date, we have only scratched the surface of the application of nucleotides in broodstock and larval feeds, an area of research that is sadly lacking. Future research into the development of better broodstock and larval diets and feed supplements will include more work on the effect of nucleotide supplementation on broodstock and larval performance.

Nucleotide-enrichment of feeds is rapidly moving from fringe to mainstream applications and there is an increasing use of nucleotide supplementation in diets for marine fish, especially salmon. This is likely to continue and, as alternative protein sources are used to replace fish meal, cost-effective raw materials with a high nucleotide content will be in increasing demand. ■

Literature Cited:

Mateo, C.D. & H.H. Stein, 2004. Nucleotides and young animal health: Can we enhance intestinal tract development and immune function? In: Nutritional Biotechnology in the Feed and Food Industries, Proceedings of Alltech's 20th Annual Symposium. Nottingham University Press, UK, pp.159-168.

Mr. Dan Fegan has been involved in commercial aquaculture for over 20 years. After graduating in Marine Biology at Heriot-Watt University in Scotland, he spent some years working in Ecuador providing technical support in the use of microencapsulated feeds for shrimp hatcheries. Since 1986 he has lived in Asia and has worked in most countries in the region. Dan spent 10 years in Thailand in commercial production of shrimp at hatchery and farm level with the Aquastar company. Following a brief spell working in Malaysia, Dan worked as a consultant, making frequent visits throughout Asia and Latin America as a consultant to international and national agencies. In 1998, Dan started work as an advisor to the Thai National Center for Genetic Engineering and Biotechnology to commercialize various research works of the Center. As a result, the Center established the Shrimp Biotechnology Business Unit to provide products and services to the aquaculture industry. Dan managed the SBBU until January 2004 when he joined the US-based biotechnology company, Alltech Inc. to further develop its products for the aquaculture industry. Dan is currently the President-Elect of the World Aquaculture Society and the immediate Past-President of the WAS Asian-Pacific Chapter as well as being on the Technical Committee of the NACA/FAO/OIE Asia-Pacific Aquatic Animal Health Program.

What factors need to be considered in selecting and applying probiotics in aquaculture, especially those applied via feed?

Probiotics in aquaculture: A commentary based on some recent observations

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In recent years, disease problems caused by Vibrio spp. and viruses have emerged as major constraints in aquaculture production. The application of antibiotics or other chemicals to culture ponds is expensive and detrimental (contamination of reared animal, antimicrobial resistance, etc).

Beneficial bacteria which compete with bacterial pathogens for nutrients and/or inhibit the growth of pathogens can be applied to the water or to the feed. These so-called beneficial bacteria are not therapeutic agents but will alter directly or indirectly the composition of the microbial community in the rearing environment and the shrimp gut.

In aquatic environments, hosts and microorganisms share the same ecosystem and the microbial community of a shrimp pond is influenced by a wide range of parameters, including microorganisms released from feces of reared animals. If a pathogen is present, its population density can increase through interactions in the intestinal tract of the animals and in its feces. Furthermore, feed pellets added to the water will adsorb or absorb bacteria from the surrounding water before being ingested by the shrimp. These feed pellets will therefore introduce bacteria, including potentially pathogenic bacteria, into the gut.

As part of an efficient farm management, regular dosing of appropriate mixtures of microorganisms can be applied into the water and with feed (top dressing or incorporation into feed pellets) in order to maintain suitable water quality and control potentially pathogenic microorganisms.

The selected probiotic strains should be able to withstand the conditions prevailing in the rearing environment, and, in the case of direct incorporation in the feed, those encountered during the manufacturing procedure.

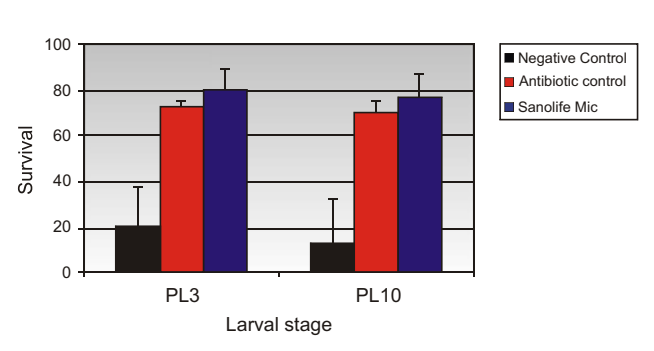

Fig.1: Penaeus monodon *survival at PL3 and PL10 in tanks receiving antibiotics as prophylactics or daily water application of Sanolife® MIC, compared to a negative control. 4 replicates per treatment. Bar indicates standard deviation.*

Bacillus species such as *B. subtilis* and *B. licheniformis* are commonly used as probiotics in aquaculture. However, the development of suitable probiotics is not a simple task and requires empirical and fundamental research, full-scale trials, as well as the development of appropriate monitoring tools. A performing mixture of probiotic strains (Fig. 1) can be designed after evaluating the ability of individual strains to grow in low/high salinity, under micro-aerophilic or anaerobic conditions, produce various enzymes and, more importantly, produce a range of inhibitory compounds.

Pelleting and extrusion processes are characterized by heat, moisture and pressure, with extrusion being the most aggressive process. In the case of hot extrusion, the higher temperatures employed facilitates the inactivation or destruction of contaminants and non-nutritional factors, but may concurrently result in the loss or damage of heat-sensitive compounds such as probiotic additives.

Although resistant to stringent conditions, *Bacillus* spores should not be included in the diet before the extrusion, expansion and drying process since the procedure has been shown to result in the loss of 99% of the *Bacillus* spores.

The same way that heat sensitive additives may be sprayed onto feed pellets after extrusion, powder coating appeared to be an efficient and convenient way to add *Bacillus* to the diet, despite some important losses (Fig. 2).

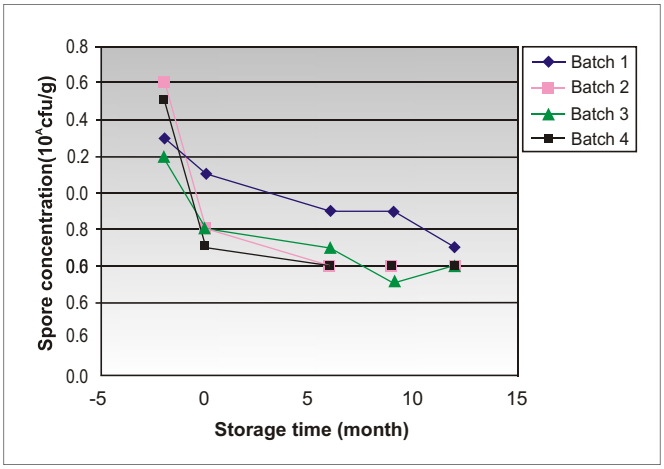

Fig.2: Effect storage time (6, 9 and 12 months) on the survival of a Bacillus added to 4 batches of commercial dry dog food as a powder coating. Unit is 106 cfu/g diet (adapted from Biourge et al. 1998. The Journal of Nutrition 128:2730S-2732S).

The following points should be considered when incorporating *Bacillus* probiotics directly into feed:

1. The dose-effect relationship has been determined for optimal efficacy, however, losses associated to incorporation (see above) in feed might lead to reduced efficacy of the probiotic;
2. Contrary to *Bacillus* that had been germinated prior to coating onto feed pellet or applied in feed, those *Bacillus* strains incorporated into feed pellet will reach the gastrointestinal tracts in a dormant stage, and not in the vegetative status, which means they would not produce inhibitory compounds at this time. However, they might still be able to compete with other bacteria for adhesion sites or act as immunostimulant. Furthermore, some of them would also be released in the environment, via the shrimp feces, where they would perform their functions of bioremediation (e.g. waste degradation) or biocontrol (e.g. competition with *Vibrio* spp.). ■

Dr. Olivier Decamp received his Ph.D. in Zoology from The Natural History Museum, London, and the University of Leicester (UK) in 1996, with focus on wastewater microbiology. After stints in Japan as a postdoctoral researcher marine microbiology and at the Oceanic Institute (Hawaii) as a research scientist in aquaculture microbiology, he joined INVE TECHNOLOGIES NV in 2002 as an R&D scientist. He is responsible for research and product development in aquaculture health.