Health benefits of dietary supplementation with nucleotides are becoming more apparent these days as scientific evidences accumulate. Nutritionists recognize nucleotides as "conditionally essential" for early life stages, in maturation and during periods of stress. This article answers some of the frequently asked questions on nucleotides.

Nucleotides

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What are nucleotides?

Nucleotides (Figure 1) are among the basic building blocks of life. They are low molecular weight biological compounds consisting of a nitrogenous base (either a purine or a pyrimidine) linked to a pentose sugar with at least one phosphate group attached (If the phosphate group is absent, the compound is referred to as a nucleoside). A group of nucleotides linked together forms nucleic acids and when the sugar is ribose, the result is ribonucleic acid (RNA) or, if 2'-deoxyribose, deoxyribonucleic acid (DNA).

Why are they important?

As nucleic acids, nucleotides are of fundamental importance in all life forms as the basis of the genetic code. Genetic information is stored in DNA (except in the case of RNA viruses) providing the basic information to code for all the proteins produced in the body whereas RNA acts as a chemical messenger relaying the information stored in DNA from the nucleus to other parts of the cell.

However, nucleotides also play major roles in almost all biological processes including:

- Storage of energy in phosphate transfer reactions, predominantly through adenosine tri-phosphate (ATP).
- As components of several important coenzymes such as Nicotinamide Adenine Dinucleotide (NAD), Nicotinamide Adenine Dinucleotide Phosphate (NADP), Flavin Adenine Dinucleotide (FAD) and coenzyme A.
- Acting as second messengers to mediate important cellular processes such as cyclic-Adenosine monophosphate (cAMP) and cyclic Guanine monophosphate (cGMP).
- Control of several enzymatic reactions.
- Serving as intermediates in biosynthetic reactions, especially in glycogen and glycoprotein synthesis.

More recent research has indicated that they are important elements in mammalian nutrition especially during periods of rapid growth or physiological stress and that they appear to play a key role in enhancing the immune system, hence their description as "conditionally essential".

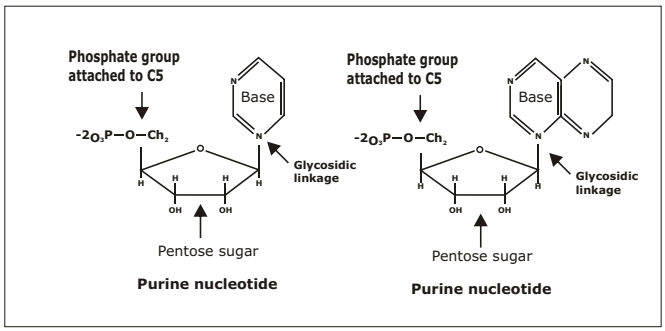

Fig.1: Nucleotide structure

How are nucleotides obtained?

Nucleotides are generally synthesized from scratch, through "salvage pathways" which form new nucleotides from existing ones, and from the diet. Although many cell types and tissues are capable of synthesizing nucleotides, some, such as immune and intestinal cells, often lack the capability to synthesize nucleotides and depend on the availability of pre-formed nucleotides.

Generally, external sources of nucleotides appear to be preferentially used since biosynthesis and salvage of nucleotides are costly in metabolic terms. Several studies have demonstrated that dietary sources of nucleotides can have beneficial effects since they may optimize the functions of rapidly dividing tissues, such as those of the immune or reproductive systems, and in times of rapid cell division such as reproduction and larval development.

What are the dietary sources of nucleotides?

Most ingredients of animal and plant origin contain nucleotides. The nucleotide content is particularly high in ingredients such as fish solubles, animal protein solubles, fishmeal, legumes (adenine content is particularly high in black eyed peas), yeast extracts and unicellular organisms such as yeasts and bacteria that are rich in RNA or DNA.

The content, proportion and availability of nucleotides differ between ingredients (see table 1). Among marine protein sources, anchovies and sardines, for example, have much higher guanine levels than squid, clams or mackerel. Availability and digestibility are also important issues. Whole yeast is much less digestible than yeast extract, possibly due to the need to digest the yeast cell wall and yeast extract having much higher levels of soluble protein. Fish and animal protein solubles are highly digestible but they do leach easily affecting overall availability.

***Table 1**: Nucleotide concentration in some common feed ingredients (from Mateo et al., 2004)*

Ingredient	Nucleotide (micrograms/g)				
	CMP	AMP	GMP	UMP	IMP
Barley	2	1	1	0	1
Casein	1	0	0	0	0
Corn	3	2	3	0	1
Fish Meal	26	11	2	1	35
Naked Oats	3	3	3	1	1
Plasma protein, spray dried	2	2	2	0	1
Red blood cells, spray dried	0	44	3	2	6
Soy protein concentrate	0	1	2	0	1
Soybean meal (44%)	16	8	3	9	2
Whey, dried	270	19	0	1	4

What benefits could be obtained from nucleotides in aquaculture?

The study of nucleotides in aquaculture has not been extensive. However, where studies have been conducted, the results suggest that the benefits reported for terrestrial animals may be similar to those found in aquatic species. Their role would appear to be in several key areas: attractants, reduction of stress, specific dietary requirements for reproduction and larval rearing, and as immune stimulants.

How do nucleotides act as attractants?

Many aquatic species have specific receptors for nucleotides on external sensory organs, suggesting that they may play a role in chemoattraction, and there are some reports on the effectiveness of nucleotides as attractants. IMP has been shown to increase diet attractability in a number of fish species, including mackerel, turbot and largemouth bass, and synthetic attractant mixes including IMP and AMP have been shown to be highly attractive to crustaceans such as lobsters and crabs.

What is the role of nucleotides in reducing physiological stress?

Aquaculture species are often subject to a great deal of physiological stress resulting in immune suppression, reduced growth rate and increased susceptibility to disease. At times of increased stress, nucleotide supplementation may provide benefits. Work carried out with salmon during transfer from fresh to salt water, a time of considerable stress, showed that fish fed a diet supplemented with nucleotides had an enhanced capacity for osmoregulation, reducing stress during transfer. More recently, the benefit of dietary nucleotide supplementation in reducing the effect of salinity stress in shrimp (*Penaeus monodon*) has been reported. In a 90-day trial in which shrimp were subjected to a regime of frequent salinity change, mortality of shrimp fed a nucleotide-enriched diet was less than 15% of that of the control group.

What role do nucleotides play in reproduction and larval rearing?

Reproduction and egg development are periods of rapid cell division. These have a high requirement for RNA and DNA and it may be expected that increasing the availability of nucleotides in broodstock diets may have a beneficial effect on egg development. Recent work using a nucleotide-enriched diet for Atlantic halibut (*Hippoglossus hippoglossus*) showed a general trend towards better spawning performance and egg quality with the nucleotide-enriched diet. Total egg yield was 30 per cent higher in fish fed with the nucleotide diet and relative fecundity, mean egg density, hatching rate and survival of yolk-sac larvae were also significantly higher. Interestingly, although egg quality and morphometry showed improvements over 2 breeding seasons, relative fecundity showed some annual variation.

The same work with halibut also showed that first feeding success in the larvae was significantly higher, contributing to a 30% improvement in larval survival at weaning. The larvae from broodstock fed the nucleotide-enriched diet were also bigger and had more advanced gut development than the control group.

What is the effect of feeding nucleotides on the immune response?

Nucleotide supplementation of diets for young mammals has been shown to increase the immune response and ability to resist disease challenges. Similar work with salmon showed that salmon fed with a nucleotide-enriched diet had elevated antibody titers following vaccination than a control group. A separate trial showed that salmon fed with a nucleotide-enriched diet were less susceptible to sea lice infection than those on a normal diet.

Work conducted with shrimp, *Penaeus monodon*, also demonstrated the effect of a nucleotide-rich yeast extract (NuPro®) on the immune response. Shrimp fed a diet containing 2% NuPro (equivalent to 0.08% nucleotide) showed an increased proportion of granular haemocytes compared to the control and an enhanced ability to clear *Vibrio harveyi* from the hemolymph following a challenge by injection (Fig. 2).

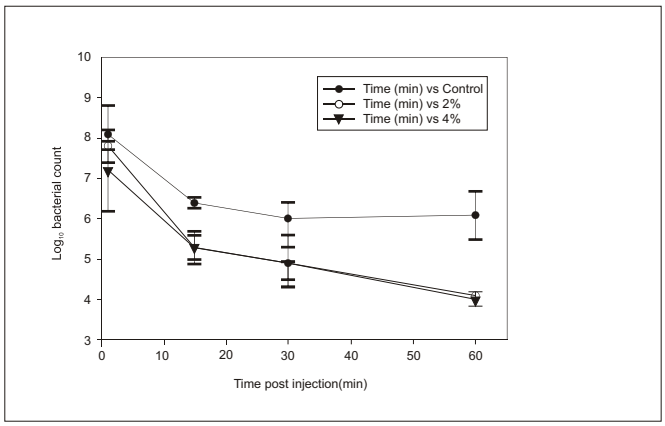

Fig.2: Clearance of V. harveyi *from hemolymph following injection challenge*

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.