Free nucleotides in the diet of animals can be a powerful ally against stressful situations occurring at certain stages of development, either with or without associated pathologies. Gaining popularity already in pig and poultry feeding, the building blocks of DNA and RNA are equally valid in aquaculture applications.

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Nucleotides are the basic constituents of nucleic acids (DNA and RNA) and are therefore absolutely necessary for any form of cell replication. Their molecular structure is based on the combination of a monosaccharide, a nitrogen base and between one and three phosphate groups (Figure 1). If the sugar is ribose, they are constituents of ribonucleic acid (RNA) and if the sugar is deoxyribose, they are constituents of deoxyribonucleic acid (DNA). Furthermore, depending on whether the base is purine or pyrimidine in nature, nucleotides can be divided into two large families with different routes of synthesis, but with the common trait of high consumption of metabolic energy (Figures 2 and 3).

In nature, there are two basic ways of obtaining nucleotides: via synthesis, which consumes large amounts of energy, and through the diet. Dietary nucleotides can be obtained in two ways: by digestion of the nucleic acids that are present in feed ingredients, or by the direct addition of free nucleotides to the feed. It is important to note that nucleotides in their non-free form or in the form of nucleic acids tend to be tremendously stable, and therefore difficult to digest. Supplementation of a well-balanced cocktail of free nucleotides can be beneficial, especially in successfully overcoming certain stressful conditions.

When extra nucleotides are required
There are certain stressful situations that can only be overcome by a high cell reproduction rate, and therefore also require a large provision of nucleotides. These situations of stress can basically be classified as:
- Digestive tract diseases that increase the turnover of enterocytes in the intestinal mucous membrane.
- Diseases in general that result in a high replication rate of cells in the immune system.
- Young animals, whose digestive and immune systems are still developing. This stage is doubly critical as they do not yet have a fully developed digestive system and so have even greater difficulty in obtaining nucleotides from the diet.
- Diets exclusively formulated with raw materials of vegetable origin: these products are generally hard to digest and low in available nucleotides.
- Early stages of fish and crustacean growth: these species have a high rate of body cell replication, compared to other animal species. These situations of deficiency can be summarised in three sections for a better understanding of their specific problems:
  - Intestinal complaints that increase the turnover of enterocytes.
  - Complaints that result in a high reproduction rate of cells in the immune system.
  - Early stages of life with a high cell reproduction rate in general.

In these cases, nucleotides can be considered as semi-essential nutrients, since endogenous synthesis, through recovery mechanisms, is not enough to respond to increased needs in situations of high cell reproduction.

Over the last few years, many experiments with animals and clinical studies in humans have been carried out in an attempt to determine the effects of supplementing the diet with nucleotides, especially on the gastrointestinal and immune system.

Intestinal development
It is well known that dietary nucleotides play an important role in the growth and development of the intestine. This hypothesis was supported by quoting the work of Uauy et al. (1990), which showed that the addition of

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nucleotides to the diet of rats during the weaning period brought about an increase in the protein and DNA content of the intestine, an increase in the length of the intestinal villi and increased maltase activity, mainly in the most proximal segments of the small intestine. The weaning period is characterised by a phase of low feed intake, which results in a negative energy balance with direct repercussions on the development of the intestinal lining. It is precisely during these critical stages that supplementing the diet with nucleotides can offer productive improvements, as they favour the reproduction of enterocytes.

**Digestive complaints**

It has been demonstrated that the inclusion of nucleotides in the diets of animals with osmotic lactose-induced diarrhoea can improve recovery of the morphology of the intestine (Bueno et al., 1994).

The induction of diarrhoea brings about a reduction in the intestine’s capacity to absorb nutrients and results in the animal being under-nourished. The lack of nutrients first affects the tissues with the highest reproduction needs such as enterocytes, so that there is a loss of integrity of the intestinal lining. This loss of integrity compromises the intestine’s function as a defence barrier, thus favouring the translocation of bacteria and, as a consequence, the appearance of digestive complaints.

In order to re-establish intestinal mucosal integrity, the enterocytes have increased needs for specific nutrients such as nucleotides. This is because enterocytes mainly depend on the recuperation of nucleotides from the intestinal lumen, as their capacity for the synthesis of new nucleotides is limited.

However, recovery of the mucous membrane is only made possible by the administration of a well-balanced source of nucleotides, and not each individual nucleotide separately (Adjei et al., 1996).

Apart from helping to repair the intestinal lining, the administration of nucleotides may also have a protective effect on intestinal health. With this in mind, it is important to note that the nucleotide supplements in formulae given to children has been shown to be capable of modulating the intestinal flora, promoting the establishment of a stable beneficial flora as regards bifidobacteria and lactobacilli, and therefore reducing the risk of the appearance of intestinal disorders (Uauy, 1995).

**Immunity function**

Diets lacking in nucleotides have been associated with lower rates of mitosis in lymphocytes, low production of interleukin-2 and a lesser degree of humoral immunity (Chandra and Kumarsi, 1994). These deficiencies in the response capacity of the immune system imply lower resistance to infections. In this aspect, nucleotides have been shown to have a positive effect on resistance to infections caused by *Staphylococcus aureus* in mice (Kulkami et al., 1986). So far, however, the way in which nucleotides work on the immune system has not been completely understood. Apart from the trophic role that nucleotides may play in the generation of immune cells, it has been suggested that they could have a regulatory effect on the development of T-lymphocytes in intestinal lymphatic tissue. Furthermore, a direct relationship has been detected between the level of nucleotides, the activity of CD4-lymphocytes and the activation of macrophages.

The beneficial effects of nucleotides have also been described in humoral immunity. After the administration of anti-*Haemophilus influenzae* and anti-diphtheria vaccines, supplemented diets were shown to promote an improvement in the production of antibodies (Pickering et al., 1998).

**Applications in animal nutrition**

The recognition of the semi-essential nature of these nutrients, until recently unknown in the field of nutrition, has justified their use in some feed formulas for children in Japan, the United States, Europe and Brazil. However, their use has not yet become widespread in animal nutrition, possibly because of a lack of experimental trials that prove...
their efficacy. With this in mind, one of the areas where a better response may be expected is that of nucleotide supplements in aquaculture, especially in situations of productive stress. Recently, studies carried out on salmonids have demonstrated that dietary supplements with nucleotides can improve their state of health, increasing the resistance of the fish to bacterial and viral infections,

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and reducing the seriousness of infestation by ectoparasites (Burrells et al., 2001a). At the same time, supplemented diets have been shown to be capable of counteracting the delayed growth caused by handling during vaccination and transfer to salt water (Burrells et al., 2001b). In these studies, and also in trials carried out in mammals, improved production is associated with better intestinal morphology and function, which could be explained by this type of feed.

Aquaculture applications

It has been widely demonstrated that nucleotides play a key role in the survival and productivity of fish and crustaceans.

Mention should be made here of some very interesting trials carried out by Dr. Estevez of CA-IRTA, where the main objective of the study was to determine the effect of nucleotides (Nucleoforce, Bioiberica, S.A., Spain) on the survival, productivity and intestinal morphology of sea bream during the pre-fattening stage. These studies show how a balanced mixture of nucleotides added to the feed promoted growth in size and weight of sea bream larvae (Figure 4). It was also observed that nucleotides increased the survival rate (Figure 5) and the number of intestinal villi (Figure 6). This increase is possibly due to the promotion of intestinal and immune system development that occurs in animals that have consumed nucleotides. This in turn implies better use of the nutrients in the feed.●

References