Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Review article doi: 10.12980/jclm.4.2016J6-165

Overview of the application of nucleotide in aquaculture

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ARTICLE INFO

Article history:
Received 29 Aug 2016
Received in revised form 13 Sep 2016
Accepted 15 Sep 2016
Available online 26 Sep 2016

Keywords:
Nucleotides
Immunostimulants
Growth performance

ABSTRACT

Although long history application in infant formula, dietary nucleotide supplementation has been used only recently in the evaluation of growth performance, stress and pathogen resistance in aquaculture species. This paper addresses the present knowledge of the use of nucleotide supplemented in the diet for culture species. Research reveals that dietary nucleotide may have significant impact and is recommended to add to the feed of aquatic species to get better performance. However, more studies should also be conducted to have better understandings on dose requirement, duration of application, impact on different life stage and under different environmental stress and pathogens. Further study should also examine the effects of dietary nucleotide supplementation of intestinal microbiota and gut morphology, and immune response of aquaculture species.

1. Introduction

Aquaculture is the global fastest growing animal food-producing sector. However, fast aquaculture development has led to the serious diseases and problems in relation to intensive culture. To counter the production decline, antibiotics have been used to control disease and improve survival but the practice carries many risks to human health and the environment[1-3]. The ban imposed against using antibiotics and other drugs as growth promoters in food animals lead to more research into replacements for growth and health enhancement[4,5]. Probiotics and prebiotics are widely used in aquaculture[6,7], but nucleotides are relatively new in aquaculture and their potential benefits and effects are not fully understood. This review discusses about the role of nucleotide supplemented in the diet for aquaculture species.

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Foundation Project: Supported by Australian Centre for International Agricultural Research (ACIAR) under grant number ACI00031.
The journal implements double-blind peer review practiced by specially invited international editorial board members.

2. Nucleotides as nutrients

Nucleotides are natural biochemical that consist of a purine or pyrimidine base, a sugar and one or more phosphate groups, which can occur as subunit nucleobases or as polymeric nucleic acids. Nucleotides can be formed either by *de novo* synthesis or recycled from dying cells via the salvage pathway. During *de novo* synthesis, nucleotides are synthesized from amino acids and other molecules. Pyrimidines are formed from aspartate or glutamine, NH_3, and CO_2, while purines are synthesized from glycine, glutamine, aspartate, and CO_2. Nucleosides are formed by attaching ribose to the purine or pyrimidine base, these nucleosides are added a phosphate groups (mono-, di- or tri-) to form nucleotides. Nucleotides can be formed to di- or triphosphates, or decayed to nucleosides intracellularly. Uracil or cytosine (pyrimidine nucleobase) or hypoxanthine (purine nucleobase) is formed by the elimination of the ribose (or deoxyribose) moiety, which can be further catabolized. Alternately, these bases possibly be recycled via use of the 5-phosphoribosyl-1-pyrophosphate to form 5' monophosphate by the phosphoribosyltranferases in the cell[8]. In the salvage process, nucleotides are synthesized from nucleosides and bases that

result from the breakdown of endogenous cellular nucleic acids[9]. This process can only happen in adults with as high as 75%–80% nucleotides derived from recycled cellular material. *De novo* synthesis is a complicated process and has a high energy requirement compared to salvage[10]. It was also stated that due to the high adverse energy of the phosphate groups, the absorption of dietary nucleotides by epithelial cells is difficult[11]. However, the phosphate groups deduct from nucleotides to form nucleosides, which are more easily taken up by intestinal epithelial cells, subsequently nucleosides are downgraded and metabolized into forms that can be salvaged by other cell types.

Nucleotide, particularly ATP is also known as source of energy in cells for metabolism including biosynthetic reactions, motility, and cell division and even nucleotide synthesis. Because ATP cannot be stored, the consumption closely follows its synthesis[8]. Furthermore, pathways for the biosynthesis of purines and pyrimidines have differing requirements for ATP, with purines requiring 6 molecules of ATP (for AMP synthesis) and 7 for guanosine monophosphate (GMP) synthesis, compared with 4 ATPs for pyrimidines biosynthesis. Therefore, nucleotide from diet might help to save energy.

It seems, therefore, that the energy requirement for obtaining nucleotides via different pathways will occur in the following order from high to low: *de novo*, salvage and exogenous supplement. Quan *et al*.[12] revealed that dietary nucleotides can shorten the route to supply requirements to cells and may be more energy efficient, particularly in damaged tissues or when the animal is sick. When nucleotides supplied in the diet, the dependence on the *de novo* pathway should lessen, reducing the energy requirements by providing nucleotides to cells more directly.

Nucleotide requirement is likely to vary with life stage. In fact, in young organisms new cell generation for growth and development greatly exceeds the very low levels of cell death[9], so the salvage pathway might not supply enough nucleotide to meet requirements. In addition, some tissues or cell types have lower capacity to synthesize nucleotides for themselves. These include the intestinal mucosa and bone marrow hematopoietic cells[13]. Furthermore, in some circumstance (*e.g.* unhealthy, diseased, stress or food shortage), exogenous nucleotide supplied via diet resulted in significant improvement[14]. Evidence of this was provided in a study that showed that nucleotides did not enhance the immune cell growth when healthy, but they did improve the immune cell development and cytokine quantity when cells were co-cultured with influenza virus antigen[15]. Also, function of immune cells were affected by the supply nucleotide derivatives[16].

In the last two decades, increasing numbers of studies have indicated potential benefits of nucleotides in mammals, including humans. Nucleotides and nucleic acids are involved in many critical pathways, including the initial process of protein synthesis[17]; improved iron absorption of intestine[18], act as hormones and neurotransmitters and are present in some co-enzymes[19]. They improve gut mucosal surface, humoral and cellular immunity[20], accelerate hepatocyte growth[21] and improving liver function[16]. Dietary nucleotides also significantly reduce DNA damage in the immune cells of chicken when exposed to T-2 toxicity, with similar findings in pigs[22]. These benefits of nucleotides, only under certain circumstances, have lead to them being termed 'semi-essential' or 'conditional' nutrients[23]. Nucleotides also have been approved by the European Commission as a food supplement to infant formula as there is insufficient nucleotide in cow milk compared with human breast milk[24]. As such, nucleotide has been added to infant formula for many years proved the benefits without any negative effects reported[25,26].

3. Dietary nucleotides in aquaculture

3.1. Use of nucleotide in aquaculture

The application of nucleotide supplements in aquaculture diets is relatively new compared to mammalian and human counterparts. Knowledge to date and prospects for nucleotides in aquaculture have been reviewed by Li and Gatlin[27] and Ringø *et al*.[28]. The underlying principles for nucleotide benefits in aquatic animals are still poorly understood, and although there are several empirical studies with varying results, it is hard to explain the benefits or otherwise without further background knowledge. Nucleotides currently used in aquaculture[29,30] are the same as those added in infant formulae[25], including 5 types of nucleotide 5'-monophosphate at sodium salts, water soluble cytidine monophosphate, uridine monophosphate, AMP, inosine monophosphate (IMP) and GMP. Nucleotides potentially improve growth, immunity, stress resistance or attractiveness of the feed[27,28].

3.2. Dietary nucleotide role in growth, immunity and stress resistance

In aquaculture, health benefits of dietary nucleotide supplement include improved survival and growth under stress[31,32]. The findings by Do Huu *et al*.[33] show that there is a requirement for nucleotide in the diet of *Penaeus monodon* (*P. monodon*) for optimal growth. It is important that nucleotide can replace fish meal in the feed of shrimp, *Litopenaeus vannamei* (*L. vannamei*)[34]. Also, *L. vannamei* fed 0.04% diet nucleotide could elevate nonspecific immune response, resistance, and growth of this species[35].

Indeed, application of nucleotide in the diet significantly enhances the growth performance in many fish species. It was reported that, nucleotide supplementation could partly replace fish meal in diet of rainbow trout, *Oncorhynchus mykiss*[36,37]. Also research revealed that nucleotide mixture supplemented at a dose range between 0.05% and 0.20% increased growth of grouper, *Epinephelus malabaricus* with maximal effect at 0.14% inclusion[30]. Nucleotide

supplementation also improved the growth of red drum, *Sciaenops ocellatus* (*S. ocellatus*) fed 0.03%–0.30%[38], Atlantic salmon, *Salmo salar*[29,39,40]. Growth, protein efficiency, and feed conversion ratio increased in *Ancherythroculter nigrocauda* fed 0.045%–0.060% nucleotide[41]. *Beluga sturgeon*, Huso huso fed 0.25% nucleotide showed significantly better growth performance[42]. Red sea bream, *Pagrus major* (*P. major*), a recommendation of 1.0–1.5 g/kg mixed nucleotides added in the diet to boost growth, immune responses and stress resistance[43]. *Hybrid tilapia*, *Oreochromis niloticus*, *Oreochromis aureus* grew faster when supplied 0.6% nucleotide in the diet[44]. Similarly, *Nile tilapia* increased growth and feed intake when fed nucleotide in the diet[45]. However, Yaghobi *et al*.[46] reported no influence of dietary nucleotide on growth, food conversion ratio and some haematological index of striped catfish, *Pangasianodon hypophthalmus*. Also, nucleotide supplementation did not significantly influence growth of fish fed diets with 30% to 50% soybean protein but could improve the non-specific immune and gut morphology of turbot[32]. In accordance to these findings, growth performance, proximate composition in juvenile turbot, *Scophthalmus maximus* did not influence by nucleotide diet[47].

Nucleotide supplementation in the diet also showed benefits to invertebrate. Growth performance, non-specific immunity of sea cucumber, *Apostichopus japonicas* increased when fed nucleotide in the diet[48]. Growth of Pacific white leg prawn *L. vannamei* was enhanced by addition of 0.04% of purified nucleotide mixture to feeds[49]. Likewise, dietary nucleotide rise growth performance, feed utilization and boosted immune against air exposure in crayfish[50]. In addition, survival rate of *Artemia* fed nucleotide and challenge with *Vibrio proteolyticus* was higher than control as demonstrated by significantly higher survival[51]. Also, there was a significant growth and survival of *Artemia* when increased nucleotide (purine and pyrimidine) was added in the diet. It is possible that *Artemia* cannot synthesize nucleotide endogenously as other species do, thus the benefit of nucleotide diets in *Artemia* is clearer than in other animals[52].

Dietary nucleotides also improve growth rate under stress conditions in Atlantic salmon[29], grouper[30], red drum[38], and Pacific white leg shrimp *L. vannamei*[49]. It was reported that 0.15%–0.20% nucleotide supplement improved growth and resistance to *Streptococcus iniae* of rainbow trout, *Oncorhynchus mykiss*[37]. Nucleotides may also improve response to osmotic stress: Atlantic salmon fed dietary nucleotide at 0.25% and 0.5% for 8 weeks and then transferred to 18 ppt salinity for 3 days showed greater immunofluorescence of Na^+, K^+-ATPase in histology samples with the highest in the group fed 0.5% nucleotide[53], perhaps evidence of higher osmoregulatory capability in fish supplied nucleotide.

Nucleotide supplement in the diets also seem to enhance the immune response of aquatic species. Neutrophil oxidative radical production is influenced by nucleotide supplement in red drum, *S. ocellatus*[38]. Carp, *Cyprinus carpio* fed with nucleotide supplemented diet for 3 days, clearance of a carp pathogen (*Aeromonas hydrophila*) was enhanced, with no bacteria in the kidney, blood or liver and an increase in serum complement and lysozyme activities of the fish treated with nucleotides. Furthermore, supplemented nucleotide in diet enhances immune responses and enahance pathogen resistance in tilapia[31,44,45]. Growth performance, immune response and tolerance upon challenge to *Aeromonas hydrophila* were higher in rohu, *Labeo rohita* fed nucleotide[54]. Supplementation of 0.25% nucleotide in the diet could boost growth, immune and stress resistance of of Caspian brown trout[55]. Although immunity in arthropod invertebrates is very different to higher animals, nucleotide effects have also been shown here: using a range of concentrations of supplement (0%, 0.15%, 0.225% and 0.3%) of nucleotide in the diet to feed giant freshwater prawn *Macrobrachium rosenbergii* for 60 days resulted in higher phenoloxidase, total haemocyte count and total haemolymph compared in prawns fed higher levels of supplement[56]. In contrary, dietary nucleotide did not improve growth and body composition in pikeperch, *Sander lucioperca*, while significantly stimulate immune and reduce mortality when exposed to *Aeromonas salmonicida*[57].

Nucleotide content in animal tissues has been found to change with variations in environment[58-60]. In penaeid prawns, levels of ATP-related compounds in tissues were impacted by harvesting methods and species. IMP level in prawns (*Penaeus japonicus*, *P. monodon*) increased with increase of temperature and time exposed to air[59]. This increase in IMP as a result of change of environment has led to tissue levels of IMP being proposed as a stress indicator[59]. Nucleotide composition in tissues of crustaceans changes in stress environments and rapid changes in salinity cause a significant variation of nucleotide composition in different tissues of the kuruma prawn, *Marsupenaeus japonicus* (*M. japonicus*)[58]. In addition, ATP is as the major source of cellular energy[61], so it is likely that during stressful conditions animals require more energy to cope with the changing environment. This energy could be supplied by guanine and adenine phosphate derivatives: levels of IMP, a major intermediate in ATP and guanosine-5'-triphosphate biosynthesis, were found to be high in stressed prawns, while adenine, particularly ATP, ADP and AMP levels dropped sharply, perhaps indicating rapid utilization in energy production[62]. It is possible that not all exogenous nucleotide addition will help stress resistance. Thus, requirement for individual concentration of each single nucleotide and their combinations as mixtures in the diet should be considered.

The changing pattern of nucleotide related to environment differs depending upon tissues. Nucleotide in gills and in hepatopancreas but not in muscle was correlated with environmental nitrite levels in *M. japonicus*, increasing in gills but reducing in hepatopancreas[60]. Additionally, change of salinity caused a significant variation of nucleotide composition in different tissue of *M. japonicus*. There was an inverse relationship between salinity and nucleotide

in the hepatopancreas, while the nucleotide in the muscles and gills increased directly with increase of salinity[58]. Because some nucleotide levels drop rapidly under stress, it is possible that exogenous dietary nucleotide can provide nucleotide rapidly to the cells reducing energy requirement so animals can resist better under stress or challenge. Oulad *et al*.[53] reported that, in histology samples from fish fed 0.5% dietary nucleotide, the highest immunofluorescence of Na^+, K^+-ATPase, which were highly correlated to osmoregulation for species that live in estuaries where environmental salinity changes with the tides[63].

3.3. Effects of high concentration of nucleotide in the diet

Most studies to date have looked at commercial mixtures of nucleotides supplied at the manufacturers recommended dose. However, there is some evidence to suggest that excessively high levels of nucleotide may cause negative effects. According to Tacon and Cooke[64] nucleic acid extracted from bacteria, when used at low concentration of 2.5% and 5% supplementation did not impact growth and feed conversion, but at 10% inclusion, feed intake, growth and feed conversion ratio of trout were negatively affected. It was also demonstrated that 23% brewer's yeast or 6% RNA could replace the nitrogen source for fish meal in the diet and could enhance growth of juvenile gilthead sea bream. However, at higher concentration of RNA growth of this species was negatively affected[65]. Terrestrial monogastric animals cannot tolerate high doses of nucleotide in the diet as heightened rates of purine metabolism produce more uric acid, resulting in toxicity and consequential harm to the animals. Uric acid accumulation could be either from an increase of purine *de novo* synthesis and catabolism or caused by the reduction of uric acid excretion from urine[66]. Furthermore, metabolism of other nutrients can also be influenced negatively at high levels of dietary nucleotide[67].

The excessive concentration of nucleotide supplementation may diminish growth of prawn and the nucleotide requirement were higher when prawn smaller[33]. In addition, excessive of exogenous nucleotide might inhibit the immune response in red drum[38]. Another study on European sea bass larvae, *Dicentrarchus labrax* supplied 3 levels (0%, 1.1% and 5.7%) of live yeast, *Debaryomyces hansenii* to the diet and showed an increase in mRNA, trypsin and lipase, with the highest enhancement of weight gain, survival rate, activities of the intestinal enzymes alkaline phosphatase, aminopeptidase N, maltase and a reduction of the malformed larvae at 1.1% yeast supplied. In contrast, the weight gain of the group fed 5.7% yeast was similar to the fish in control[68]. These numerous studies indicate that animals only need a certain amount of nucleotide supplement and over dosage may cause a negative effect. As a consequence, the dosage response of dietary nucleotide supplementation requires substantial investigation and optimization.

3.4. Effect of nucleotide on offspring and young stage animals

As stated earlier, nucleotide requirement is likely to vary with life stage as new cell generation for growth and development in young organisms greatly exceeds the very low levels of cell death thereby reducing the availability of nucleotides via salvage[9]. However, research into requirements of early developmental stages, or effect of parental supplement on offspring in aquaculture, is very limited. Gonzalez-Vecino *et al*.[69] reported that dietary nucleotide supplement of haddock, *Melanogrammus aeglefinus* broodstock resulted in larvae with higher feeding success, low mortality, intestinal improvement and size compared to larvae from broodstock without dietary supplement. However, comparative studies between differing sizes and lifestages of the same species are few. Preliminary small-scale research on *P. monodon* and *Penaeus japonicus* indicated differences in effect of supplementation with commercial nucleotides on small and large animals in terms of haemocyte counts (total and differential) and growth[70]. Similarly, mitogenic response of B lymphocytes of rainbow trout fed nucleotide for 60 days was enhanced but not for 120 days. Perhaps the bigger somatic size of the fish at 120 days, and their slower metabolic rates reduced the requirement for nucleotides compared to younger stages[71]. More extensive studies to elucidate the requirements of animals of differing ages are required.

3.5. Effects of nucleotide feeding duration on animals

The time required to detect any effect is still not known in most aquatic species and is likely to depend upon what parameters are under investigation. For example, it is likely to take longer to detect any effect on growth than it will to detect physiological changes such as immunity. For instance, a supplementation of 0.5% of Ascogen P in the diet of hybrid striped bass, feeding for 6 or 7 weeks, did not influence growth, whole body composition, hematocrit or serum lysozyme. However, there was significant increase in neutrophil oxidative radical production and survival rate after challenging with *Streptococcus iniae*. In addition, the commercial nucleotide mixture Optimun fed for 8 weeks to juvenile red drum, *S. ocellatus* did not show any response in terms of weight gain, feed efficiency, cortisol level related to density stress or survival rate following a parasite challenge with *Amyloodinium ocellatum*[72].

3.6. Role of individual nucleotides in diets

Almost all research to date in aquatic animals has investigated commercial mixtures that are high in nucleotides but are complex in their makeup. Few studies have investigated the roles of individual nucleotides though it is likely that different nucleotides have differing effects on immunity and cell development in aquatic animals. For example, cyclic nucleotides, particularly cAMP and cGMP

regulate molting in crustacean, but this could be species specific[73]. Research by Lin *et al*.[30] on grouper, *Epinephelus malabaricus* fed diets supplemented with 0.5, 1.0, 1.5 and 2.0 g/kg mixture of inosine monophosphate, AMP, GMP, uridine monophosphate and cytidine monophosphate (1 : 1 : 1 : 1 : 1) or each single nucleotide at 1.5 g per kg of diet for 8 weeks indicated that single or mixed nucleotide are beneficial to growth and immunity in these fish, with 0.15% mixed nucleotide addition diet being optimal. However, in addition, AMP at 0.15% nucleotide enhanced the immunity of grouper more than other single nucleotides, while the nucleotide mixture improved both growth and immune response. Similarly, it was reported that supplemented 0.15% IMP and mixed-NTs could enhanced the growth and immune (the nitroblue tetrazolium and lysozyme activities) in juvenile red seabream, *P. major*[74]. An inclusion of 0.4% dietary inosine and 0.2% and 0.4% AMP significantly increased growth, immune response, oxidative stress resistance and intestinal health condition in red sea bream, *P. major*[75,76]. However, Rumsey *et al*.[67] found a significant reduction on growth rate, feed intake, and other parameters of rainbow trout when fed free nucleotide adenine (1.54%), whereas the remaining purines (1.85% guanine, 2.17% xanthine and 1.94% hyponanthine) did not influence on feed intake or growth. Supplemented individual nucleotide in diet for prawn, *P. monodon* for 52 days confirmed the importance of guanine, along with inosine and adenine, in prawn diets, perhaps reflecting the relative energy (ATP) requirements for synthesis of purines compared to pyrimidines. Although individual nucleotide showed benefits to aquaculture species, the mixture seems to gain better response in grouper[30]. Likewise, the commercial nucleotide mixture provided a better response in prawn, *P. monodon*[77] and in red sea bream, *P. major*[43].

More extensive research on individual nucleotides, and their structural derivatives, has been done in mammals: Brule *et al*.[78] fed rats with diets supplemented with free purine (adenine, guanine, xanthine and hypoxanthine), their nucleosides (adenosine, guanosine and inosine) and nucleotides (adenosine monophosphate, guanosine monophosphate and inosine monophosphate) at a concentration of 30 mmol/kg diet for 14 days. The urinary allantoin excretion was higher in rats fed xanthine and hypoxanthine, nucleosides and nucleotides in comparison to the control and there was no effect on weight and structure of kidney, urine volume, serum uric acid and creatinine. Adenine caused toxicity in rats such as reduction in weight, bigger kidney, higher urine volume, greater blood urea nitrogen amount, however; the toxicity caused by adenine only found as free form, but not when fed as nucleoside or nucleotide[78]. It was also found the same negative effects of excessive adenine diets on growth reduction and health of rats including kidney expansion, urine and plasma nitrogen quantity raise and confirmed that the removal of adenine from the diet show the recovery on health and growth of rats after 9 days. In contrast, guanine, xanthine and hypoxanthine did not show any influence on growth, while adenine and guanine diets could enhance the levels of those free nucleotides in the liver of rats[79]. It is possible that the increase in intracellular cAMP levels is the toxic cause of adenine to cells[27]. The existence of high levels of adenine is reported in soybean in some countries, which has led to a possible explanation for poor health associated with diets high in soybean[80].

4. Future direction

Whilst research on potential benefits of dietary nucleotide inclusion is progressing in many aquatic species[27], there are some areas that require further research and improved understanding, particularly against a background of changing natural ingredients in aquaculture feeds as a result of environmental and economic pressure. Optimum dosage of nucleotide supplemented in the diet shoud be determined for each species. In addition, the duration of feeding nucleotide diet to gain highest growth performance is not fully understood. Moreover, although it seems logical that there will be higher nucleotide requirement for mass tissue development in young stages of life, there is little research on nucleotide requirement at different stages of life. Further study should also examine the effects of dietary nucleotide supplementation of intestinal microbiota and gut morphology, and immune response of aquaculture species.

Additionally, a major omission in previous research is that the level of nucleotides in the basal diets or in individual ingredients has not been determined, thus the actual nucleotide requirement for any species has not really been accurately determined. This is important because there are high concentrations of nucleotide found in food products such as seafood, beef as well as from plant sources such as corn and wheat[81,82] or seaweed[83]. Many of these, particularly fish and krill meal, are used core ingredients in aqua feeds. Most previous studies used ingredients such as fishmeal[38] or commercial mash as a base for experimental diets[84] without any determination of the nucleotide content in the finished diets used to feed animals in their experiments. So, the actual effects of the total nucleotides on animals are still not clear.

In addition, most studies on dietary nucleotides to date used mixtures of nucleotides with one dose[29,38,40,49,72]. Some research on the effects of individual nucleotides on various species[75-77,85]. However, those studies only applied one dose of nucleotide in the diet. Therefore, it is still limited understandings on determination of requirement of individual nucleotides and at different life stages, duration of administration for each species. This is a serious omission in many aquatic species, as there are likely to be differing requirements for different nucleotides, reflecting the differing energy requirements for their synthesis or salvage, and differing absolute requirements for the end product in the relevant tissues.

Furthermore, under different stresses, macronutrient requirements of aquaculture animals are different. Furthermore, cells involved in immune response do not synthesize nucleotides. Thus, nucleotides from dietary sources are preferably used at times of rapid growth or physiological stress. It is therefore likely that, along with the higher

requirement for other nutrients under stress conditions, nucleotide requirements will be higher under stress.

Finally, any study on inclusion of small water-soluble molecules into aquaculture feeds should also investigate their stability and retention within the pellet. This is particularly important for aquaculture species as feed expose to water, allowing time for water-soluble molecules to leach into the environment, thereby reducing the amount available for the culture animal.

Acknowledgments

The study supported by Australian Centre for International Agricultural Research (ACIAR) under grant number ACI00031. Many thanks to Assoc. Prof. Andrew Barnes, Simon Tabrett, Simon Irvin and David M. Smith for valuable advice on this study.

Conflict of interest statement

I declare that I have no conflict of interest.

References

[1] Berendonk TU, Manaia CM, Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, et al. Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol* 2015; **13**: 310-7.

[2] Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ Microbiol* 2006; **8**: 1137-44.

[3] Martínez JL. Antibiotics and antibiotic resistance genes in natural environments. *Science* 2008; **321**: 365-7.

[4] Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J Antimicrob Chemother* 2003; **52**: 159-61.

[5] Marshall E. Scientists endorse ban on antibiotics in feeds. *Science* 1983; **222**: 601.

[6] Do Huu H, Jones CM. Effects of dietary mannan oligosaccharide supplementation on juvenile spiny lobster *Panulirus homarus* (Palinuridae). *Aquaculture* 2014; **432**: 258-64.

[7] Do Huu H, Sang HM, Thanh Thuy NT. Dietary β-glucan improved growth performance, *Vibrio counts*, haematological parameters and stress resistance of pompano fish, *Trachinotus ovatus* Linnaeus, 1758. *Fish Shellfish Immunol* 2016; **54**: 402-10.

[8] Henderson JF, Paterson ARP. *Nucleotide metabolism: an introduction.* New York: Academic Press; 1973.

[9] Rudolph FB, Kulkarni AD, Fanslow WC, Pizzini RP, Kumar S, Van Buren CT. Role of RNA as a dietary source of pyrimidines and purines in immune function. *Nutrition* 1990; **6**: 45-52; 59-62.

[10] Barness LA. Dietary sources of nucleotides–from breast milk to weaning. *J Nutr* 1994; **124**(Suppl 1): 128S-30S.

[11] Sanderson IR, He Y. Nucleotide uptake and metabolism by intestinal epithelial cells. *J Nutr* 1994; **124**(Suppl 1): 131S-7S.

[12] Quan R, Barness LA. Do infants need nucleotide supplemented formula for optimal nutrition. *J Pediatr Gastroenterol Nutr* 1990; **11**: 429-34.

[13] Savaiano DA, Clifford AJ. Adenine, the precursor of nucleic acids in intestinal cells unable to synthesize purines *de novo*. *J Nutr* 1981; **111**: 1816-22.

[14] Carver JD. Dietary nucleotides: effects on the immune and gastrointestinal systems. *Acta Paediatr Suppl* 1999; **88**: 83-8.

[15] Holen E, Bjørge OA, Jonsson R. Dietary nucleotides and human immune cells. II. Modulation of PBMC growth and cytokine secretion. *Nutrition* 2006; **22**: 90-6.

[16] Cai X, Bao L, Wang N, Xu M, Mao R, Li Y. Dietary nucleotides supplementation and liver injury in alcohol-treated rats: a metabolomics investigation. *Molecules* 2016; **21**: 435.

[17] López-Navarro AT, Ortega MA, Peragón J, Bueno JD, Gil A, Sánchez-Pozo A. Deprivation of dietary nucleotides decreases protein synthesis in the liver and small intestine in rats. *Gastroenterology* 1996; **110**: 1760-9.

[18] Cosgrove M. Perinatal and infant nutrition. Nucleotides. *Nutrition* 1998; **14**: 748-51.

[19] Joule JA, Mills K. *Heterocyclic chemistry*. 5th ed. Chichester: John Wiley & Sons, Ltd.; 2010, p. 664.

[20] Carver JD. Dietary nucleotides: cellular immune, intestinal and hepatic system effects. *J Nutr* 1994; **124**: 144S-8S.

[21] Ohyanagi H, Nishimatsu S, Kanbara Y, Usami M, Saitoh Y. Effects of nucleosides and a nucleotide on DNA and RNA syntheses by the salvage and *de novo* pathway in primary monolayer cultures of hepatocytes and hepatoma cells. *JPEN J Parenter Enteral Nutr* 1989; **13**: 51-8.

[22] Salobir J, Rezar V, Pajk T, Levart A. Effect of nucleotide supplementation on lymphocyte DNA damage induced by dietary oxidative stress in pigs. *Anim Sci* 2005; **81**: 135-40.

[23] Maldonado J, Navarro J, Narbona E, Gil A. The influence of dietary nucleotides on humoral and cell immunity in the neonate and lactating infant. *Early Hum Dev* 2001; **65** Suppl: S69-74.

[24] Carver JD, Stromquist CI. Dietary nucleotides and preterm infant nutrition. *J Perinatol* 2006; **26**: 443-4.

[25] European Commission. Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC Text with EEA relevance. 2006. [Online] Available from: http://eur-lex.europa.eu/eli/dir/2006/141/oj [Accessed on 20th May, 2016]

[26] Wang L, Liu J, Lv H, Zhang X, Shen L. Effects of nucleotides supplementation of infant formulas on plasma and erythrocyte fatty acid composition: a meta-analysis. *PLoS One* 2015; **10**: e0127758.

[27] Li P, Gatlin DM III. Nucleotide nutrition in fish: current knowledge and future applications. *Aquaculture* 2006; **251**: 141-52.

[28] Ringø E, Olsen RE, Vecino JLG, Wadsworth S, Song SK. Use of immunostimulants and nucleotides in aquaculture: a review. *J Mar Sci Res Dev* 2012; **1**: 104.

[29] Burrells C, William PD, Southgate PJ, Wadsworth SL. Dietary nucleotides: a novel supplement in fish feeds: 2. Effects on vaccination,

salt water transfer, growth rate and physiology of Atlantic salmon (*Salmo salar* L.). *Aquaculture* 2001; **199**: 171-84.

[30] Lin YH, Wang H, Shiau SY. Dietary nucleotide supplementation enhances growth and immune responses of grouper, *Epinephelus malabaricus*. *Aquac Nutr* 2009; **15**: 117-22.

[31] Shiau SY, Gabaudan J, Lin YH. Dietary nucleotide supplementation enhances immune responses and survival to *Streptococcus iniae* in hybrid tilapia fed diet containing low fish meal. *Aquac Rep* 2015; **2**: 77-81.

[32] Peng M, Xu W, Ai QH, Mai KS, Liufu ZG, Zhang K. Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.). *Aquaculture* 2013; **392-5**: 51-8.

[33] Do Huu H, Tabrett S, Hoffmann K, Köppel P, Lucas JS, Barnes AC. Dietary nucleotides are semi-essential nutrients for optimal growth of black tiger shrimp (*Penaeus monodon*). *Aquaculture* 2012; **366-7**: 115-21.

[34] Zhao LB, Wang WG, Huang XX, Guo TF, Wen W, Feng LF, et al. The effect of replacement of fish meal by yeast extract on the digestibility, growth and muscle composition of the shrimp *Litopenaeus vannamei*. *Aquac Res* 2015; doi: 10.1111/are.12883.

[35] Manoppo H, Sukenda. Enhancement of nonspecific immune response and growth performance of *Litopenaeus vannamei* by oral administration of nucleotides. *J Vet* 2013; **14**: 430-6.

[36] Hunt AÖ, Yılmaz FÖ, Engin K, Berköz M, Gündüz SG, Yalın S, et al. The effects of fish meal replacement by yeast based nucleotides on growth, body composition and digestive enzyme activity in rainbow trout juveniles (*Onchorchyncus mykiss*). *Israeli J Aquac Bamidgeh* 2014; 1-10.

[37] Tahmasebi-Kohyani A, Keyvanshokooh S, Nematollahi A, Mahmoudi N, Pasha-Zanoosi H. Dietary administration of nucleotides to enhance growth, humoral immune responses, and disease resistance of the rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Fish Shellfish Immunol* 2011; **30**: 189-93.

[38] Li P, Gatlin DM III, Neill WH. Dietary supplementation of a purified nucleotide mixture transiently enhanced growth and feed utilization of juvenile red drum, *Sciaenops ocellatus*. *J World Aquac Soc* 2007; **38**: 281-6.

[39] Burrells C. Nucleotides aid dietary louse control. *Fish Farmer* 2001; **24**: 62.

[40] Burrells C, Williams PD, Forno PF. Dietary nucleotides: a novel supplement in fish feeds: 1. Effects on resistance to disease in salmonids. *Aquaculture* 2001; **199**: 159-69.

[41] Yin HC, Huang J, Yang DH, Zhao HY, Jia F, Zhang Y. Effects of yeast nucleotide on growth performance, serum immune index and muscle composition of *Ancherythroculter nigrocauda* Yih & Wu. *Iran J Fish Sci* 2015; **14**: 646-59.

[42] Abtahi B, Yousefi M, Kenari AA. Influence of dietary nucleotides supplementation on growth, body composition and fatty acid profile of *Beluga sturgeon* juveniles (*Huso huso*). *Aquac Res* 2013; **44**: 254-60.

[43] Hossain MS, Koshio S, Ishikawa M, Yokoyama S, Sony NM. Dietary nucleotide administration influences growth, immune responses and oxidative stress resistance of juvenile red sea bream (*Pagrus major*). *Aquaculture* 2016; **455**: 41-9.

[44] Xu L, Ran C, He SX, Zhang JL, Hu J, Yang YL, et al. Effects of dietary yeast nucleotides on growth, non-specific immunity, intestine growth and intestinal microbiota of juvenile hybrid tilapia *Oreochromis niloticus* ♀ × *Oreochromis aureus* ♂. *Anim Nutr* 2015; **1**: 244-51.

[45] da Silva Berto R, do Vale Pereira G, Mouriño JLP, Martins ML, Fracalossi DM. Yeast extract on growth, nutrient utilization and haemato-immunological responses of Nile tilapia. *Aquac Res* 2016; **47**: 2650-60.

[46] Yaghobi M, Dorafshan S, Paykan Heyrati F, Mahmoudi N. Growth performance and some haematological parameters of ornamental striped catfish (*Pangasianodon hypophthalmus*) fed on dietary nucleotide. *Iran J Vet Res* 2014; **15**: 262-5.

[47] Fuchs VI, Schmidt J, Slater MJ, Zentek J, Buck BH, Steinhagen D. The effect of supplementation with polysaccharides, nucleotides, acidifiers and *Bacillus* strains in fish meal and soy bean based diets on growth performance in juvenile turbot (*Scophthalmus maximus*). *Aquaculture* 2015; **437**: 243-51.

[48] Wei Z, Yi L, Xu W, Zhou H, Zhang Y, Zhang W, et al. Effects of dietary nucleotides on growth, non-specific immune response and disease resistance of sea cucumber *Apostichopus japonicas*. *Fish Shellfish Immunol* 2015; **47**: 1-6.

[49] Li P, Lawrence AL, Castille FL, Gatlin DM III. Preliminary evaluation of a purified nucleotide mixture as a dietary supplement for Pacific white shrimp *Litopenaeus vannamei* (Boone). *Aquac Res* 2007; **38**: 887-90.

[50] Safari O, Shahsavani D, Paolucci M, Atash MMS. The effects of dietary nucleotide content on the growth performance, digestibility and immune responses of juvenile narrow clawed crayfish, *Astacus leptodactylus leptodactylus* Eschscholtz, 1823. *Aquac Res* 2015; **46**: 2685-97.

[51] Madalla N, Wille M, Sorgeloos P. Effects of dietary nucleotides on growth rate and disease resistance of crustaceans using axenic *Artemia* culture tests. *Tanzan J Agric Sci* 2013; **12**: 37-42.

[52] Hernandorena A. [The patterning function of purines in the brine shrimp *Artemia*]. *C R Seances Acad Sci III* 1999; **322**: 289-301. French.

[53] Oulad S, Abedian AM, Khodabandeh S. Effect of dietary nucleotide on the osmoregulatory function of pyloric caeca in Caspian Sea salmon, *Salmo trutta caspius*. *Comp Biochem Physiol A Mol Integr Physiol* 2009; **153**: s81.

[54] Murthy HS. Effect of nucleotide on growth, immune responses and resistance of *Labeo rohita* to *Aeromonas hydrophila* infection. *J Aquac Mar Biol* 2015; **2**: 00037.

[55] Kenari AA, Mahmoudi N, Soltani M, Abediankenari S. Dietary nucleotide supplements influence the growth, haemato-immunological parameters and stress responses in endangered Caspian brown trout (*Salmo trutta caspius* Kessler, 1877). *Aquac Nutr* 2013; **19**: 54-63.

[56] Shankar R, Murthy HS, Sujatha HR, Jayaraj EG, Tejpal CS, Chinthamani VS. Effect of nucleotide on growth, immune responses and resistance of *Macrobrachium rosenbergii* (De Man) to *Macrobrachium*

rosenbergii nodavirus (MrNV) and extra small virus (XSV) and *Aeromonas hydrophila* infection. *Aquac Int* 2012; **20**: 1-12.

[57] Jarmołowicz S, Zakęś Z, Siwicki A, Terech-Majewska E, Kowalska A, Partyka K, et al. Immunomodulatory effect of dietary brewer's yeast extract in *Sander lucioperca* juveniles against the challenge of *Aeromonas salmonicida*. *Aquac Int* 2013; **21**: 939-45.

[58] Cheng SY, Lee WC, Chen JC. Increase of uricogenesis in the kuruma shrimp *Marsupenaeus japonicus* reared under hyper-osmotic conditions. *Comp Biochem Physiol B Biochem Mol Biol* 2004; **138**: 245-53.

[59] Paterson BD. The rise in inosine monophosphate and $_{L}$-lactate concentration in muscle of live penaeid prawns (*Penaeus japonicus, Penaeus monodon*) stressed by the storage out of water. *Comp Biochem Physiol B Comp Biochem* 1993; **106**: 395-400.

[60] Cheng SY, Lee WC, Chen JC. An increase of uricogenesis in the Kuruma shrimp *Marsupenaeus japonicus* under nitrite stress. *J Exp Zool A Comp Exp Biol* 2005; **303**: 308-18.

[61] Hardie DG, Hawley SA. AMP-activated protein kinase: the energy charge hypothesis revisited. *Bioessays* 2001; **23**: 1112-9.

[62] Chen HC, Moody MW, Jiang ST. Changes in biochemical and bacteriological quality of grass prawn during transportation by icing and oxygenating. *J Food Sci* 1990; **55**: 670-3.

[63] Lucu C, Towle DW. Na^+ + K^+-ATPase in gills of aquatic crustacea. *Comp Biochem Physiol A Mol Integr Physiol* 2003; **135**: 195-214.

[64] Tacon AGJ, Cooke DJ. Nutritional value of dietary nucleic acids to trout. *Nutr Rep Int* 1980; **22**: 631-40.

[65] Oliva-Teles A, Guedes MJ, Vachot C, Kaushik SJ. The effect of nucleic acids on growth, ureagenesis and nitrogen excretion of gilthead sea bream *Sparus aurata* juveniles. *Aquaculture* 2006; **253**: 608-17.

[66] Yamaoka T, Itakura M. [Metabolism of purine nucleotides and the production of uric acid]. *Nihon Rinsho* 1996; **54**: 3188-94. Japanese.

[67] Rumsey GL, Winfree RA, Hughes SG. Nutritional value of dietary nucleic acids and purine bases to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 1992; **108**: 97-110.

[68] Tovar-Ramírez D, Infante JZ, Cahu C, Gatesoupe FJ, Vázquez-Juárez R. Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. *Aquaculture* 2004; **234**: 415-27.

[69] Gonzalez-Vecino JL, Cutts CJ, Batty RS, Greenhaff PL, Wadsworth S. The effects of nucleotide-enriched broodstock diet on first feeding success and survival of haddock (*Melanogrammus aeglefinus* l.) larvae. In: ICES symposium on Gadoid mariculture: development and future challenges; 2004 Jun 13-16; Bergen, Norway. 2004.

[70] Ancieta-Probstl DK, Smullen RP, Barnes AC. Enhancing growth performance of shrimp with nucleotide supplemented diets. *Aquac Asia Pac* 2005; **4**: 26-8.

[71] Leonardi M, Sandino AM, Klempau A. Effect of a nucleotide-enriched diet on the immune system, plasma cortisol levels and resistance to infectious pancreatic necrosis (IPN) in juvenile rainbow trout (*Oncorhynchus mykiss*). *Bull Eur Assoc Fish Pathol* 2003; **23**: 52-9.

[72] Li P, Burr GS, Goff J, Whiteman KW, Davis KB, Vega RR, et al. A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum (*Sciaenops ocellatus*). *Aquac Res* 2005; **36**: 1120-7.

[73] Covi JA, Chang ES, Mykles DL. Conserved role of cyclic nucleotides in the regulation of ecdysteroidogenesis by the crustacean molting gland. *Comp Biochem Physiol A Mol Integr Physiol* 2009; **152**: 470-7.

[74] Song JW, Lee KJ. Effects of dietary nucleotide supplementation on the growth performance, feed utilization, hematological parameters and innate immunity in red seabream *Pagrus major*. *Korean J Fish Aquat Sci* 2013; **46**: 785-92.

[75] Hossain MS, Koshio S, Ishikawa M, Yokoyama S, Sony NM, Usami M, et al. Inosine supplementation effectively provokes the growth, immune response, oxidative stress resistance and intestinal morphology of juvenile red sea bream, *Pagrus major*. *Aquac Nutr* 2016; doi: 10.1111/anu.12463.

[76] Hossain MS, Koshio S, Ishikawa M, Yokoyama S, Sony NM. Dietary effects of adenosine monophosphate to enhance growth, digestibility, innate immune responses and stress resistance of juvenile red sea bream, *Pagrus major*. *Fish Shellfish Immunol* 2016; **56**: 523-33.

[77] Do Huu H, Tabrett S, Hoffmann K, Köppel P, Barnes AC. The purine nucleotides guanine, adenine and inosine are a dietary requirement for optimal growth of black tiger prawn, *P. monodon*. *Aquaculture* 2013; **408-409**: 100-5.

[78] Brulé D, Sarwar G, Savoie L, Campbell J, Van Zeggelaar M. Differences in uricogenic effects of dietary purine bases, nucleosides and nucleotides in rats. *J Nutr* 1988; **118**: 780-6.

[79] Claramunt D, Gil-Peña H, Fuente R, García-López E, Loredo V, Hernández-Frias O, et al. Chronic kidney disease induced by adenine: a suitable model of growth retardation in uremia. *Am J Physiol Renal Physiol* 2015; **309**: F57-62.

[80] Kaneko K, Aoyagi Y, Fukuuchi T, Inazawa K, Yamaoka N. Total purine and purine base content of common foodstuffs for facilitating nutritional therapy for gout and hyperuricemia. *Biol Pharm Bull* 2014; **37**: 709-21.

[81] Mateo CD, Stein HH. Nucleotides and young animal health: can we enhance the intestinal tract development and immune function? In: Lyons TP, Jacques JK, editors. Nutritional biotechnology in the feed and food industries. proceedings of Alltech's 20th annual symposium. United Kingdom: Nottingham University Press; 2004, p. 159-68.

[82] Brulé D, Sarwar G, Savoiet L. Purine content of selected Canadian food products. *J Food Compos Anal* 1988; **1**: 130-8.

[83] Newton RP, Kingston EE, Overton A. Identification of novel nucleotides found in the red seaweed *Porphyra umbilicalis*. *Rapid Commun Mass Spectrom* 1995; **9**: 305-11.

[84] Hill J, Smullen R, Ancieta D, Barnes AC. Highly purified dietary nucleotide supplements improve growth performance and health status of penaeid shrimp. In: AQUA 2006 WAS meeting abstract. 2006 May 10-13; Florence, Italy.

[85] Hossain MS, Koshio S, Ishikawa M, Yokoyama S, Sony NM, Ono S, et al. Comparison of the effects of inosine and inosine monophosphate on growth, immune response, stress resistance and gut morphology of juvenile red sea bream, *Pagrus major*. *Aquaculture* 2016; **458**: 64-74.