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ROLE OF NUCLEOTIDES IN FISH NUTRITION

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Abstract

Study of nucleotide role in fish metabolism has been sparingly studied for over 25 years. Nucleotide s play vital role in diet palatability, fish feeding behaviour and biosynthesis of non-essential amino acids besides this it has been recently revealed that it enhances immunity and disease resistance in fishes. Role of nucleotide in fish research shown that they improve larval growth enhance larval quality via broodstock fortification, alter intestinal structure, increase stress tolerance as well as modulate innate and adaptive immune response, Despite some inconsistent results of using nucleotide supplementation in feed it shows beneficial effect on various fish species. However, there are lacuna in existing research about exogenous supplementation of nucleotide feed of fishes i.e. digestion, absorption, metabolism and influence on various physiological responses especially expression of immunogene and modulation of immunoglobulin production. Apart from this there is a gap of knowledge about age /size related responses, appropriate dose and timing of administration of nucleotide. Thus further research in these areas should be carried out.

Introduction

Nucleotides plays an important role in essential physiological and biochemical function which includes encoding and deciphering genetic information ,mediating energy metabolism and cell signalling beside this it serves as an component of coenzymes, allosteric effectors and cellular agonistics (Carver and Walker,1995; Cosgrove,1998). Inclusion of nucleotide exogenously was debated for many years. In both human and animal model there seems no deficiency symptom of nucleotide due to this reason previously it was considered as non-essential nutrients. But in mean course it was challenged by successive researchers that dietary nucleotide deficiency leads to impair liver, heart, intestine and immune function

(Grimble and Westwood,2000a). Gil, 2002 revealed that dietary nucleotide has modulatory effects on lymphocyte maturation, activation and proliferation, macrophages phagocytosis, immunoglobulin response as well as genetic expression of certain cytokines in animals and human beings. Dietary supplementation of nucleotide in fishes are traced back to 1970s, reason was mainly focused on chemo attractive effect of this compound. (Mackie, 1973; Kiyohara et al., 1975; Mackie and Adron, 1978)

Nucleotide Biochemistry

Purine or a pyrimidine base, a ribose or 2' deoxyribose sugar and one or more phosphate groups contained in nucleotide.Nucleotide term is not refers to a specific from of the compound but to all form of the compound but to all form of compound that contains purine or pyrimidine bases. As per Rudolph,1994 adenine, guanine, hypoxanthine and xanthine are major purine bases which form nucleotides through a glycosidic bond between the N-8 nitrogen and C-1 carbon of the pentose, while phosphoryl groups is esterified with C-5' hydroxyl group. In same way uracil, thymine and cytosine are pyrimidine major bases form nucleotide.

Reviewed by Rudolph, 1994; Carver and Walkar, 1995; Grimble and Westwood, 2000 as purine and pyrimidines are synthesized from de novo pathway or obtained from salvage pathways. In cystosol of mammalian cells purine rings are synthesized from glycine, aspartate, glutamine, tetrahydrofolate derivatives and Co_2 with considerable energy, while aspartate, glutamine and Co_2 synthesized pyrimidine in cystosol and mitochondria of mammalian cells. It is presumed that these pathway are operative in fishes. Maintenance of nucleotide homeostasis and conserving energy is done by salvage pathway.

Salvage and de novo pathways varied among different tissues and it may be influenced by physiological functions and metabolic needs are concluded from mammalian research. These finding are to the confirmed in fish, as salvage pathway supply nucleotide turnover in erythrocyte, lymphocytes, heart and brain of mammals. As per Lopez –Navarro etal., 1995 liver metabolism has been modulated by dietary nucleotide.

Grimble and Westwood, 2000a revealed that liver is adapted to rapid induction of nucleotide supply and also important organ of nucleotide storage and transport nucleotide to other organ to meet physiological needs.

<u>Metabalism of nucleotides and related metabolites</u>

All food of animal and vegetable origin are natural source of nucleotide as in the form of free nucleotides and nucleic acid. Gill, 2002 find out that concentration of RNA and DNA in food mainly depends on cell density. Purine and RNA content in some food stuffs like various organ meats, seafoods and dried legumes.(Clifford and Story, 1976). Purine and pyrimidine bases in common aqua feed ingredient such as fish meal (1.4%), press cake fish meal (0.4%), Fish soluble (2.8%), yeast (0.9%) yeast extract (2.3%) and single cell protein (2.1%) has been reported by Devresse (2000).

Brewers yeast *Saccharomyces cerevisiae* a single cell protein which is readily used for aqua feeds contains 12-20% of total nitrogen and RNA nitrogen (Mainly consist of nucleoprotein *i.e.* Purine and Pyrimidine) as reported by Rumsey *etal.*, 1992.

Nucleoproteins are degraded by proteases to peptides and nucleic acids as per the finding based on mammalian research. (Reviewed by Quan and Uauy, 1991)

Nucleic acids are cleaved by enzyme nucleases to nucleotides. Intestinal enzyme alkaline phosphatase remove phosphate groups of nucleotides to from nucleosides. Nucleosidases are cleaved sugars to produce free purine and pyrimidine bases. Gut mucosa absorbs nucleosides and nitrogenous bases.

As per Sonoda and Tatibana, 1978 combination of various nucleotides intermediates are absorbed by humans while nucleotides in the form of nucleosides are absorbed by humans while nucleotides in the form of nucleosides are absorbed by mice.

Research on digestion and absorption of nucleotides by fish is in infant stage. Presence and characterisation of alkaline phosphotises and proteases enzymes are well studied in fish intestine. Nuclease, the most important enzyme in nucleotide digestion in fish is poorly understood although its presence has been reported in temperate fish Rainbow trout (Roald,1978) Therefore, the digestion capacity of nucleotides by various fishes remains unknown at this time.

Why exogenous supplementation of nucleotide required by the fishes ?

Two main hypothesis are raised to address the issue of exogenous supplementation of nucleotides by the fishes.

I. Nucleotides in non free form or in the form of nucleic acid are stable and difficult to digest due to this it is recommended a well balanced mixture of free nucleotides to overcome certain stressful conditions (Borda etal., 2003)

II. There is an adequate supply of exogenous nucleotides in standard commercial fish feed when fish tends towards rapid growth under normal, non-stressful condition (Burrells etal., 200lb and Low etal., 2003)

Digestibility and bioavailability nucleic acids in natural feed ingredients such as marine protein sources or brewers yeast for fishes remain unknown, however fish such as rainbow trout can utilize yeast nucleic acid extract for growth, nitrogen retention and possibly nonessential amino acid synthesis (Rumsey etal., 1992)

Environmental contaminants such as lingnosulphonates inhibit nucleases as well as proteases of rainbow trout. This shows that various environmental and or physiological factors influence digestion and absorption of nucleotides in fishes.

There is scanty scientific literature on nucleotide content of the feed ingredients and bioavailability to the fishes also it is not confirmed yet how environmental factors such as stressors and pollutants exert their effects on digestion of nucleotides.

These are issues are fundamental for understanding of role of nucleotide in fish nutrition and for this further research in this direction to be done.

Role of nucleotide in fish feed as chemo attractant and feeding stimulant

Low molecular weight fractions of squid and hypothesized nucleotides (AMP) and nucleoside (inosine) components are the maim chemo attractants for squid and animals like lobster (Mackie, 1973). Forty seven nucleosides and nucleotides are screened by Mackie and Adron 1978. They identified that inosine and IMP as the most potent gustatory feeding stimulants for turbot fish while performing dietary experiment.

Nucleotide (UMP) was most effective based on gustatory sensitivity of various marine teleost including aigo rabbit fish, isaki grunt, kampochi amberjack, maaji jack mackerel and masaba chub mackerel (Ishida and Hidaka, 1987)

Supplementation of 2.5% and 4.1% yeast RNA extract or 1.85% guanine or 2.17% xanthine significantly increased cumulative feed intake of rainbow trout over a 12 week feeding duration. (Rumsey *etal.*, 1992.)

Gustatory responses of fishes to exogenous nucleotides may be species specific i.e aigo rabbit did not respond to any nucleotides as did most other marine teleosts (Ishida and Hidaka, 1987)

Research publication pertaining to the stimulatory effect of inosine or IMP on various fishes is not consistent. Dietary inosine enhances growth of turbot larvae. (Person – Le Ryet etal., 1983). In subsequent publication they reported that turbot larvae fed a diet supplemented with both betaine and inosine showed significant higher growth and larvae fed diet supplemented only with betain or inosine and a reduced amount of betaine. (Metailler etal., 1983).

Use of jack mackerel as an experimental model it was found that IMP,GMP,UMP,UDP,UTP were effective feeding stimulants while nucleosides (including inosine, adenosine, guanosine, uridine) and other nucleotides (AMP, ADP, ATP, IDP, ITP, GDP, GTP, xanthosine, 5'– mono phosphate 3' IMP, 3' UMP, 2-deoxy –IMP, allyltio-IMP) were not.

IMP is reported to be effective stimulatory effects than inosine on feeding of fish species including jack mackerel (Kubitza etal., 1997)

Supplementation of IMP@2800mg kg -1 enhanced feed intake of largemouth bass by 46% compared to the non-supplemented soyabean meal based diet.

Soybean meal diet incorporated with either 2800 or 5600 mg kg^{-1} IMP was inferior to the fish feed containing 10% fish meal as fishmeal has relatively high concentration of IMP. IMP may serve as primary candidate for feed attractant research for complete replacement of fish meal in aqua feed nucleotide on.

Effect of Nucleotide on Gastro intentional tract of fishes

There was multiple effect of nucleotide on gastrointestinal tract in animal models which includes physiological, morphological and microbiological influences. Uauy etal.,1990 revealed that dietary nucleotides or AMP alone can significantly increase growth and differentiation of Gastro Intestinal (GI) tract. Nucleotides have ameliorating effect on intestinal injury and facilitate bifidobacteria predominance in intestine. (Carver and Walker ,1995)

Influence of dietary nucleotide in human and terrestrial animal GI tracts includes increased villus height (Uauy etal, 1990), increased jejunum wall thickness and villus cell number (Bueno etal., 1994). There is limited research on gastro intestinal responses of fishes to dietary nucleotides.

Histological studies of Atlantic salmon intestine showed first time the morphological responses under the influence of dietary nucleotides in fishes. (Burrells etal., 2001b.) In this study mean fold height of proximal, mid and distal intestine as well as total gut surface area of fish fed a nucleotide supplemented diet was significantly greater than those of fish fed the control diet. Borda etal., 2003 reveled that in juvenile sea bream dietary nucleotides can beneficially influence intestinal health of fish .

Significant amount of dietary nucleotide are retained in the GI tract and intestine is very important immune organ. In nucleotide nutrition research Mucosa Associated Lymphoid

Tissue (MALT) regarded as prioritized area although there is very limited knowledge on MALT in fish.

Dietary nucleotide influence intestinal micro flora such as bifidobacteria in human as well as other animals. Biofidobacteria have not been reported as probiotics in fish, possible effect of dietary nucleotide on microbial ecology of fish GI tract is an interesting topic of research.

Influence of nucleated on fish Growth

Cosgrove, 1998 revealed that under normal condition de novo nucleotide synthesis is sufficient to support growth. Gyorgy,1971 studied that supplementation of nucleotides enhance growth of weaning rats fed on low-protein diets. Borda etal.,2003 reviewed that exogenous supply of nucleotides to seabream larvae may promote growth of fish and crustaceans in early stage to meet their high rate of cell replication. Sea bream larvae growth of fish and crustaceans in early stages to meet their high rate of cell replication. Turbot larvae fed with inosine containing (100 mg /fish) results enhanced growth and survived after 55 day feed trial. Subsequent study revealed that diet supplemented with 0.77% inosine also significantly increased weight gain of tar bat larvae. (Person -Le Ruyet etal.,1983). It was hypothesized that inosine enhance growth due to improved enhance feed intake at the stage of weaning enhanced food intake that reduce nutrient leaching into water or playing role in metabolism (Metailler etal., 1983). Apart from inosine growth enhancing effects of nucleotide metabolite mixture i.e. Ascogen R, Chemoform Co., Basal, Switzerland) occasionally have been observed in fish such as tilapia larva (Ramadan and Atef, 1991) and juvenile rainbow trout (Adamek etal., 1996). The growth enhancing effect on most juveniles or sub adult fishes enhancing effect on most juveniles or sub-adult fishes appears to be rather marginal (Li etal., 2004a)

Role of nucleotide in fish reproduction

Most recognized use of nucleotide supplementation in human nutrition is related to infants Research on dietary supplementation of nucleotide. There was a scanty literature regarding effect of nucleotide supplementation on maternal strategy. Brood stock of haddock when feed with nucleotide fortified feed showed that first feeding success of larvae was significantly higher than that of larva from brood stock fed a basal diet without supplemented nucleotide. Survival rate is 30% greater in nucleotide supplemented brood stock then the brood stock fed without supplement action of nucleotide . Brood stock of haddock when fed with nucleotide supplement diet shows significant effect on gut development and size of larvae as compare to that of larvae from brood stock a basal diet without supplemented nucleotide.

Nucleotide effects on innate immunity.

Grimble and Westwood 2000b;Gil; 2002 recognized that nucleotide can influence macrophage activity i.e. phagocytosis and natural killer cell activity (Carver etal.,1990) Nucleotide research on fishes shows that exogenous nucleotide influence bath homoral and cellular component of the innate immune system. Exogenous nucleotide influence increase in serum complement (alternative pathway) and lysozyme activity as well as phagocytosis and superoxide anion production of head kidney phagocytes of *Cyprinus carpio* (Sakai etal.,2001)

In hybrid striped bass fed Ascogen PR, Canadian Biosystem, Alberta, Canada supplemented diet had higher blood neutrophil oxidative radical production that fish fed the diet without supplementation . (Li etal.,2004a). Burrells etal., 2001a demonstrated that dietary nucleotide supplementation has no effect on respiratory burst of head kidney cells of salmonids. Exogenous nucleotides are key nutrients for shrimp immune system but this hypothesis has to be tested .(Devresse,2000). There is a change in immune gene expression induced by dietary nucleotide supplementation and found that nonspecific immune components such as lysozyme expression were significantly decreased in the spleen and kidney of turbot but no effect was observed in the gill.(Low etal.,2003). Due to supplementation of nucleotide there was significant increase in expression in the kidney whereas non supplemented fish shows no effect on expression of transferrin and transforming growth factor h. (Low etal.,2003)

Nucleotide effect on adaptive immunity

Dietary Nucleotides has greater impact on modulating immune system by production of immunoglobulin. (Jyonouchi etal.,1993;1994 and Navarro etal.,1996.) There is marked immunopotentiating effect on both humoral and cell mediate immune response in tilapia fed with nucleotide (Ascogen) after intramuscular injection or direct immersion with formalin killed *Aeromonas hydrophila*. (Ramadan etal., 1994). Hybrid striped bass fed on aligonucleotide supplemented diet subjected to vaccination with formalin killed *Streptococcus iniae* showed three times higher antiboby titer than that of fish fed with no supplementation of nucleotide (Li etal.,2004a). Dietary nucleotides enhanced expression of immunoglobulin M and recombinase activating gene in gill and spleen of turbot but reduced the expression in kidney (Low etal.,2003). Nucleotide fed Atlantic Salmon when vaccinated reduces mortality from 6% to 2%. There has consistent results in various fish species where modulation of adaptive immunity due to exogenous nucleotides are been used.

Nucleotide effect on stress response

There is a beneficial effect of exogenous supply of nucleotide in aquaculture feed for mitigating stressors associated with normal aquaculture condition and practices such as poor water quality crowding and handling (Burrells etal., 2001b;Low etal.,2003). Dietary nucleotide influence the fish immune system by partially offsetting the inhibitory effect of cortisol released associated with stress.

Atlantic salmon when fed with nucleotide supplemented diet enhance stress tolerance i.e. osmo regulatory capacity and growth performance when subjected to sea water transfer. (Burrells etal .,2001b). This observation of Burrells etal., 2001b was not fully confirmed untill Leonardi etal.,2003 observed that there was reduction of serum cortisol level of infections pancreatic necrosis virus when fed with exogenous nucleotides for 90-120 day. It is unknown about the involvement of exogenous nucleotide in signalling pathway associated with stress responses or there is a specific response of stressors on nucleotide metabolism in fishes. Due to extreme variation among individual fish as per Li and Gatlin Un published data there is a failure in confirmation of this phenomena with juvenile red drum.

Nucleotide role for resistance to infectious diseases

Investigation of disease resistance of fish is still limited therefore survival after challenge with certain pathogens is usually assessed as a measure of disease resistance. Exogenous dietary nucleotide can enhance resistance against pathogens including viral, bacterial and parasitic pathogens indicating a promising use for health management in aquaculture .

Atlantic Salmon fed with nucleotide supplemented diet for 2weeks there was a cumulative total mortality of 35.7% compared to fish fed the basal diet (48%) as these fishes are previously injected with Infections Salmon Anaemia (ISA) virus. (Burrels etal., 2001a)

Rainbow trout fed for 60days with exogenous nucleotide supplemented diet survived after injection with infectious pancreatic necrosis (IPN) virus, whereas there was total mortality of fishes fed with basal feed.(Leonardi etal.,2003). Enhanced resistance to various pathogenic bacteria has been reported in several species i.e. Salmonids (Burrells etal.,2001a) ,Common carp (Sakai etal.,2001) and hybrid striped bass (Li etal.,2004a).when they are fed with nucleotide supplemented feed. Rainbow trout after bath challenge with *Vibrio anguillarum*, it was fed with nucleotide supplemented diet had cumulative mortality of 31% while the fish with basal diet and h-glucan supplemented diet were 49% and 43% respectively.(Burrells etal.,2001a). Cohabitation of coho salmon with fish infected with *Piscirickettsia salmonis*, a rickettsia-like intracellular g-proteobacteria resulted in 76.9% mortality in fish fed a basal diet; whereas only 46.9% mortality was observed in fish fed the nucleotide supplemented diet. The mechanism regarding the significant enhancement in survival after *P.salmonis* challenge was not fully defined. Burrells etal.,2001 revealed that besides viral and bacterial pathogens, dietary nucleotides significantly reduced the number of sea lice infecting Atlantic salmon. Nucleotide dietary supplement in conjunctions with cypermethrin affect the development potential of early chalimus stages of sea lice, thereby reducing the number of mobile pre-adult lice (Burrells etal.,2001). Consistent results from different researches that dietary nucleotide enhances resistance of fish against numerous different pathogens.

Dose, administration, timing and type of dietary nucleotides

As per Sakai, 1999 dose is a primary consideration in administration of immunostimulant. Dose dependent effect of exogenous nucleotides on macrophage phagocyte c activity (Sakai;etal.,2001) Rumsey etal.,1992 revealed that terrestrial monogastric animals not able to tolerate high level of dietary nucleotides due to high level of uric acid from purine metabolism and associated toxicity; as well as adverse effect on other nutrient metabolism. Fishes like salmonids and sea bass can able to tolerate high level of nucleic acids and yeast by virtue of their active liver uricase (Kinsella etal., 1985;Rumsey etal.,1992;Olive-Teles and Goncalves,2001.) Incorporation of Yeast RNA extract upto 4.1% in diet of rainbow trout did not depress growth (Rumsey etal., 1992) Tacon and Cooke, 1980 revealed that there is growth depressing effect of bacterial RNA extract (10% of diet) on rainbow trout due to increased serum urea and carcass ash content in the diet did not reduce growth. There is increasing trend in growth of rainbow trout when the Ascogen (dietary nucleotide) was incorporated in the diet @0.62 and 2.5g/kg while 5g/kg diet led to growth depression of rainbow trout and goldfish after 37 days of feeding.(Adamek etal.,1996). Feeding dietary nucleotide for 60 days of feeding to rainbow trout showed mitogenic response of B Lymphocyte but not after 120 days (Leonardi etai.,2003). Li et al., 2004a showed that hybrid striped bass fed an Ascogen PR supplemented diet for 8weeks shows enhancement of innate immune response including blood neutrophil oxidative radical production, serum lysozyme and extracellular and neutracellular superoxide anion production of head kidney cells but same result was not fount after 16 weeks of feeding the same diet. These results suspects that length or regime of dietary nucleotide administration need to be taken into consideration Research work should be undertaken to clear the ambiguity regarding prolonged administration of dietary nucleotide in feed on immunity and disease resistance in fishes .

Dietary nucleotide research has focused mainly on a mixture of nucleotides, rather than specific type of nucleotides exception inosine and IMP for feeding stimulation research. Free base adenine in diet (1.54%) shows undesirable effect as it severely retards feed intake, weight gain, nitrogen retention and increased mortality of rainbow trout; in contrast other purine bases such as guanine (1.85%), xanthine (2.17%) and hypoxanthine (1.94%) did not show adverse effects. (Rumsey etal., 1992). Adenine toxicity to cell might related to increased intercellular c AMP levels. Additional research is needed to explore the effects of various nucleotides.

Conclusion

In most of the nucleotide research, patented nucleotide mixture are been used so the proportion of individual nucleotides are un known Due to this there is a deficient in knowledge about the effect of nucleotide on immune system. Nucleotides can experimentally use as alternative nitrogen sources and feeding stimulants, based on the current research on dose, administration regime , even nucleotide type is rather limited to ensure the efficacy of these biochemical under various physiological and ecological situations. Research is inadequate on age related responses of fish i.e. sub-adult fishes studies are to be undertaken on digestion and absorption process of dietary nucleotides. Nucleotides play important role in cell signaling pathway and also serve as nutrients for biosynthesis. Deciphering genomic information about fishes shed light on development of powerful analytical tools access the effect of nucleotides. The nucleotide research in fishes are still in nascent stage, so tremendous efforts are needed to establish the exact requirement of nucleotide by fishes..

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