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## Environmental and immunonutrition effects on public health linked to water pollutants and fish disease

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### Abstract

Freshwater aquatic environment are indeed in a bad state worldwide, due to human activities and there is an urgent need to save them and their biodiversity. Water plays a key role in diluting pollutants and because of that superiority as a solvent, it also means that water-soluble wastes pollute water easily. For instance, runoff from nearby lands provides freshwater life zones with an almost constant input of organic material, inorganic nutrients, and other pollutants. These cause undesirable changes in the physicochemical or biological factors of ecosystem, which in turn directly or indirectly affect the ecological balance of the environment, and ultimately affect human beings. And also advances in culture techniques and the introduction of new species have contributed to the rapid growth of the aquaculture industry. However, treatment methods including approved antibiotics and chemotherapeutants are directly or indirectly affected the consumer/environment-friendly. This review paper tried to indicate how agricultural activities and unbalanced fish nutrition affect fish health and water quality at large the environment.

**Keywords:** Environmental immunonutrition, effects on public health, water pollutants, fish disease

### Introduction

Water pollution can have a drastic impact on aquatic life in any body of water, which can result in the complete elimination of certain species. Chemical compounds can be toxic to aquatic life, and heat pollution can create an environment that causes aquatic animals to have difficulty breathing, and maintain the correct level of oxygen to survive. Additionally, water tainted by pollution will likely be toxic to land animals that attempt to drink it, meaning pollution reduces the amount of safe drinking water that humans are able to use. As the population of humanity increases, and demand for water goes up, the importance of clean drinkable water will continue to rise, so water pollution is likely to become an increasingly critical issue.

The contribution of aquaculture to world food production has increased significantly over the last few decades and this sector now supplies nearly half of the total fish and shellfish used for human consumption (FAO, 2010). Advances in culture techniques and the introduction of new species have contributed to the rapid growth of the aquaculture industry. Considering its importance in the world food sector, it is widely recognized that the industry should become sustainable from every angle. However, the downside of intensification of the farming operations has been economic losses, primarily due to infectious diseases, particularly during the early production stages. Treatment methods including approved antibiotics and chemotherapeutants are more often neither effective nor consumer/environment-friendly. Preventive measures are deemed to be sustainable and Food and Agriculture Organization (FAO) of the United Nations wants the scientific community to investigate further on: the role of good nutrition in improving aquatic animal health, use of immunostimulants and non-specific immune-enhancers to reduce susceptibility to disease, to reduce the use of chemicals and drugs in aquaculture. It is now widely accepted that nutritional approaches are essential to alleviate diseases among farmed aquatic animals. The concept that better nutrition leads to improved health is very familiar in humans and is applicable to aquatic animals too. Efforts have been made over the past two decades especially in the case of farmed fish species to understand the link between nutrition, immune response and resistance to diseases.

Most of the earth's surface is covered by water, and most of the human body is composed of water – two facts illustrating the critical linkages between water, health and ecosystems. The introduction by man into the environment of substance or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structure or amenity or interference with legitimate uses of the environment is known as Pollution. There are a few natural sources of pollutants present in aquatic ecosystems. But most freshwater ecosystems may become unbalanced by factors due to human activities such as agricultural activities. Human activities affect the bioavailability of chemicals to organisms, cause temperature fluctuations, and modify rainfall, pH and salinity. This sometimes results in habitat destruction and extinction of local aquatic populations.

Scientific evidence shows that ecosystems are under unprecedented pressure, threatening prospects for sustainable development. While the challenges are daunting, they also provide opportunities for local communities, business and government to innovate for the benefit of communities, economies and the global environment. However, in order to secure the environmental conditions for prosperity, stability and equity, timely responses that are proportionate to the scale of the environmental challenges will be required. In creating such responses, governments, the international community, the private sector, civil society and the general public all have an important role to play.

### **Impact of agriculture pollutants on freshwater and freshwater ecosystems**

Water quality is fundamental to the health and sustenance of aquatic ecosystems and hydrology. The benefits of renewable freshwater to humans include water for drinking, irrigation, industrial uses, production of fish, and for such in-stream uses as recreation, transportation and waste disposal (Jackson *et al.*, 2001) [57]. Water also plays a major role in the cycling of materials and can be a vector if it becomes a source that spread harmful substances and diseases. The quality and stability of river water depends on such factors as lithology of catchment, climatic conditions, atmospheric and anthropogenic inputs etc. (Markich and Brown, 1998; Bellos *et al.*, 2004) [35, 12]. Magnificent Increases in agricultural and industrial activities in an area directly influence the quality of water. In other words, water reservoirs are collectors of all materials spread by human industrial and agricultural activities. Heavy metals penetrate into water reservoirs via atmosphere, drainage, soil waters and soil erosion. As the concentration of heavy metal in the environment increases, these metals inevitably enter the biogeochemical cycle (Riget *et al.*, 2004, Kendrick *et al.*, 1992 and Mansour & Sidky, 2002) [41, 28, 34]. Having contaminated water, heavy metals accumulate in organisms, which are consumed by fish or penetrate into fish directly through skin and gill.

Water can be stressed with heavy metal load sourced from weathered soils/rocks, Agricultural activities, mining and metallurgical releases and industrial emissions (Adams, 2001) [3]. This may exert unsustainable demand on freshwater by aquatic microflora/microfauna, other aquatic life forms and humans who depend on such waters and may be exposed to contamination by heavy metals (Edorh, 2007) [2]. Heavy metals enter a river from a variety of sources; either natural or anthropogenic (Adaikpoh *et al.*, 2005;

Akoto *et al.*, 2008) [2, 7]. Usually in unaffected environments, the concentration of most of the metals in rivers is very low and is mostly derived from weathering of rock and soil (Reza and Singh, 2010) [5].

The main anthropogenic sources of heavy metal contamination are mining and smelting activities, disposal of untreated and partially treated effluents contain toxic metals, as well as metal chelates from different industries and indiscriminate use of heavy metal containing fertilizer and pesticides in agricultural fields (Macklin *et al.*, 2006; Nouri *et al.*, 2008; Reza and Singh, 2010) [33, 55, 56]. Chemicals derived from agricultural operations (pesticides and herbicides) and industrial effluents, such as metals, ultimately find their way into a variety of different water bodies and can produce a range of toxic effects in fish and other aquatic organisms (Bernet *et al.*, 1999) [13].

Although heavy metals do not remain in water for long, water catchments can become a source of concern if the levels of heavy metals in them exceed health guideline concentration criteria (Salomon and Forstner, 1984) [42]. But having contaminated waters, heavy metals can accumulate in organisms, which are consumed by fish or penetrate into fish directly through skin and gills. The concentration of any pollutant in any given organ/tissue of the fish depends on its rate of absorption and the processes associated with its elimination by the fish (Al-Kahtani, 2009) [9].

Metal contamination in aquatic environments has received much concern due to its toxicity, abundance and persistence in the environment, and subsequent accumulation in aquatic inhabitants. Heavy metal residues in contaminated habitats may accumulate in microorganisms, aquatic flora and fauna, which, in turn, may enter into the human food chain and result in health problems (Cook *et al.*, 1990; Deniseger *et al.*, 1990; Sin *et al.*, 2001) [19, 58, 59]. The deleterious effects of metals on aquatic ecosystem necessitate the continuous monitoring of their accumulation in key species, since it affords indication of temporal and spatial extent of the process and impact on organism's health (Kotze *et al.*, 1999) [60]. The heavy metals contamination is one of the vital factors for decline of water quality that has an obvious impact on fish diversity. Consumption of fish from the contaminated site poses a higher health risk to human. Many studies are being carried out to determine the level of metals in the fish since it is considered as one of the richest sources of protein and unsaturated omega-3 fatty acid for human (Chandrasekar *et al.*, 2003; Karadede *et al.*, 2004; Licata *et al.*, 2004; Amaraneni 2006; Calvi *et al.*, 2006; Agarwal *et al.*, 2007) [18, 27, 31, 10, 17, 5]. Heavy metals can be bioaccumulated and biomagnified via the food chain and finally assimilated by human consumers resulting in health risks (Agah *et al.*, 2009) [4].

### **Effects of heavy metals pollution on Fish**

Fish is an important component of the human diet. It is generally appreciated as one of the healthiest and cheapest sources of protein and it has amino acid compositions that are richer in cysteine than most of the other sources of protein (Eletta *et al.*, 2003) [61]. These facts often place Fishes at the top of the aquatic food chain. Fishes may concentrate large amounts of some metals from the water (Mansour and Sidky, 2002) [34]. And they are one of the most indicative factors in freshwater systems, for the estimation of trace metals pollution and risk potential of human consumption (Barak and Mason, 1990; Papagiannis

*et al.*, 2004) [11, 39]. The aquatic organisms exposed to the heavy metal from the run-off water tend to accumulate in their body but fishes are more commonly affected than other species (Güven *et al.*, 1999; Henry *et al.*, 2004) [24, 62].

Species in relatively low trophic levels are exposed to comparatively lower contamination. On the other hand, fish in the upper food web position are prone to accumulate metals (Terra *et al.*, 2008) [47]. Usually, many toxic compounds affect organisms in nature at the same time, each of them having a specific effect on physical and chemical processes that influence an organism's condition and reactions. Therefore, in order to maintain the quality of food it is important to regularly monitor and evaluate the pollution levels in fish as well as in water reservoirs. Heavy metals cause the mutation of fish inner organs, disturb immune reactions, change blood parameters, reduce an organism's adaptation qualities, vitality, resistance to diseases, loss of fry and degeneration and diminution of valuable varieties of fish are observed as a result of heavy metal pollution (Blasco *et al.*, 1999,) [14]

### **Fish nutrition and dietary nutrients**

The role of dietary nutrients or additives in fish nutrition and/or fish culture has been investigated since the 1980s. As a result, a vast amount of information on fish nutrition has been generated during the past decades and these have been collated in several books including that of Halver and Hardy (2002) [51]. A concrete nutritional categorization similar to that available for farmed terrestrial animals is not possible in the case of farmed fish as there are wide differences in the anatomy and physiology of their digestive systems. The current practice is therefore to use the nutritional facts from a well-studied species for other fishes. Further, the details available are not complete, particularly on the micronutrients of the major farmed species. The requirement of both macronutrients and micronutrients have generally been based on growth and deficiency symptoms (Lim and Webster, 2001; Halver and Hardy, 2002) [63, 51]. Moreover, the requirements are often described based on a controlled laboratory environment in contrast to the stressful and unfavorable environmental conditions in farms, which will certainly demand increased amounts of nutrients to cope with the needs of the defense mechanisms. This has been highlighted in some of the earlier reviews on nutrients and health of fish (Blazer, 1992) [15]. It is important not only to have a sound knowledge on nutrient requirements, but also to formulate feeds optimally using the appropriate ingredients. This has been a challenge for the aquatic feed industry, mainly because the prime ingredients, fishmeal and fish oil, have always been a constraint due to their high demand and pricing, not to mention the increased awareness on their unsustainability. Besides selection of appropriate raw materials, correct formulation and processing ensure that feeds attain physical and chemical properties suitable for the farmed animal. Any imbalance in formulation or the inferior nature of an ingredient may inadvertently impair the health status of the fish and increase their susceptibility to diseases. Proper feeding practices also have a key role in keeping the culture environment clean and reducing the chances of disease outbreaks. Nutrients, essential or non-essential, either singly or in combination, directly or indirectly can influence immune functions and fish health.

### **Immuno-nutrition**

Fish health is dependent on what fish eat or better it depends on what they are fed with in the case of aquaculture. An appropriate feed and feeding regime give optimum health; this conjecture is based on our comprehension of the linkage between the nutrition and immunology. The teleost immune system is well-developed to operate an efficient defense procedure against unfavorable situations in farms that could be either a stress factor or a pathogen invasion. Under such circumstances, biological processes are activated in the fish to create a hostile milieu for the pathogen or to resolve the imbalances resulting from a stressor. The protective mechanisms such as the regulatory cytokines, the antioxidant defenses, acute phase proteins or the cellular responses are initiated to tackle the situation and later terminated upon achieving their objectives. The endogenous sources of nutrients supply the basic requirements for the immune system to realize its functions as well as to protect tissues from collateral damage. Immunonutrition is aimed to provide the animal with additional resources/molecules that would support one or more of the processes broadly outlined above, to finally obtain a higher degree of protection. The existing knowledge on immune responses of fish to a nutrient, a feed ingredient or an additive is skewed towards those based on a few selected substances. As mention on the above, macronutrient deficiencies may inadvertently cause micronutrient imbalances that could compromise the functioning of the immune system of cultured fish. Indeed, artificial feeds should scientifically formulate, optimally processed, and judiciously supplied, considering the specific nutritional needs of the cultivated species and the intensity of cultural operations. Current studies have been reported that dietary immunostimulants (Protein-amino acids, lipid-fatty acids, antioxidants nutrients (vitamins C) and Immunonutrition-additives (herbs)) were significantly influenced the growth and health of blunt snout bream.

### **Dietary protein**

Dietary protein is the most important and expensive macronutrient in prepared feeds and thus it should be carefully formulated to meet the nutritional needs of the cultured organism for achieving efficient production based on age/size of the fish. Dietary protein is the most important factor affecting growth performance of fish and feed cost (Lovell, 1989) [32]. Generally, increasing protein levels in diets can lead to improved fish production, especially for carnivorous fish (Lee *et al.*, 2002) [29]. However, when dietary protein level exceeds the requirement, the fish excretes more ammonia nitrogen into the surrounding environment (Gomez-Montes *et al.*, 2003; Habte-Tsion *et al.*, 2013; Habte-Tsion, 2013) [23, 25], thus reducing the quality of the culture water and retarded fish growth (Monentcham *et al.*, 2009; Abdel-Tawwab *et al.*, 2010; Habte-Tsion *et al.*, 2013; Habte-Tsion, 2013) [36, 1, 25]. On the contrary, a protein-deficient diet results in reduced growth of the fish (Wilson, 2009; Habte-Tsion *et al.*, 2013; Habte-Tsion, 2013) [51, 25]. Moreover, dietary protein provides animals' need for essential amino acids, nitrogen required for the synthesis of non-essential amino acids as well as nitrogen-containing physiologically relevant molecules (Young, 2000) [54]. Insufficient intake of proteins or amino acids ultimately affects the cells' protein content and eventually incapacitates them. In fact, protein level usually reaches 35% in practical diets. High protein content proportionally increases feed cost. Besides, the ammonia



excreted after amino acids are metabolized leads to deterioration of water quality (Kim and Lee, 2005) <sup>[64]</sup>. Therefore, it is important to improve protein utilization for body protein synthesis rather than for energy purposes (Lee *et al.*, 2002) <sup>[29]</sup>. It is well known in carnivorous fish that protein utilization can be improved by partially replacing protein with lipid (Kim and Lee, 2005) <sup>[64]</sup>. Habte-Tsion *et al.* (2013) <sup>[25]</sup> studied “Effects of Dietary Protein Level on Growth Performance, Muscle Composition, Blood Composition, and Digestive Enzyme Activity of Wuchang Bream (*M. amblycephala*) Fry”. The purpose of the study was to determine the dietary protein requirement and effect of dietary protein levels on growth performance, muscle composition, blood composition, and digestive enzyme activity in *M. amblycephala* fry. The study indicated that the dietary protein requirement of *M. amblycephala* fry on the bases of the broken-line regression analysis of weight gain rate (WGR), specific growth rate (SGR) and feed conversion ratio (FCR) against dietary protein level was estimated to be between 32 and 33% (P/E = 20 and 21 mg/KJ, respectively). Within this dietary protein level range; maximum growth, lowest feed conversion ratio, high muscle protein content and optimum physiological performance were obtained in the fish during the 10 weeks feeding trial. However, the increase/ decrease of dietary protein level above/ below this range would result poor growth, feed utilization and physiological performance in *M. amblycephala* fry (Habte-Tsion *et al.*, 2013) <sup>[2]</sup>. Moreover, a dietary protein level between 320 g kg<sup>-1</sup> and 340 g kg<sup>-1</sup> could increase protein synthesis, reduce blood cortisol level and enhance immunity of blunt snout bream fry exposed to temperature stress (Habte-Tsion, 2013) <sup>[25]</sup>.

### Dietary lipid

Lipids are the energy dense macronutrients in feeds that fulfill both energy and the essential fatty acid (EFA) requirements. Investigations conducted during the early part of the twentieth century revealed that dietary fat is essential for the health of warm-blooded animals (Ziboh, 2000) <sup>[66]</sup>; similar observations on cold-blooded animals appeared thereafter (Castell *et al.*, 1972) <sup>[67]</sup>. Like other vertebrates, fish should obtain the precursor fatty acids (linoleic acid, LA, 18:2n-6 in the case n-6 family and linolenic acid, LNA, 18:3n-3 in the case n-3 family) or their highly unsaturated metabolic derivatives from dietary sources. It is known that in mammals, several polyunsaturated or monounsaturated fatty acids are involved in different immune functions, exerting their influence through changes in membrane fluidity, eicosanoid synthesis, formation of lipid peroxides, regulation of gene expression, apoptosis, alteration of antigen presentation, or modulation of intestinal microbiota (Puertollano *et al.*, 2008) <sup>[68]</sup>. In a recent review, Trichet (2010) <sup>[48]</sup> highlighted the significance of fatty acids in building the immunocompetence of farmed fish. Though most of the studies on lipids and fish health have considered the impact of alternate oil sources, there is a dearth of information on the effects of individual fatty acids. Improved immune responses were noted in rainbow trout that were offered purified fatty acids in diets fish that received n-3 PUFA had enhanced phagocytic activity, antibody production and better resistance to virus infection. Li *et al.* (2010) <sup>[65]</sup> studied “Effects of dietary protein and lipid levels in practical diets on growth performance and body composition of blunt snout bream (*M. amblycephala*)

fingerlings”. The experiment was conducted to evaluate the protein sparing potential of lipid for *M. amblycephala*. Besides, to determine whether an interaction of protein and lipid exists for growth performance, protein utilization and body composition of blunt snout bream fingerlings. Finally, Li *et al.* (2010) <sup>[65]</sup> found that an increase in the dietary lipid level from 4% to 7% had a protein-sparing effect, and the diet containing 31% protein with 7% lipid (18.57 MJ/kg diet) is optimal for growth and effective protein utilization of blunt snout bream fingerlings.

### Indispensable amino acids

Ten indispensable amino acids are required by fish, approximately in proportions found in their bodies (Hardy, 2002) <sup>[51]</sup>. Feeds based on good quality fishmeal satisfy the amino acid needs of fish. However, as replacement levels of fishmeal in feeds rise, the content and bioavailability of amino acids from alternative sources may cause concern. The inclusion of plant proteins in feeds of carnivorous fish, particularly from a source that does not appear in the food chain, could lead to amino acid imbalances. Further the endogenous ant nutritional factors present in feedstuffs from plants, which if not eliminated through processing and biotechnological methods, may be toxic to fish (Tacon, 1995) <sup>[46]</sup>. These issues may cause immune dysfunctions even though such effects of ant nutritional factors need convincing evidence. For instance, in gilthead sea bream, *Sparus aurata* the inclusion of plant proteins at over 75% replacement caused liver steatosis, accompanied by a decrease in complement activity (Sitjà-Bobadilla *et al.*, 2005) <sup>[44]</sup>. All fish species studied to date have been shown to require 10 indispensable amino acids (IAA) in their diet for maximum growth (Wilson, 1985) <sup>[50]</sup>. An IAA deficiency may cause reduced growth and poor diet conversion (Wilson and Halver, 1986) <sup>[49]</sup>. Amino acids have a central role in the defense mechanisms since they are involved in the synthesis of an array of proteins such as antibodies and in the control of key immune regulatory pathways. In human and animal nutrition, arginine, glutamine, threonine and cysteine have received greater attention, though several amino acids are involved in sustaining immune competence and disease resistance. Amino acid imbalances as well as their antagonisms could affect nutrient utilization and can have a direct consequence on immune organs and responses. The role of dietary amino acids on fish immune response has hardly received attention with the exception of arginine. For instance, in a study on channel catfish, though the dietary arginine levels did not distinctly correlate with nitric oxide (NO) generation in macrophages, it was pointed out that the plasma arginine may partially regulate the intracellular availability of arginine and sequentially affects the ability of NO production by macrophages (Buentello and Gatlin III, 1999) <sup>[16]</sup>. To formulate amino-acid balanced, cost-effective and eco-friendly practical diet for intensive aqua farming of a candidate fish, species specific understanding of the ten dietary indispensable amino acid needs is essential. Besides, fishmeal substitution in aqua-feed is the imbalanced amino acid profiles, which may cause symptoms of disease or sub-healthy status, especially lack of some functional amino acids. Therefore, many studies were hammered at searching the appropriate rations of amino acids in aqua feeds and investigating the relationship between amino acids in the fish nutrition research (Alam *et al.*, 2005) <sup>[8]</sup>. Some

functional amino acids are important regulators of key metabolism pathways that are necessary for maintenance, growth, reproduction, and immunity in organisms, therefore maximizing efficiency of food utilization, enhancing protein accretion, reducing adiposity, and improving health (Wu, 2009) [52].

### Dietary methionine

Methionine is usually the first limiting amino acid in some plant protein sources to replace fish meal (Sardar *et al.*, 2009). Methionine deficiency results in slow growth and reduced feed efficiency in Atlantic salmon (*Salmo salar* L.) (Opstvedt *et al.*, 2003) [37], juvenile cobia (*Rachycentron canadum*) (Zhou *et al.*, 2006) [69], juvenile rockfish (*Sebastes schlegelii*) (Yan *et al.*, 2007) [53], juvenile black sea bream (*Sparus macrocephalus*), Indian major carp fry (*Cirrhinus mrigala*). The presence of the non-essential amino acid cystine in the diet represents a sparing effect in that it reduces the requirement for methionine (Goff *et al.*, 2004) [72]. In this case, cystine can only be synthesized from methionine so if cystine is present in the diet, methionine may be spared from cystine synthesis (Goff *et al.*, 2004) [72]. Therefore, it is important to consider the dietary cystine content to quantify the methionine requirement of the cultured species for maximum growth and efficient feed utilization. Nutrient substance is the foundation of immune organ development and contribution; deficiencies of nutrient substance result in immunity descent (Susanna *et al.*, 2005) [45]. Methionine is an essential dietary nutrient for normal growth of fish (Ahmed *et al.*, 2003) [6] and normal growth is often associated with immune response (Meeker *et al.*, 1987) [70]. Liao *et al.* (2013) [30] in press studied "Dietary methionine requirement of juvenile blunt snout bream (*M. amblycephala*) at a constant dietary cystine level". A 9-week feeding trial was conducted to investigate the dietary methionine requirement of juvenile *M. amblycephala* at a constant dietary cystine level. Liao *et al.* (2013) [30] in press reported that analysis of dose response with broken-line regression on the basis of specific growth rate (SGR) and protein productive value (PPV) versus dietary methionine level, the optimum dietary methionine requirement of juvenile *M. amblycephala* was estimated to be between 8.4g kg-1 and 8.5g kg-1 of the diet (24.71g kg-1 and 25g kg-1 of protein), respectively.

### Conclusion

Marginal deficiencies and nutrient imbalances would impact an optimal growth performance, physiological and biochemical responses (including immune and metabolic response). Very few attempts have been directed to examine the underlying mechanisms as influenced by a nutrient or its interactions with other nutrients/additives. The interaction between nutrition and metabolic and immune system of fish involves myriad physiological and biochemical processes occurring in different organs under different levels of regulation. Since feeds are a complex mixture that delivers various nutrients and beneficial substances to support multiple physiological responses (Panserat and Kaushik, 2010) [71], therefore an integrative approach is necessary to analyze them.

Moreover, aquatic feeds have evolved over the last half a century and the feed industry is aiming to optimize the quality of their products and to develop alternate ingredients that meet the physiological requirements of the animal,

besides having minimal environmental impact. As functional ingredients are being increasingly used to add value to feed, vigilance should be exercised on those that are labeled immune-modulators. Extensive and rigorous research is still needed to validate the benefits of such ingredients to each target fish species. As more tools and markers become available for the major fish species, it would be possible to have a fair assessment on the benefits of the ingredients. Through this knowledge it should be possible to improve growth performance, enhance immune system and implement preventive health care strategies based on nutritional principles for aquaculture operations, besides having minimal environmental impact, particularly for blunt snout bream (*M. amblycephala*) culture.

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