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The Role of Humic Acids in Aquaculture: A Review

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Review Article

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Abstract

Water conditions that offer balanced values are ideal environments for fish. Sudden or fluctuating changes in water parameters such as pH, oxygen, and especially temperature interact and destabilize other relevant parameters and cause stress to the fish. When fish encounter such stressful situations, their defense mechanisms do not work properly, which makes them easily susceptible to pathogenic microorganisms and secondary infections. Because such an impact that affects the physical condition, physiological state, and behavioral status of fish, deteriorates general welfare. Various antibiotics and similar medications have been used in controlling or eliminating stress-related diseases to overcome such adverse conditions. However, despite the proper use of these medications, various measures have been taken and the use of some antibiotics has been limited or stopped, as the efficiency of treatments decreased over time, and some antibiotics caused antibiotic-resistant bacteria in fish. As an alternative approach, the use of various natural or synthetic substances has become popular and widespread today in commercial and experimental applications to take preventive measures against pathogens and provide a solid background to fish welfare. Humic acids, one of the substances belonging to the natural environment of fish are among the natural and organic components used for this purpose. Since humic substances are part of the natural habitat of fish, the defense mechanism of fish does not recognize this foreign matter as a threat, so that the fish maintain its usual activities without any reaction. Humic acids that generally known to have positive effects on many organisms, also enhance some vital parameters of fish such as healing of lesions, inhibiting pathogen load, boosting the immune system, and promoting growth when used as a feed additive. Moreover, humic acids have been reported to reduce inverse effects of stress on physiological and histopathological parameters of fish, toxic effects of heavy metals and organic pollutants, and to repair the disease-based injuries faster. In this review, the latest knowledge on the effects of humic substances on two of the primary problems of aquaculture systems, which are fish diseases and relevant pathogens, is discussed.

Keywords: Humic Acid; Fish Health; Fish Parasites; *Amyloodinium*; Aquaculture

Introduction

Although aquaculture has not had high growth rates as in the 1980s and 1990s since 2000, it is still growing faster than other food production sectors; and it is stated that this sector plays an important role in improving food safety and human nutrition according to the 2030 hunger struggle agenda of FAO [1].

Parasitic factors that cause fish diseases are among the most common problems in aquaculture applications. In this sense, fish parasites are one of the primary reasons of economic losses, in densely stocked environments such as aquaculture facilities [2]. Because, adverse conditions such

as poor water quality, malnutrition, and stress make it easier to increase and spread parasites in these intensive systems [3]. The use of antibiotics and chemotherapeutics developed to struggle with such situation are also unsustainable due to their undesirable effects on the environment [4]. Therefore, alternative approaches to prophylactic stimulation of the defense system of the fish have become more necessary [5]. Dietary supplements, medicinal and aromatic plants, non-specific immunostimulants, vaccines, probiotics, and prebiotics are some of these alternative methods for the prevention and control of diseases [6].

The use of alternative feed additives in animal husbandry has been increasing in recent years as alternative additives have the ability to enhance the growth and reduce the risk of diseases. Besides, their contribution to production and ecology by lowering the feed expenditures are also among the reasons for the preference of these substances [7]. Therefore, it has been stated in studies that humate compounds can be used as feed additives due to their growth-promoting properties [8].

Although humic acids are widely studied in agriculture and terrestrial animals, a limited number of studies have been carried out on aquaculture. In particular, there are almost no studies encountered on marine fish. Therefore, in this study some literature was reviewed to enlighten the current status of the use of humic acid on fish, to provide a source for future studies on its positive and negative effects, and to refer to its use in aquaculture.

What is Humic Acid?

Humic substances are the main constituents of natural organic substances that arise from the physical, chemical, and microbiological transformation of biomolecules. These substances are found in debris and soil. It is stated that approximately 95% of dissolved organic matter in water is composed of humic substances [9-12], and those substances constitute nitrogen (N) and carbon (C) reserves [13,14].

Humic substances; are divided into three major fractions which are; humic acid, fulvic acid, and humin [9,15]. The distinction in these substances is resulting from the differences in pH levels and molecular weights [9,16]. While humic acids dissolve at higher pH, they do not dissolve when pH level is below two. It is also stated in the literature that fulvic acids dissolve at all pH values, whereas humine does not dissolve at any value [13,14]. Furthermore, humic acids differ from substances such as fulvic acid and huminic in that they dissolve in alkali environments, partially dissolve in water, and are insoluble in acidic environments [17].

Benefits of Humic Acid

Humic acids prevent the increment of pathogen presence by optimizing the pH in the digestive tract, inhibit the adherence of viral particles to the cell by antiviral action, promote the utilization of calcium and trace elements, and reduce the toxic effect of heavy metals [18-20]. It is also reported that those are also effective in recovering the damages arising from bacterial and parasitic sources, especially from fungal pathogens [5]. Humic acids are known as important antioxidants due to their powerful properties on free radical scavenging activities [4]. They are also expressed to stabilize the intestinal flora, thus enable efficient use of nutrients in animal feed [21]. Considering humic acids are part of the natural environment of the fish and the defense systems of fish are accustomed to these natural xenobiotics, those can be ideal candidates for use in aquaculture [9].

As well as regulating immunity and blood sugar [18,22], the humic substances are stated to have antiviral, antimicrobial, antibacterial, antioxidant, antitumor, anti-inflammatory, anticlastogenic, antiallergic, antitoxic, antiulcerogenic, antirheumatismal, antiangiogenic, antipyretic, antimutagenic, and analgesic properties. Taking into account the abovementioned features, together with their anti-HIV and immuno-stimulatory properties, it seems viable to use the humic acids as feed additives in the diets of aquatic organisms [4]. No allergic, anaphylactogenic, teratogenic, embryotoxic, or carcinogenic effects are reported as humic substances are used in recommended doses as well as they do not have harmful effects on humans and animals [12,23,24].

The Use of Humic Acids in Aquaculture

Humic acids can be used as alternative natural feed additives due to their positive effects on the parameters such as larvae and egg yield, growth and feed intake, disease resistance, survival rate, suppression of secondary infections, detoxification of harmful metals and chemicals in water, and rapid recovery from ectoparasites [18,21,25]. Humic acids; can increase the physiological condition of the organisms, reduce physiological and histological damages caused by stress, and quickly repair damages caused by fish pathogens. Since humic acids have different advantages over stress, their ecophysiological relationship in aquaculture is emphasized [5].

In the present work, some of the studies investigating the effects of humic compounds on aquatic organisms were listed (Table 1) and reviewed briefly.

Species	Treatment	Pathogen	Effect	Reference
Carassius aurata	Sodium	-	-Reduced mortality rate	29
Cyprinus carpio	humate		-Improvement in skin, fins and gills	
Cyprinus carpio	Silver humate	-	-Bactericidal activity on *Pseudomonas* and *Aeromonas* cultures	23
Ictalurus punctatus	Potassium humate		-No effect on the intestinal microbial flora	
Cyprinus carpio	Humic acid	*Aeromonas hydrophila*	increased weight gain and specific growth -Reduced mortality	26
Cyprinus carpio	Humus extract	*Aeromonas salmonicida*	-Reduced mortality -Increased survival rate -Improvement in skin lesions	30
Cyprinus carpio	Humic acid	-	-Promoted growth and feed utilization -Detrimental effect on gill, liver and kidney	33
Dicentrarchus labrax	Humic acid	*Amyloodinium* sp.	-Increased hemoglobin, hematocrit and NBT activity -Improvement in gill filaments	18
Litopenaeus vannamei	Fulvic acid	-	-Increased weight ratio -Increased SOD, CAT and GSH values in hepatopancreas and hemolymph -Decreased HSP70 levels	37
Mugil cephalus O. niloticus	Humate compounds	*Saprolegniosis*	-Increased survival rate	32
Oncorhynchus mykiss	Humic acid	*Yersinia ruckeri*	-Increased activities of pepsin, trypsin and lipase -Increased survival rates	4
Oncorhynchus mykiss	Humic substance	*Yersinia ruckeri*	-Positive effects on immune and serum biochemical parameters -Increased survival rates	35
Oreochromis niloticus	Humic acid	-	-Decreased growth performance -Reduced occurrence of degeneration and vacuolization	38
Oreochromis niloticus	Sodium humate	*Aeromonas hydrophila*	-Increased survival rate -Increase in hepatosomatic index (HIS) with increasing doses	40
Paramisgurnus dabryanus	Fulvic acid	*Serratia, Acinetobacter, Aeromonas Edwardsiella*	-Increase in the number of beneficial *Lactobacillus* sp. -Increased growth -Enhancement on the wellness of intestine	28
Plecoglossus altivelis	Humus extract	*Flavobacterium psychrophilum*	-Suppression of mortality -Improvement in skin lesions	31
Poecilia reticulata	Humic substance	*Gyrodactylus turnbulli Dactylogyrus sp.*	-Reduced ratio of infected fish and parasites per fish	34
Procambarus clarkia	Fulvic acid	*Aeromonas hydrophila*	-Increased body weight gain and survival rate -Increased protease, lipase and amylase activities	36
Rhamdia quelen	Humic acid	-	-Reduced weight gain and feed intake	27
Tambaqui juveniles	Humic acid	*Salmonella hadar*	-Reduced shedding of *S. hadar*	39

Table 1: List of some studies investigating the effects of humic compounds on aquatic animals.

The effects of different humic substances in carp (*Cyprinus carpio*) and channel catfish (*Ictalurus punctatus*) were investigated [22]. According to the results, potassium humate did not show a significant effect on the intestinal microbial flora of fish. The antimicrobial effect of *Pseudomonas* and *Aeromonas* cultures and ten bacterial strains isolated from fish has been investigated and silver humate has been reported to be the most effective bactericide.

In a study with common carp (*Cyprinus carpio*), four different diets were prepared using humic acid, which incorporated into diets at 0.0%, 0.4%, 0.8%, and 1.0% [26]. Fish were fed with those diets for 45 days; the growth performance, immune response, and disease resistance against *Aeromonas hydrophila* were evaluated at the end of the study. It was stated that the weight gain, specific growth rate, and lysozyme activity were found to be significantly higher in the 0.4% and 1% humic acid supplemented groups compared to the control group. According to challenge test results, the highest mortality rate was reported to be in the control group while the lowest mortality was in the group fed with 0.8% and 1% humic acid supplemented feeds.

The effects of dietary humic acid on the growth performance of silver catfish (*Rhamdia quelen*) juveniles were tested at different pH levels (5.5 and 6.5) [27]. For this purpose four diets were prepared to contain humic acid at 0.0, 10.0, 25.0, and 50.0 mg L-1, and fed the fish for 40 days. At the end of the trial, the authors reported humic acid to be highly detrimental in the tested concentrations for juvenile silver catfish growth as it lowers the weight gain and the feed intake of fish.

In an experiment conducted to determine the effects of fulvic acid on growth performance and intestinal health of juvenile loach *Paramisgurnus dabryanus*, fish were fed with fulvic acid supplemented feeds which are incorporated into the diet at 0.0%, 0.5%, 1%, 1.5%, and 2% for 60 days [28]. It was stated that an elevating effect of fulvic acid was achieved on growth rates of fish with increasing doses and the maximum weight gain and specific growth rates were observed to be in the fish fed with the 1.5% fulvic acid supplemented diet. It was also reported that the fulvic acid supplementation decreased the presence of pathogenic bacteria *Serratia, Acinetobacter, Aeromonas,* and *Edwardsiella*, whereas an increase in the abundance of beneficial *Lactobacillus* was achieved in the intestine. The authors concluded that the fulvic acid might be an immune enhancer on loaches considering it promotes growth performance and enhances intestinal wellness of juvenile loach.

The effects of sodium humate on pathogen-exposed goldfish (*Carassius auratus*) and carp (*Cyprinus carpio*) was investigated [29]. It was stated at the end of the experiment that the sodium humate treatment reduced the mortality rate of infected fish and the fish treated with sodium humate showed significant improvement in skin, fins, and gills.

Six different diets were formulated to contain %0.0, 0.2%, 1.0%, 5.0%, 10.0%, and 20.0% humus extract for common carp *Cyprinus carpio* fry [30]. The fish were infected with *Aeromonas salmonicida* and fed with experimental feeds 30 days prior to the bacterial challenge and 22 days immediately after the challenge. At the end of the experiment, it was reported that the mortality and development of skin lesions such as ulcer and hemorrhages were significantly suppressed in the groups fed with the diets containing 1.0%, 5.0%, and 10.0% humus extract whereas the survival rates were higher in the fish fed with 5.0% and 10.0% humus extract supplemented feed. The authors have concluded that the humus extract treatment has been found to be effective to prevent *A. salmonicida* infection.

The preventive effects of orally administered humus extract on Ayu Fish (*Plecoglossus altivelis*) against the bacteria *Flavobacterium psychrophilum* was investigated [31]. For this purpose, the humus extract was incorporated into the diet at %0.0, 1.0%, 5.0%, 10.0%, and fed to fish for 30 days prior to the challenge and 21 days afterward. The feeds containing humus extract at 1.0%, 5.0%, 10.0% significantly suppressed the death rates, hemorrhages, and other skin lesions of fish challenged with *F. psychrophilum*. The mechanism of action on the protective feature of the extract was not expressed as the direct killing of bacteria or antibiotic activity, considering no distinct reduction in the bacterial count was achieved. It was reported at the end of the study that the humus extract was an effective feed additive against cold water disease.

In another study the effects of humate compounds on the control of Saprolegniosis, which leads to significant economic losses in fish farming were tested on Nile tilapia [32]. In the trial, fish were subjected to 5%, 10%, and 15% humate, and it was reported that the treatment of humate at 5% increased survival rates of the fish.

Dietary humic acid was incorporated into diets of common carp (*Cyprinus carpio*) whether it has an impact on growth performance and histopathology (gill, liver, and kidney) of fish [33]. At the end of the study, it was reported that despite dietary supplementation of humic acid (180 and 360 mg.kg-1) slightly promoted growth and feed utilization of fish; its detrimental effect on the tissues was found to be more evident.

Five different diets were supplemented with humic acid (HA) at 0.00%, 0.25%, 0.50%, 0.75%, and 1.50% and fed to sea bass *Dicentrarchus labrax* for 15 days to investigate the effects of humic acid on some immunological and

histological parameters of fish [18]. For this purpose the parasite *Amyloodinium* sp. was introduced to the culture water. It was reported that the hemoglobin, hematocrit levels, and NBT activity increased in the group of fish fed with 0.50% HA supplemented feed. It was also stated that the serum NBT and LA significantly decreased in the fish infested with *Amyloodinium* sp. while MPO activity did not change. According to histopathological results, 0.50% HA supplemented feed caused an improvement in gill filaments compared to other experimental groups against *Amyloodinium* sp. infestation (Figure 1) [18]. It was concluded that the HA has positive effects on some health-promoting parameters of *Dicentrarchus labrax* juveniles challenged with *Amyloodinium* sp.

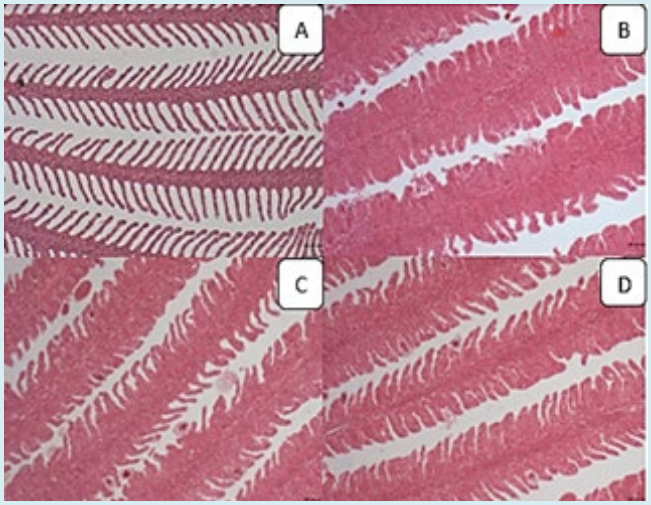

Figure 1: *Dicentrarchus labrax* gill: (A) control treatment, (B) 0.25% HA treatment; (C) 0.50% HA treatment, (D) 0.75% HA treatment. Scale bar 100 µm, H&E. Abbreviations: HA=humic acid.

In a different study, humic-rich culture water and synthetic humic supplemented feed both reduced the ratio of infected fish (*Poecilia reticulata*) when challenged with parasites *Gyrodactylus turnbulli* and *Dactylogyrus* sp [34]. The authors have concluded that the humic-rich water and sludge derived from a recirculating aquaculture system has the ability to protect fish from ectoparasites, whereas humic compounds do not exhibit a toxic effect on the parasites used.

The effects of dietary humic acid on growth performance, haemato-immunological, and physiological parameters of rainbow trout juveniles were investigated in another experiment [4], in which, the authors fed the experimental fish with humic acid incorporated diets at 0.0%, 0.3%, 0.6%, and 1.2% for 60 days. At the end of the experiment, it was stated that the growth performance and haematological parameters of fish were not affected by humic acid supplemented diets whereas 0.6% humic acid supplementation significantly increased the activities of pepsin, trypsin, and lipase. After 20 days of exposure to the bacteria *Yersinia ruckeri*, to test the resistance of fish, significantly higher survival rates were reported to be in the 0.6% humic acid group. It was concluded that the dietary humic acid might be a good alternative to antibiotic treatments of *Yersinia ruckeri* infected rainbow trout (*Oncorhynchus mykiss*).

The effects of humic substances containing products FARMARIN®XP and INFISH-AQUA® on growth performances, immune responses, digestive enzyme activities, and disease resistance of juvenile rainbow trout challenged with the bacteria *Yersinia ruckeri* was investigated [35]. The diets were supplemented with FARMARIN®XP at 0.0%, 0.1%, 0.2%, 0.4% while a fifth experimental group was additionally formed and treated with INFISH-AQUA® (sulphadiazine 20% + trimethoprim 4%). The diets were given to the fish for 60 days and mortalities were recorded after the challenge test. It has been stated in the study that the dietary FARMARIN®XP may exhibit positive effects on immune and serum biochemical parameters of rainbow trout juveniles, increase the survival rates against *Y. ruckeri*, and therefore can be used as a non-antibiotic additive to prevent Yersiniosis disease.

In the fulvic acid study on red swamp crayfish (*Procambarus clarkia*), body weight gain and survival rate of crayfish were found to be higher in treatment groups according to the control group [36]. While protease, lipase, and amylase activities were significantly increased in the mid-dose group, the high dose did not affect these rates. As a result of the challenge of crayfish with *Aeromonas hydrophila*, the survival rate in all treatment groups was found to be higher than the control.

Fulvic acid incorporated diets were fed to Pacific white shrimp (*Litopenaeus vannamei*) at varying levels in a different study [37]. It was reported that the final wet weights were higher in moderate levels of fulvic acid (0.9% and 1.2%) than the control, whereas no statistically significant differences were observed at lower levels. Moreover, the HSP70 value in hemolymph and hepatopancreas reported to be decreased as the level of fulvic acid increased. According to the antioxidant parameters, SOD, CAT, and GSH values in the hepatopancreas and hemolymph was reported to be significantly higher in the treatment groups compared to the control.

The effects of humic acid and *Yucca schidigera* extract were examined whether they promote the quality of culture water under low water-exchange [38]. It was stated that the humic acid has a considerable effect on the availability

of phosphorus compounds to benefit, and both substances were reported to reduce the effect of ammonia stress.

In a study conducted to evaluate possible control strategies against *Salmonella hadar* in local tambaqui farms of Brazil, fish were fed with humic acid and probiotic supplemented diets [39]. It was reported at the end of the experiment that after 20 days feeding with experimental diets caused a 50% reduction in *Salmonella hadar* shedding in guts.

Another study was carried out on *Oreochromis niloticus* in which dietary sodium humate was fed to fish [40]. Increasing levels of sodium humate were reported to possess a linear relationship with the hepatosomatic index (HIS) at the end of the study. No statistically significant differences in feed intake, protein efficiency ratio, and viscerosomatic index (VSI) were noticed, and the survival rates of treatment groups were reported to be higher than the control when challenged with *Aeromonas hydrophila*.

Conclusion

When humic acid used in appropriate levels

- It might be a better alternative to chemical treatments
- It may improve growth performance.
- It may reduce mortality.
- It may prevent parasites from penetrating and spreading to the body.
- It may repair the damages caused by diseases and may reduce their physical effects.
- It may enhance immunity and reduce the effects of stressors.
- Its side effects might be less than chemicals.

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