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Review Article

Potential Benefits of Humic Substances in Aquaculture

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Abstract

Balanced water conditions are crucial for fish health. Sudden changes in pH, oxygen levels, and temperature can stress fish, making them vulnerable to diseases. This stress weakens their defense mechanisms and can lead to infections. Traditional antibiotics have been used to combat these stress-related diseases, but their effectiveness has waned over time, and they can promote antibiotic-resistant bacteria in fish. As an alternative, natural and synthetic substances like humic substances are gaining popularity in commercial and experimental fish farming. Humic acids, part of fish's natural environment, do not trigger a defensive response. Instead, they offer several benefits, including healing wounds, reducing pathogens, boosting immunity, and promoting growth when used in fish feed. Additionally, humic acids mitigate the adverse effects of stress, heavy metals, and pollutants on fish physiology, histopathology, and disease recovery. This review explores how humic substances address primary challenges in aquaculture.

Keywords: Aqacuture; Humic acids; Fluvic acids; Antibiotics; Antistress

Introduction

Aquaculture, the fastest-growing food segment in the global economy, faces growing progressive pressure regarding finfish and shellfish-intensive farming. These intensive farming practices resulted in diseases accounting for up to 50% of production loss in aquaculture [1], posing a significant obstacle in the sector. These estimated economic losses due to diseases exceed 6 billion US dollars annually [1]. In finfish aquaculture alone, losses range from 1.05 to 9.58 billion US dollars annually [2]. Bacterial infections are the most common (54.9%), followed by viral (22.6%), parasitic (19.4%), and fungal (3.1%) diseases affecting aquatic animals during production [3,4]. Therefore, aquaculture often uses antibiotics to treat and prevent these diseases. Commonly used antibiotics worldwide include tetracycline, oxytetracycline, oxolinic acid, flumequine, sarafloxacin, enrofloxacin, amoxicillin, erythromycin, sulfadimethoxine, ormetoprim, and florfenicol [5]. Each country has regulations concerning antibiotic approval, usage practices, and residue limits in aquaculture products. However, these synthetic antibiotics have faced criticism for residues, bacterial resistance, and toxicity, leading many countries to restrict their use. As an alternative to antibiotics, humic substances such as fluvic acid, humic acid, and humin offer promising natural immunostimulants. Hence, this article aims to review existing literature that explores the advantages of using humic substances in aquaculture.

Humic substances

Humic substances form through plant and animal matter's chemical and biological humification, facilitated by microorganisms. This process gives rise to a transformation product known as humus. Humic substances exhibit chemical reactivity and resistance to microbial reactions, leading to beneficial effects in soils and plants [6]. Humic acids, a significant fraction of humic substances, possess long-term stability and amphiphilic properties, enabling them to form complex cations [7]. They contain approximately 60% organic carbon (C), which plays a crucial role in the growth of soil microorganisms [8]. Humic acids also contain nitrogen (N), oxygen (O), hydrogen (H), and sulfur (S). It serves as the biological core of humus and is primarily composed of phenols, carbohydrates, and amino acids.

Moreover, humic substances, being natural organic compounds, are found in natural water systems and are compatible with various aquatic life forms. Adding humic substances to rearing water can modulate fish-associated bacterial communities, potentially reducing the presence of harmful pathogens. While scientific literature primarily focuses on livestock, particularly egg production, and traits in laying hens, studies on using humic acid in aquaculture are limited.

Benefits of the use of humic substances in aquaculture

In aquaculture, water pollution commonly occurs through eutrophication and heavy metal contamination. Soil pollution also includes soil acidification, salinization, and heavy metal pollution. Chemical fertilizer leaching is a major contributor to nutrient enrichment, but combining humic acid with chemical fertilizer can reduce the use of chemical fertilizer by 30% to over 50%. Humic substances have proven beneficial in aquaculture systems, improving water quality by reducing total ammonia and nitrate nitrogen levels. They also alter microbial communities and strengthen nitrification, improving nitrogen utilization efficiency. In addition to aquaculture, humic substances are used in various fields such as ecology, soil remediation, restoration, drilling operations, and pond preparations. They contain humic acids, humates, polysaccharides, peptides, amino acids, fulvic and hematogenic acids, and micro and macro elements like potassium and phosphorus [9].

Furthermore, the humic substance's proteins, vitamins, and enzymes are easily accessible to living organisms, can influence cell metabolic processes, enhance immune function, and exhibit other vital properties [10]. Overall, they serve as natural growth promoters and productivity stimulants without harming the health of fish and aquatic plants. Unlike antibiotics, humic substances leave no undesirable residues in the water. Therefore, adding humic acids as feed additives in aquaculture can effectively enhance the immunity of cultured animals, reducing reliance on antibiotics. Additionally, humic substances improve the physiological condition of organisms, reduce histological damage caused by stress, and facilitate rapid repair of damage caused by fish pathogens.

Growth and immune responses

In their 1996 study, Schreckenbach., *et al*. highlighted the positive impact of synthetic humic acids (HS1500) on the overall health of fish. Their findings revealed a range of beneficial effects: (1) An enhancement of physiological processes, (2) an amplification of immune response, and (3) a hindrance to pathogens through decreased adherence and penetration capabilities. Firstly, humic substances support metabolism and strengthens the mucosal barrier, enhancing the defense system against pathogens. Secondly, humic substances decreases pathogen viability, lowering its pathogenicity. According to Wiegand., *et al*. (2004), exposure to humic substances triggers non-specific defense responses in invertebrates and fish. These reactions include the expression of chaperones (hsp70) and increased activity of biotransformation enzymes. Although these reactions consume energy, they simultaneously stimulate metabolism. Moreover, these studies suggested that stimulating metabolism and defense mechanisms, along with a reduced energy expenditure for pathogen defense, may result in higher net growth of fish.

Treatment to fish diseases/Challenge studies

The potential application of humic substances in treating fish diseases has been relatively unexplored by researchers. However, studies conducted on fish eggs and larvae have shown promising results. [11] and [13] conducted studies that revealed humic substances enhance defense mechanisms against pathogenic fungi (Saprolegnia) and various microorganisms. Incorporating a humic substance into the water used for fish culture decreased the attachment and penetration of pathogens into the fish skin [11].

[14] discovered that humic substances suppressed inflammatory responses in goldfish gills and fins affected by saprolegniasis. Another study by [15] examined the impact of humate compounds on controlling Saprolegniosis, a condition causing substantial economic losses in fish farming. The study specifically tested Nile tilapia and found that treating the fish with a 5% humate increased survival rates.

When common carp were exposed to *Aeromonas salmonicida*, their infection rates significantly decreased after oral administration of humic-rich sludge from a recirculating aquaculture system, synthetic humic acid, or an extract derived from Leonardite [16]. In 2007, [14] demonstrated the protective effect of humus extract against atypical *Aeromonas salmonicida* infection, specifically ulcer disease, in common carp.

Research on *Oreochromis niloticus* involved feeding the fish sodium humate [17]. The study found a direct correlation between higher concentrations of sodium humate and the hepatosomatic index (HSI). Furthermore, a sodium humate concentration of 0.27%-0.38% improved growth and higher survival rates when exposed to *Aeromonas hydrophila*.

A study conducted on red swamp crayfish (*Procambarus clarkia*) investigated the effects of fulvic acid [18]. It was observed that all treatment groups (0.1-1 g/kg of diet) exhibited higher body weight gain and survival rates compared to the control group. In the mid-dose group (0.5g/kg of diet), protease, lipase, and amylase activities were significantly increased, while the high dose (1g/kg of diet) did not impact these rates. Additionally, when the crayfish were challenged with *Aeromonas hydrophila*, all treatment groups had a higher survival rate.

Furthermore, when fish were exposed to fulvic acid, there was an increase in mucosal lysozyme activity. [19] reported that the inclusion of FARMARIN®XP in the diet, a product containing humic substances, could potentially have beneficial effects on immune and serum biochemical parameters in rainbow trout juveniles. The study also found that it increased survival rates against *Yersinia ruckeri*, suggesting it could be a non-antibiotic additive to prevent Yersiniosis disease.

In a study by [20], adding 1% humus extract to the feed of ayu fish (*Plecoglossus altivelis*) resulted in reduced development of skin lesions during *Flavobacterium psychrophilum* infection. The high concentration of humus extract created an acidic condition that effectively killed *F. psychrophilum*. According to Soytaş (2015), a 0.50% humic acid-supplemented feed had positive effects on some health-promoting parameters of *Dicentrarchus labrax* juveniles [21].

Lifespan

When Daphnia *Magna straus* females were fed a high-quality diet (*Pseudokirchneriella subcapitata*) and exposed to a commercial humic substance preparation (Humin Feed®), their offspring production decreased, even at concentrations as low as 0.5 mg/L DOC and their lifespan unchanged [22]. However, when provided with a low-quality diet (yeast), lifespan and offspring production increased for this species [22]. Another study demonstrated that humic substances had a gender-specific effect on the lifespan of *Daphnia magna*, increasing it for males while decreasing it for females [23]. Steinberg., *et al*. (2010) also conducted a comparative study showing that two closely related species, *Moina macrocopa* and *Moina micrura*, responded differently to exposure to Humin Feed® [22]. *Moina micrura* exhibited an increased lifespan and decreased offspring production, while no effects were observed in *Moina macrocopa*.

Antistress

Few studies, viewed humic substances as directly inert to aquatic organisms and these had indirect effects such as light attenuation, binding of metals and organic pollutants, acidification, and serving as carbon sources for bacterial and photochemical degradation [24-26]. However, some studies proved that humic substances, or some of their building blocks, are taken up by organisms and interact with biochemical constituents and signaling pathways [26,27]. Consequently, humic subtances exposure causes organisms to exhibit oxidative stress symptoms, such as the accumulation of reactive oxygen species, reduced antioxidant capacity (even at low humic substances concentrations), membrane lipid peroxidation, and increased activity of antioxidant enzymes [22,28,29]. Steinberg., *et al*. (2003) coined the term "natural xenobiotics" for humic substances, highlighting their interactions with biota and comparing them to secondary plant compounds. However, unlike secondary plant compounds, humic substances is not synthesized to defend against herbivory but to challenge any exposed organism. Further studies have shown that mild chemical stress induced by humic substances or their building blocks can extend lifespan and enhance resistance to multiple stressors such as high temperatures and intensive handling [30-33]. On the other hand, increasing salinity and concentrations of heavy metals or other pollutants have detrimental effects on lifespan, somatic and population growth rates and may delay sexual maturity in aquatic organisms [34,35].

Meinelt., *et al*. (2004) conducted a study that revealed positive effects on swordtail fish (*Xiphophorus helleri*) when exposed to an artificial humic substance in their water [30]. For 21 weeks, the substance, with a concentration ranging from 5 to 180 mg/L DOC, increased vigor, growth, lifespan, and stress resistance. Similarly, [36] study found that a natural humic substance from a Brazilian coastal lagoon reduced osmotic stress in the cladoceran *Moina macrocopa* when present at 10 mg/L DOC [36]. This stress resistance was inherited by the subsequent generation, likely through DNA methylation, as [31] suggested. Despite causing some stress symptoms, exposure to natural humic substances alleviated stress caused by elevated salinity in *M. macrocopa*, even across generations.

Humic acids as a feed binder

Humic acid is a cost-effective substance derived from various sources, known for its powerful adsorption force on the surface of iron ore. The strength of this adsorption force depends on factors like the concentration of humic acid, solution pH, and the presence of metal cations. A modified humic acid pellet binder (MHA) with excellent adhesion and viscosity properties has been developed by separating and purifying solid wastes such as lignite and weathered coal and subjecting them to chemical modification. This MHA has been successfully employed in the production of iron ore pellets. Industrial tests have demonstrated that MHA significantly enhances the strength of green and dry pellets, reduces residue after high-temperature roasting, minimizes metallurgical pollution, and produces highly durable fired pellets. Moreover, MHA can be used as a partial or complete substitute for bentonite.

Conclusion

Aquaculture production is currently adversely impacted by stress induced by pathogens and unfavorable environmental conditions. This review highlighted the potential of humic substances to enhance the physiological well-being of organisms, mitigate physiological and histological damage caused by stress, and facilitate rapid recovery from fish pathogens. However, some important aquaculture species still need to thoroughly investigate the beneficial effects of humic substances and their optimal dosage. Additionally, there is a need to explore efficient sources of humic substances and determine the most effective process for incorporating them into fish feed.

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