**DOI:** https://doi.org/10.54083/978-81-980121-3-5_09

*Chapter* 9

# Ferulic Acid: A Key Player in Enhancing Growth and Immunity in Fish

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## Abstract

Aquaculture has emerged as a vital solution to meet the global demand for animal protein, offering a highly efficient means of producing easily digestible and nutritious protein. Increased fish demand intensified aquaculture systems, which face several challenges, including stress, environmental degradation frequent disease outbreaks. To mitigate disease risks, antibiotics synthetic chemicals are often overused in aquaculture, contributing to the emergence of antimicrobial resistance (AMR) and long-term soil and water quality degradation. A sustainable approach to address these challenges is needed, aligning with the One Health framework by reducing reliance on antibiotics and chemicals. Plant-based bioactive compounds are gaining attention due to their potential to support the health and growth of aquatic species. Ferulic acid (FA), a plant phenolic compound, has shown considerable promise. This compound possesses antioxidant, growth-promoting, anti-inflammatory, wound-healing antimicrobial properties, making it a suitable alternative for enhancing aquaculture productivity. Research has demonstrated that FA promotes growth, alleviates stress, enhances metabolism supports muscle growth in various freshwater and brackish-water species, including Common carp, Trout, GIFT, Nile tilapia, White leg prawn, hybrid grouper, *etc.* Additionally, it boosts antioxidant potential, helping aquatic species combat oxidative stress in intensified farming systems. However, while the initial findings are promising, further scientific studies are essential to standardize appropriate dosages, understand the underlying mechanisms evaluate potential negative effects. Through comprehensive research, the full potential of Ferulic acid can be harnessed, contributing to sustainable disease management and improved health outcomes in aquaculture.

***Keywords*** Antioxidant, Ferulic acid, Plant based nutraceuticals, Phenolic compound, Sustainability

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***How to cite:***
Bhadra, S., Aklakur, M., Debroy, S., Roy, U., 2025. Ferulic acid: A key player in enhancing growth and immunity in fish. In: *Aquaculture Reimagined: Modern Approaches to Sustainable Fish Farming.* (Eds.) Saini, V.P., Paul, T., Singh, A.K., Biswal, A. and Samanta, R. Biotica Publications, India. pp. 112-125. DOI: https://doi.org/10.54083/978-81-980121-3-5_09.

## 1. Introduction

By 2050, the world's population is expected to have grown to 9.8 billon, at a pace of 1.6% (United Nations, 2022). This rapid growth has intensified the demand for animal protein, placing pressure on food production systems, particularly aquaculture. Aquaculture industry with its fast growth rate *i.e.*, 7.5% since 1970 which is the only answers to the question of how we can meet global animal protein demand in future. Despite its significant contribution to global food security, the sector confronts obstacles such finite natural resources, such as water and land and an increasing prevalence of infectious diseases. Disease outbreaks remain one of the primary constraints in aquaculture sector, causing high mortality rates and substantial economic losses in different kinds of aquafarming. Intensive farming practices, which are increasingly adopted to meet the rising demand for fish, often lead to overcrowding, environmental degradation and disease proliferation (Lu *et al.*, 2024). The widespread use of antibiotics as a preventive and therapeutic measure has been widely criticized due to its contribution to antimicrobial resistance (AMR), posing a threat to both human health and environmental sustainability (Bhadra *et al.*, 2024). In response to this, there is a growing preference for "back to nature" approaches, utilizing medicinal plants as alternative, eco-friendly solutions for disease management in aquaculture. These natural products can serve as growth promoters, immune boosters and stress resistance enhancers, offering a safer and more sustainable option compared to synthetic chemicals and antibiotics.

Ferulic acid (FA), a plant based phenolic compound, has garnered attention as a promising functional feed additive in aquaculture (Li *et al.*, 2021). Known for its potent immunomodulatory properties, FA has been extensively studied for its potential to enhance fish growth and immunity (Fu *et al.*, 2022). It is obtained from sources like vegetables, fruits and herbal plants and is characterized by its ability to reduce oxidative stress in fish cells (Dawood *et al.*, 2020; Li *et al.*, 2021; Lu *et al.*, 2024). These properties make FA an effective agent for mitigating stress-related damage and improving overall fish health.

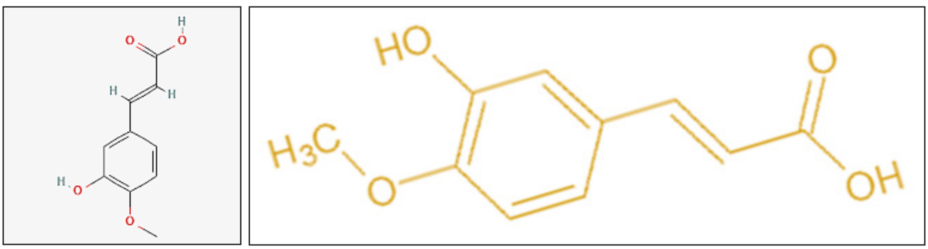
The inclusion of FA in fish diets has demonstrated significant benefits, including improved growth, immune responses and increased resistance to stress and disease (Xu *et al.*, 2022; Chen *et al.*, 2022a). Studies have reported that FA can positively impact fish metabolism, digestive efficiency and physiological functions, ultimately contributing to better survival rates and higher productivity in aquaculture systems (Lu *et al.*, 2024). However, it is crucial to establish appropriate dosage levels to maximize its benefits while avoiding potential negative effects, such as nutrient malabsorption or oxidative damage in tissues.

Given its multifunctional properties and growing relevance in aquaculture, FA presents a promising solution to some of the industry's most pressing challenges. It's potential to improve both the growth and immunity of fish positions FA as a key component in the development of sustainable, healthconscious aquaculture practices. This chapter will explore the multifaceted role of ferulic acid in aquaculture, focusing on its application as a growth and immunity booster in fish, its mechanisms of action and its potential to enhance the sustainability of the aquaculture sector.

## 2. FA: Structure and Sources

### *2.1. Structure*

FA, commonly known as 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid, is also referred to by several other names such as 3-methoxy-4-hydroxycinnamic acid, coniferic acid and caffeic acid 3-methyl ether (de Oliveira *et al.*, 2021). The form of FA isolated from plants is typically the trans isomer, which is relatively stable in organic solvents. According to Kundu *et al.*, (2016), plants can also produce a related compound called isoferulic acid, or 3-hydroxy-4-methoxycinnamic acid (Figure 1). However, in water, ferulic acid may slowly degrade through thermal decarboxylation, producing 4-vinylguaiacol (Dorfner *et al.*, 2003). For example, heating ferulic acid at 100 °C and pH 4.0 for one hour has results in 5.2% decarboxylation. Additionally, iron can catalyze its oxidation, leading to the formation of a dilactone.




(a) Ferulic Acid (b) Isoferulic Acid

**Figure 1:** Figure 1: Molecular structure of ferulic and isoferulic acid (Ou and Kwok, 2004)

### *2.2. Sources*

FA is present in various plants, including rice (Fujimaki *et al.*, 1977), wheat (Fulcher *et al.*, 1972; Smart and O'Brien, 1979; Pussayanawin and Wetzel, 1987; Pussayanawin *et al.*, 1988; Wetzel *et al.*, 1988), barley (Fincher, 1976; Smart and O'Brien, 1979), oats (Durkee and Thivierge, 1977) sorghum (Cherney *et al.*, 1991), tree bark (Laver and Fang, 1989; Putman *et al.*, 1989; Balde *et al.*, 1991), poplar buds (Bankova *et al.*, 1989), roasted coffee (Klocking *et al.*, 1971), tomatoes (Qureshi *et al.*, 1976), olives (Nergiz *et al.*, 1991), asparagus (Smith and Stanley, 1989; Pedersen *et al.*, 1991), berries (Mosel and Herrmann, 1974), peas (Mendez *et al.*, 1971) and citrus fruits (Wheaton and Stewart, 1965). In seeds, FA is primarily concentrated in the bran, where it contributes to the autofluorescence observed in tissues like wheat aleurone layers and barley scutella (Fulcher *et al.*, 1972; Smart and O'Brien, 1979; Pussayanawin *et al.*, 1988). In wheat, FA is predominant phenolic acid, with concentrations of 50 μg $g^{-1}$ in white flour to 500 μg $g^{-1}$ in whole wheat (Pussayanawin and Wetzel, 1987).

## 3. Application of FA in Aquaculture

### *3.1. FA as Growth and Immunity Booster of Aquatic Animals*

Dietary supplementation of Ferulic acid (FA) can improve zootechnical performance of aquatic species, as evidenced by Yu *et al.* (2018). Additionally, FA plays a pivotal role in bolstering the immune response of fish, with Ahmadifar *et al.* (2019) demonstrating its ability to enhance immune responses and overall health in fish. Furthermore, FA is recognized for its capacity to augment antioxidant defences, contributing to the overall well-being of fish. Yu *et al.* (2017) highlighted that FA supplementation increases antioxidant capacity, thereby reducing oxidative stress and promoting a healthier physiological state. Collectively, these findings suggest that FA is a multifaceted bioactive compound with the potential to improve not only growth and immunity but also the antioxidant status in fish, warranting further exploration of its applications in aquaculture.

#### *3.1.1. Application in Finfish*

##### 3.1.1.1. Zebra Fish (*Danio rerio*)

In a study conducted by Wen and Ushio (2017), FA was shown to have significant effects on growth parameters in adult zebrafish (*Danio rerio*). Diet supplemented with FA led to a marked increase in body weight, indicating an overall enhancement in growth performance. Furthermore, FA treatment resulted in improvement of muscle mass, confirmed by histological analysis showing an enlarged cross-sectional area of myofibers. This suggests that FA promotes muscle hypertrophy in zebrafish, likely through mechanisms involving anabolic pathways. The findings indicate the potential use of FA in diet to improve growth and muscle development in aquatic species, making it an important feed additive for use in aquaculture.

##### 3.1.1.2. Genetically Improved Farmed Tilapia (GIFT)

FA in fish diet has been shown to significantly enhance zootechnical performance and positively influence the physical characteristics of flesh in GIFT juveniles, as reported by Yu *et al.* (2018). The study demonstrated that FA plays a critical role in promoting growth by supporting the metabolic functions essential for tissue development and nutrient assimilation. Additionally, the antioxidant properties of FA contributed to improved oxidative stability in the fish, reducing the harmful effects of reactive oxygen species (ROS) and supporting overall health.

The research also highlighted improvements in the flesh quality of GIFT juveniles, noting that FA supplementation contributed to desirable physical characteristics, such as increased firmness and enhanced colour, which are important factors for consumer preference and market value. These findings suggests FA as a beneficial feed additive in aquaculture, not only for enhancing growth rates but also for improving fish product quality, making it an attractive option for optimizing fish farming practices.

3.1.1.3. Common Carp (*Cyprinus carpio*)

The effects of *Lactobacillus fermentum* (LF) and/or ferulic acid (FA) on enhancing the resistance of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila* infection were thoroughly evaluated (Ahmadifar *et al.*, 2019). The study observed significant improvements in final weight (FW), weight gain (WG) and specific growth rate (SGR), in groups that received either LF, FA, or a combination of both. Notably, these fish exhibited a lower feed conversion ratio (FCR), indicating more efficient nutrient utilization compared to control groups. Moreover, the study highlighted the positive impact of LF and FA supplementation on immune parameters. Fish fed diets containing LF, FA, or both showed a marked enhancement in serum respiratory burst activity and lysozyme activity, two critical indicators of innate immune function. This suggests that these supplements bolster the fish's ability to mount an effective immune response, potentially improving their capacity to fight off infections. In addition to immune modulation, the antioxidant defence system of the common carp was also significantly enhanced. The fish supplemented with FA or both LF and FA demonstrated improved levels of serum antioxidant enzymes, particularly catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD). These enzymes play vital roles in neutralizing oxidative stress, which is often elevated during infections, thereby reducing cellular damage and supporting overall fish health.

Collectively, the findings of Ahmadifar *et al.* (2019) indicate that the combined use of Lactobacillus fermentum and ferulic acid can effectively protect common carp from the detrimental effects of *Aeromonas hydrophila* infection. The observed benefits are likely mediated through the stimulation of both immune responses and antioxidative defences, suggesting that these supplements hold promise as functional dietary additives in aquaculture to enhance disease resistance and improve fish health.

3.1.1.4. Nile tilapia (*Oreochromis niloticus*)

In recent studies, FA has been identified as a promising dietary supplement that significantly enhances growth performance, immune response and antioxidative capacity in Nile tilapia. Research conducted by Dawood *et al.* (2020) demonstrated that the inclusion of FA in the diet of Nile tilapia positively influenced various physiological parameters, leading to improved overall health and growth outcomes.

This study indicates that the optimal level of FA ranges from 81.25 to 98.75 mg $kg^{-1}$ of nile tilapia feed. At these concentrations, FA appears to enhance nutrient absorption and utilization, which are critical factors in promoting growth. Additionally, FA's role as a natural antioxidant contributes to enhanced oxidative stress resistance, supporting the fish's immune system and overall vitality.

By incorporating ferulic acid into their diets at the specified optimal levels, aquaculture practitioners can potentially improve welfare of Nile tilapia, ultimately leading to more sustainable and productive farming practices. These results underscore the importance of further exploration into the use of phytochemicals in aquaculture diets, particularly in their capacity to support fish growth and immunity.

3.1.1.5. Hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus polyphekadion* ♂)

FA has enhanced growth, antioxidant capacity and innate immunity in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus polyphekadion* ♂), a commercially important species in aquaculture. FA's beneficial effects are largely attributed to its potent antioxidant properties, which help mitigate oxidative stress, thereby improving the overall physiological health of the fish. Additionally, FA strengthens the fish's immune response by modulating key immune pathways and reducing the expression of pro-inflammatory markers, further contributing to enhanced resilience against diseases.

In terms of growth performance, FA supplementation promotes better nutrient absorption and utilization, leading to improved weight gain and feed efficiency in hybrid grouper. The compound's ability to activate the TOR signaling pathway, coupled with its role in stimulating growth-related hormones underscores its contribution to accelerated growth in fish.

However, while FA supplementation at moderate levels is beneficial, excessive doses can have deleterious effects on hybrid grouper. Studies by Fu *et al.* (2022) have demonstrated that high concentrations of FA (above 640 mg $kg^{-1}$) may impair growth performance and reduce feed utilization efficiency. This negative impact is likely due to the compound's interference with nutrient digestibility and its potential to disrupt the balance of intestinal microbiota. Furthermore, excessive FA intake may lower the expression of growth-related genes, such as GH and GHR2, potentially inhibiting the fish's growth mechanisms. These findings highlight the importance of optimizing FA dosage to maximize its beneficial effects while avoiding potential adverse outcomes in hybrid grouper aquaculture.

3.1.1.6. Grass carp (*Ctenopharyngodon idella*)

In grass carp, supplementation with ferulic acid (FA) has been demonstrated to significantly improve the zootechnical performance. These results indicate that FA plays a critical role in promoting efficient growth in this species, particularly when incorporated into cottonseed meal (CSM)-based diets. According to a study by Chen *et al.* (2022), the optimal level of FA supplementation in such diets is 204 mg $kg^{-1}$ of feed. At this concentration, FA not only maximizes growth performance but also supports better nutrient assimilation and metabolic efficiency. The study highlights the importance of precise FA dosing to achieve optimal results, as inappropriate dosages can potentially diminish the growth-promoting effects. The data indicate that FA at the recommended level can effectively enhance feed efficiency, contributing to the sustainable aquaculture of grass carp.

3.1.1.7. Rainbow trout (*Oncorhynchus mykiss*)

In rainbow trout, FA resulted in enhancement of overall welfare. Recent research by Habibnia *et al.* (2024) highlights the synergistic effects of FA when combined with *Pediococcus pentosaceus*, a probiotic strain, on the antioxidative and immune responses of the fish. The study demonstrated that trout receiving a diet supplemented with both FA and *P. pentosaceus* exhibited the highest levels of antioxidative activity and immune system function compared to control groups or those treated with either FA or *P. pentosaceus* alone. This combined treatment not only boosted growth metrics, such as weight gain and feed conversion ratio, but also improved the fish's physiological welfare by enhancing their resistance to oxidative stress and immune challenges. These findings suggest that the co-administration of FA and *P. pentosaceus* could serve as an effective dietary strategy for optimizing both the growth and health of rainbow trout in aquaculture systems.

*3.1.2. Application in Shellfish*

3.1.2.1. Oriental River Prawn (*Macrobrachium nipponese*)

FA has resulted in promoting growth and health of *M. nipponense* by improving both biochemical and antioxidant capacities. According to Liu *et al.* (2022), inclusion of FA at concentrations ranging from 163.99 to 183.33 mg $kg^{-1}$ diet led to notable improvements in growth performance. When the prawns were challenged with the pathogen non-O1 Vibrio cholerae GXFL1-X, this impact was followed by a significant decrease in fatality rates indicating enhanced disease resistance.

In addition to its growth-promoting effects, FA also positively influenced the immune function of *M. nipponense*. It significantly enhanced the prawn's non-specific immunity, a critical component of the organism's defence against pathogens. This improvement in immune response was linked to FA's capacity to modulate key immune-related pathways. Specifically, FA down-regulates the mRNA of several NF-κB-related genes, which are inflammation and immune responses regulatory genes. The suppression of these genes suggests that FA may exert an anti-inflammatory effect, contributing to the overall enhancement of the prawn's immune capacity.

The combination of improved growth, enhanced antioxidant and immune responses and increased disease resistance indicates FA as a beneficial feed additive for *M. nipponense*. These findings underscore the relevance of FA in aquaculture, where its application can support not only the health and growth of the species but also improve their resilience to disease, making it a promising tool for sustainable aquaculture practices.

3.1.2.2. White Leg Shrimp (*Litopenaeus vannamei*)

Lu *et al.* (2024) recently reported that FA 50 to 100 mg $kg^{-1}$ in the shrimp diet, or combining FA 50 mg $kg^{-1}$ with 100 mg $kg^{-1}$ of dihydromyricetin (DMY) has been shown to significantly improve growth performance and digestive enzyme activities in *L. vannamei*. This improvement is attributed to the bioactive properties of both FA and DMY, which support more efficient nutrient digestion and assimilation, thereby contributing to enhanced growth outcomes. Additionally, the FA-DMY complex plays a crucial role in promoting the efficient accumulation of nutrients by regulating lipid metabolism. This regulatory function ensures optimal lipid utilization, which is essential for maintaining energy balance and supporting the physiological growth processes in crustaceans.

Furthermore, the dietary inclusion of FA or the FA-DMY complex has been found to bolster the antioxidant capacity and immune defense mechanisms of *L. vannamei*. Both compounds possess strong antioxidant properties, which mitigate oxidative burst activity by neutralizing free radicals and reducing damage at cellular level. This enhancement of antioxidant capacity not only improves the overall health status of *L. vannamei* but also strengthens its immune defenses, helping to protect against various pathogens and environmental stressors. Notably, this study is the first to demonstrate the synergistic effects of FA and DMY on the antioxidant capacity and immune defense in crustaceans, highlighting the potential of combining these compounds to achieve superior health and growth benefits. The results suggest new prospective into the use of natural bioactive compounds in aquaculture to promote both growth and resilience in crustacean species.

### *3.2. Mechanisms of FA Action in Fish Physiology*

In recent years, the application of phytochemicals sourced from plants has attracted significant interest in both aquatic and terrestrial farming, largely due to their potential to promote growth and health. Among these compounds, phenolic acids such as ferulic, vanillic, caffeic, chlorogenic and p-coumaric acids stand out. Ferulic acid, in particular, is notable for its superior bioavailability, remaining in the bloodstream longer than other phenolic acids (Bezerra *et al.*, 2017; Tee-ngam *et al.*, 2013). Consistent research findings demonstrate that FA supplementation in animal diets enhances zootechnical performance across different species, such as genetically improved farmed tilapia (GIFT) (Yu *et al.*, 2017), zebra fish (Wen and Ushio, 2017), common carp (Ahmadifar *et al.*, 2019), Nile tilapia (Dawood *et al.*, 2020), hybrid grouper (Fu *et al.*, 2022), grass carp (Chen *et al.*, 2022), *M. nipponense* (Liu *et al.*, 2022) and rainbow trout (Habibnia *et al.*, 2024).

The mechanisms underlying FA's growth-enhancing effects are multifaceted. One prominent hypothesis is that FA elevates growth hormone (GH) and testosterone levels in the bloodstream, which, in turn, stimulate growth (Yu *et al.*, 2017). Additionally, this natural antioxidant also plays a important role in modulating immune response, as it down-regulates the expression of key inflammatory markers such as Dorsal, HSP70 and Toll by activating the NF-κB pathway. This activation enhances organism's antioxidative capacity and bolstering immunity, ultimately leading to improved growth performance. Safari *et al.* (2020) further suggest that FA enhances growth by boosting digestive enzyme activity, stimulating the synthesis of proteins and regulating the composition of gut microbiota. Growth in fish, much like in mammals, is predominantly governed by the endocrine system, particularly the growth hormone-insulin-like growth factor (GH-IGF) axis (Picha *et al.*, 2008). GH is crucial for growth, immune function, cellular differentiation and metabolism, as it binds to growth hormone receptors (GHRs) (Amenyogbe *et al.*, 2020). Furthermore, GH help in synthesis and release of IGF-1 (Triantaphyllopoulos *et al.*, 2019), a key growth factor. Notably, GHR1 and GHR2, two distinct growth hormone receptors, differ structurally, with GHR2 lacking several critical intracellular and extracellular components (Li *et al.*, 2007). The synthesis and release of GH and IGF-1 are influenced by several factors, including nutrient availability, hormones, growth stage and environment (Cameron *et al.*, 2007). Polyphenolic phytochemicals, including FA, have been shown to stimulate the production of GH and IGF-1 (Midhun *et al.*, 2016). Interestingly, FA increased GH levels in serum, while low-dose FA *i.e.*, 80-160 mg $kg^{-1}$ has been linked to the upregulation of growth-related genes (Gorewit, 1983). Conversely, excessive FA doses (above 640 mg $kg^{-1}$) resulted in reduced GH and GHR2 expression (Fu *et al.*, 2022).

The FA is mainly known for its antioxidant potential and the mechanism is complex, primarily involving the inhibition of reactive oxygen species (ROS) and nitrogen formation, along with neutralizing free radicals. It also chelates metal ions like Cu (II) and Fe (II), preventing the formation of harmful hydroxyl radicals (Rice-Evans *et al.*, 1996; 1997). FA not only scavenges free radicals but also inhibits enzymes that generate them and boosts scavenger enzyme activity (Bezerra *et al.*, 2017). Its chemical structure enables it to form stable phenoxyl radicals, disrupting the chain reaction of free radical production (Kiewlicz *et al.*, 2015). Additionally, FA can donate hydrogen atoms to protect cell membrane lipids from oxidation (Sheu *et al.*, 2006). By binding transition metals, it further prevents oxidative stress-related damage and it has been shown to reduce the activity of enzymes like xanthine oxidase and cyclooxygenase-2, both involved in ROS production (Nile *et al.*, 2016).

The FA is also found to promote collagen synthesis, quality fish muscle development and wound healing (Cao *et al.*, 2022). The compound is involved in synthesis of hydroxyproline and hydroxylysine which are essential amino acids for promoting collagen synthesis, growth and immunity in fish (Zduńska *et al.*, 2018).

From the discussion it is understood that, Ferulic Acid (FA) presents significant potential as a growth booster in fish, particularly through its influence on the GH-IGF axis, its ability to maintain tight junction integrity and its enhancement of antioxidant, wound healing, quality muscle development capacity. However, more research is essential to fully understand its mechanisms and optimal dosages across different species to standardize its use and understand its untapped potential in welfare of the cultured organism.

## 4. Conclusion

Ferulic acid presents significant potential as a valuable antioxidant in aquaculture due to its ability to mitigate oxidative stress and enhance the health and resilience of aquatic organisms. Its multifunctional role in scavenging free radicals, inhibiting harmful enzyme activity and protecting cell membranes from lipid peroxidation positions it as a promising additive for improving the immune response and overall well-being of cultured species. Furthermore, ferulic acid's capacity to chelate transition metals and suggests its utility in managing oxidative damage caused by environmental stressors and intensive farming practices. Incorporating ferulic acid into aquaculture systems could contribute to more sustainable and healthy fish farming practices, supporting the industry's growth while safeguarding aquatic animal health. With further research the use of ferulic acid can be optimized and untapped potential can be explored with other bioactive compounds in aquaculture feed formulations.

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