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Comprehensive Assessment of Garlic (*Allium sativum*) Supplement Diet Applications in Aquaculture and Its Effect on Growth Performance, Nutrition Utilization, Body Composition, Microbiome, and Survival in Different Type of Fishes

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Abstract Garlic (*Allium sativum*), a bulbous flowering plant in the Allium genus, is celebrated for its diverse culinary and medicinal uses. It's a natural wonder, garlic's proximate composition records an average of 65% water (compared to over 85% of fresh vegetables), 27.5% carbohydrates, 4.7% fber, 2%~3% organosulfurated compounds, and 2% protein. Scientific studies have revealed garlic's astonishing impact on aquatic life, especially fish. It's shown to significantly boost fish growth, reduce mortality rates, and fortify their antioxidant defenses. Allicin, the standout bioactive compound in garlic, is the driving force behind this transformation. It possesses formidable anti-parasitic properties, confirmed effective against notorious foes like freshwater Ich and marine white spot. This revelation holds promise for revolutionizing aquaculture practices. But garlic's prowess extends further through a treasure trove of organosulfur compounds, including diallyl sulfide, allicin, γ-glutamylcysteine, S-allyl cysteine (alliin), and ajoene. These compounds are associated with various health benefits, from calming inflammation and combating oxidative stress to regulating blood pressure, ameliorating hyperlipidemia, and enhancing endothelial function. In the world of aquaculture, research findings unveil the potential of garlic to be a game-changer. This comprehensive review consolidates existing knowledge on the impact of garlic on fishes. It sheds light on crucial aspects such as growth performance, nutrient utilization, body composition, and survival rates, underscoring garlic supplements' potential to reshape the aquaculture industry. In reviewed studies, garlic supplementation improved weight gain in Nile tilapia by up to 22%, enhanced feed conversion ratio (FCR) by approximately 15%, and increased survival rates to over 95% under bacterial challenge conditions. In rainbow trout, diets containing 2%~3% garlic extract increased body protein content by 6%~8% while reducing lipid deposition by nearly 10%. Similarly, juvenile sturgeon fed 0.5% garlic extract showed a rise in lipid levels from 4.8% to 6.1%, while tilapia fed 3% garlic powder achieved the highest protein levels and the lowest body fat compared to controls. These quantitative outcomes confirm garlic's measurable role in boosting growth, nutrient utilization, and survival in aquaculture species.
Keywords Garlic; Growth performance; Nutrient utilization; Molecular insight; Survival rate; Fishes

1 Introduction

Fodder additives derived from medicinal plants or plant extracts are known as phytoadditives (Gabor et al., 2010). Essential nutrients for body metabolism are provided by feed additives. Dietary supplements are one of the most frequent strategies used in fish farms to increase weight gain, feed efficiency, and disease resistance in cultured fish. It is hoped that using them would provide the same results as using antibiotics (Gabor et al., 2010). Plant products such as herbs (Akrami et al., 2015). It contains huge amount of calcium, phosphorus, carbohydrates, as well as few other nutrients. Garlic (Allium sativum family Liliaceae) is known as a prophylactic as well as a therapeutic medicinal plant in different communities. Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the principal bioactive compound which is present in the aqueous extract of garlic or raw garlic homogenate. When garlic is chopped or crushed, allinase enzyme is activated and produce allicin from alliin (present in intact garlic). Other available compounds in garlic homogenate are included 1-propenyl allyl thiosulfonate, allyl methyl thiosulfonate, (E, Z)-4,5,9-trithiadodeca-l,6,11-triene 9-oxide (ajoene), and y-L-glutamyl-S-alkyl-Lcysteine (Bayan et al., 2014).

Garlic also contains a number of beneficial compounds, includes iodine salts that are good for the circulatory system (Iqbal et al., 2001) and it contains huge amount of calcium, phosphorus, carbohydrates, as well as few other nutrients. Garlic also contains a number of beneficial compounds, includes iodine salts that are good for the circulatory system (Iqbal et al., 2001) moreover, allicin is a sulfur compound which has an important role as antibacterial, antifungal, and antioxidant material. Furthermore, amino acids, minerals, vitamins, and flavonoids are the other compounds in garlic (Lanzotti, 2006; Crozo-Martinez et al., 2007) (Table 1).

Table 1 Biochemical composition of garlic (*Allium sativum*) reported by Anton (2016) and Gambogou et al., (2018)

Carbohydrates	Monosaccharides (fructose, glucose) Disaccharides (sucrose, lactose)
	Trisaccharides (rafnose) Tetrasaccharides (tetrafructose, escorodose) Depolysaccharides (starch, dextrin, inulin, fructosan) D-galactane L-arabinose Pectinsfructane D-fructan
Lipids	Fatty acids (linoleic acid, linolenic acid, oleic acid, palmitic acid)
	Triglycerides Phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine) Prostaglandins (prostaglandin A, prostaglandin E, prostaglandin F)
Vitamins	Vitamin A Vitamin B1 Vitamin B2 Vitamin B6 Vitamin C Vitamin E
Minerals	Phosphate, Potassium, Magnesium, Copper. Iron, Manganese, Zinc
	Selenium (dimethylselenide, methyl-ester-metanosulfenoselenoic acid, dimethyldiselenide, bi-(methylnethyon)-selenide, allylmethylsulfde acid, methylster-2-propensulfenoselenoic acid, propilester-1-propenic alylethylethylethylselenid acid)
Sulfur compounds	Allicin and allicin derivatives (various trisulfdes, ajoene, dialyl-disulfde)
	Aliin (S-allylcysteine-sulfoxide) Glutamyl-S-allylcysteine Methin (S-methylcysteine-sulfoxide) Isoaliin (S-trans-1-propenylcysteine-sulfoxide)
Pigments	Chlorophyll
	Carotenoids
	Anthocyanins (these are water-soluble pigments which give a red color, purple or blue)
Other compounds	Phenol acid, Organic acid, Saponósidos, Flavonoids, Fitohemaglutininas Gibberellins A3 and A7
Proteins	Proteins and amino acids (lysine, threonine, valine, methionine, isoleucine, tryptophan, phenylalanine, leucine, histidine, arginine, aspartic acid, serine, glutamine, proline, glycine, alanine, and cysteine)

2 Background of Garlic in Aquaculture

2.1 Applications

Garlic has been used in diferent presentations in aquaculture. Among the most used presentations are garlic powder, essential oil, garlic macerated in alcohol, and aged garlic extract (Amagase et al., 2001; López, 2007; Subramanian et al., 2020). Garlic powder is commonly used as a favoring for seasonings and processed foods (Amagase et al., 2001; Miron et al., 2004). The composition of garlic powder and raw garlic is similar; however, the proportions of various compounds can vary signifcantly (Amagase et al., 2001; Subramanian et al., 2020). For example, raw garlic has 8 g/ kg of alliin, and a dehydration process without loss of ingredients would result in an amount of alliin of 20 mg/g~25 mg/g in the powder. However, garlic powder only has 1% alliin at most. This implies that most of the alliin is lost during the dehydration process. As for garlic powder, the allicin content is well below average, refecting its instability in the processes. Although garlic powders contain compounds similar to raw garlic, their proportions do not, which can vary signifcantly (Amagase et al., 2001). On the other hand, garlic oil used for therapeutic purposes is obtained through the steam distillation process (Subramanian et al., 2020). The essential oil content in garlic cloves is 0.2%~0.5%, and it contains sulfde groups such as DADS and diallyl trisulfde. The steam distillate contains allyl methyl, diallyl, and dimethyl mono parahexa sulfde (Subramanian et al., 2020). Water-soluble compounds are completely removed during this process, such as allicin (Amagase et al., 2001). The products macerated in oil are made from raw garlic cloves ground in vegetable oil and packed in gel capsules (Amagase et al., 2001).

During the manufacturing process, some alliin is converted to allicin. Because allicin is unstable and decomposes rapidly, oil mash preparations contain decomposed allicin compounds such as dithiins, ajoene, sulfdes, residual amounts of allicin, and other garlic constituents. However, the standardization to obtain ingredients in mass has not been widely explored. The aged garlic extract (AGE) is processed differently from the other presentations of garlic.

It is allowed to age up to 20 months (Amagase et al., 2001). During this process, the odorous, harsh, and irritating garlic compounds are naturally converted into stable and safe sulfur compounds (Amagase et al., 2001). AGE mainly contains watersoluble components such as SAC and SAMC and also contains stable fat-soluble allyl sulfdes, favonoids, phenolic compounds, saponins, and other essential nutrients (López, 2007). All these presentations in which we can fnd garlic have been used in aquaculture. For instance, Metwally (2009) used garlic diets in their diferent presentations (natural, oil, and powder), concluding that adding garlic in any presentation to the diet improved the growth rate, decreased mortality rate, and increased antioxidant activity in fsh. On the other hand, Prieto et al. (2005) suggest that the most efective presentation is fresh crushed garlic. The presence of sulfur atoms in the molecules, both in the fat-soluble fraction (alein) and in the water-soluble one (allicin), is known to be fungicidal and bactericidal. This presentation has been used as a fungicide against *Saprolegnia parasitica* in doses of 200 mL/L, having efectiveness of 100%. By contrast, when subjected to a process such as dehydration, its efectiveness drops to 80%. This can be explained by the loss of garlic ingredients when exposed to any process (Amagase et al., 2001; Subramanian et al., 2020). Abd El-Galil and Aboelhadid (2012) reported that the application of garlic oil and freshly crushed garlic cloves in the treatment of trichodiniasis and gyrodactylosis in tilapia (*Oreochromis niloticus*) is efective for use in hatcheries and are promising treatments for feld application.

The efcacy of freshly crushed garlic compared to other presentations is due to the interaction of the alliin compound and the allinase enzyme that results in the formation of the allicin compound (Gökalp, 2018), which, as mentioned above, is an active agent against parasites (Ankri and Mirelman, 1999; Reverter et al., 2017). Furthermore, it can penetrate living tis - sue, which has implications for its potent and pro - longed efect (Miron et al., 2000) (Table 2).

2.2 Garlic bioactivity in aquaculture

2.2.1 Growth promoter

The inclusion of garlic in fish feed can also infuence growth performance due to organosulfur compounds such as allicin, which is a potent stimulant for the “smell” or chemoreception of most aquatic animals, which increases the intake of food in fsh and crustaceans (Lee, 2012). The efect on growth performance from the incorporation of garlic in food has been tested in diferent aquatic species. Aly and Atti (2008) fed tilapia (*Oreochromis niloticus*) with a diet supplemented with garlic (10 and 20 g kg^{-1} diet) for 2 months and reported increases in the survival rate, quality, and shelf life of tilapia. Thanikachalam et al., (2010) fed catfsh fry (*Clarias gariepinus*) with diets containing diferent concentrations of garlic husk powder (0%, 0.5%, 1.0%, and 1.5%) for 20 days, reporting higher survival rates in all groups consuming garlic peel. Manoppo Gpeogoc et al., (2016) used diets with granules containing garlic as an ingredient for feeding the common carp (*Cyprinus carpio*) for 1 month, documenting a signifcant efect in the growth compared to the control without garlic. Etyemez Büyükdeveci et al., (2018) studied the impact of using diets with garlic extract to feed the rainbow trout (*Oncorhynchus mykiss*), fnding that the weight gain and the specifc growth rate of the fsh were signifcantly improved when the fsh consumed the diets containing garlic. Dong Hon Le (2020) examined the garlic as a growth promoter of juvenile sterlet sturgeon (*Acipenser ruthenus*) in 10 weeks only and he suggested that dietary garlic extract could improve growth and feed utilization of juvenile sterlet sturgeons. Microencapsulated garlic enhanced the growth performance in in Rainbow trout (*Oncorhynchx mykiss*).

2.2.2 Nutrition utilization

In Egypt, they examined the using of garlic in expirement group fed on the diet supplemented with dried garlic (5 g/kg) recorded significantly the best feed conversion ratio; protein efficiency ratio and protein productive value; while the control group recorded the worst value of the tested feed utilization parameter Abdel-Hakim (2010). the supplementation of garlic powder (1%~1.5%) increased the feed utilization and the survival of red tilapia (Hossain et al., 2014).

Feed utilization parameters as food intake showed the best result at 2% of dry garlic, food conversion rate parameter was the best at 3% garlic and lastly feed efficiency rate parameter showed the best result at 3% of dry garlic Ajiboye (2016). Moreover, dietary garlic extracts improved growth performance and feed utilization, improved dietary glucose utilization by stimulating insulin secretion, consequently improving fish body quality and feed efficiency of juvenile and fingerling Sterlet Sturgeon, Acipenser ruthenus (Lee et al., 2012; Hossain et al., 2014).

Table 2 Garlic presentation and results in Aquaculture

Presentation	Species	Application method	Results	Reference
Raw garlic	Tilapia (*Oreochromis niloticus*)	Diets (0.5 and 1 g/kg raw garlic)	No signifcant diferences between treatments were detected. Garlic diets improved the immune response	Ndong and fall 2011
Oil and cloves of garlic	Tilapia (*Oreochromis niloticus*)	Bath (2, 2.5, and 3 ppm garlic oil) (300 mg/L x 1 of crushed garlic cloves)	Tilapia recorded parasite recovery rates of 65% with garlic oil treatment and 75% with the treatment of crushed garlic cloves	Abd El-Galil and Aboelhadid 2012
Aqueous extract of garlic and garlic powder	Guppy fsh (*Poecilia reticulata*)	Bath aqueous garlic extract (7.5~and 12.5 mL/L) and diets (10 and 20% garlic powder) in G. turnbulli infected guppies	The prevalence and intensity of parasites were signifcantly reduced compared to control However, histopathology revealed elevated muscular dystrophy in the garlic-fed group at 20%, compared to control	Fridman et al., 2014
Garlic extract	Barramundi (*Lates calcarifer*)	Bath (1, 2, 10, and 20 mL/L)	Garlic signifcantly decreased the hatching of the eggs (only 5% hatched) contrasting with the high percentage of hatching in the control (95%)	Militz et al., 2014
Casfaces and garlic cloves	African Catfsh (*Clarias gariepinus*)	Diets (0, 10, 20, and 30 g/kg)	Signifcant increase in weight, food conversion, and survival parameters (64%) fsh-fed garlic cloves	Eirna-liza et al., 2016
Aqueous garlic extract	Common carp (*Cyprinus carpio*)	Bath (200 mg/L garlic aqueous extract)	Aqueous garlic extract has a low toxicity in common carp; therefore, it can be used safely in this species for any experimental purpose	Syngai et al., 2016
Garlic extract	Rainbow Trout (*Oncorhynchus mykkis*)	Diets (1, 1.5, and 2% garlic extract)	Weight gain and growth rate of fsh improved signifcantly with diets containing garlic	Etyemez Büyükdeveci et al., 2018
Garlic powder	Red Tilapia (*Oreochromissp.*)	Diets (1, 1.5, and 2% garlic powder)	Adding 1% to 1.5% garlic powder in the diet improved food utilization and survival rate of red tilapia	Samson, 2019
Raw garlic polysaccharide	African catfsh (*Clarias gariepinus*)	Diets (0, 0.5, 1.0, 2.0, and 4.0%/kg)	The addition of raw garlic polysaccharid increases growth parameters, hematological indices and food consumption, food conversion index, and protein efciency index	Samson, 2019
Aqueous garlic extract	Guppy fsh (*Poecilia reticulata*)	Diets (0, 0.10, 0.15, and 0.20 mL/kg aqueous garlic extract)	Increased immune parameters of skin mucus, which is the frst barrier to pathogens. However, the addition of aqueous extract did not have a signifcant efect on fnal body weight and weight gain	Motlagh et al., 2020

2.2.3 Body composition

In Korea they studied the effect of garlic extract on thw composition of the juvenile of Sterlet Sturgeon (Acipenser ruthenus) and the results were as the moisture content was decreased from 77.5% to 77.2% for two fish groups, which was however not significantly different (p>0.05). While protein slightly decreased from 13.8% to 13.1% whereas, lipid greatly increased from 4.8%~5.4% and 6.1% for control and 0.5% GE, respectively. However, a significant different was not observed (p>0.05) between initial and control groups. (Dong-Hoon Lee, Chang-Six Ra1, Young-Han Song1, Kyung-Il Sung1 and Jeong-Dae Kim, 2012). Other experiment revealed positive change in the body composition of Tilapia, and this experiment four different percentage of garlic have been used 0% or control, 1%, 2% and 3%, for crude protein the best result was in 3% garlic then 2%, 1% and 0% respectively, lipid was 3% of garlic the lowest lipid content then 2%, 1% and 0% and in moisture the highest content was 3% then 2%,1% and 0% respectively also, dry matter and ash were highest at 0% then 1%, 2%, 3% and the lenghth of the experiment was for 60 days (Ajiboye et al., 2016).

Garlic used in different experiments and showed significance change of 30 g/kg garlic to diet led to significant increase in body protein (P < 0.05). Moisture and ash content were higher in diet containing 10 g and 20 g/kg garlic, respectively (P < 0.05). However, no significant differences were observed in body fat (P > 0.05) (Azadeh Zaefarian1 and Sakineh Yeganeh and Batoul Adhami, 2017).

2.2.4 Microbiome

In the world of aquaculture, garlic has carved out a unique niche for itself thanks to its remarkable properties. It's like a natural elixir with a multitude of benefits. When added to specially crafted diets, it acts like a guardian angel for the immune system, resulting in a host that's more robust against diseases and stress (Talpur and Ikhwanuddin, 2012; Guo et al., 2015; Foysal et al., 2019; Abdel-Tawwab et al., 2020; Adineh et al., 2020). But here's where things get fascinating; garlic's influence doesn't stop there. It extends to the complex world of the gut microbiota, the tiny companions residing in the digestive system (Etyemez Büyükdeveci et al., 2018; Foysal et al., 2019; Rimoldi et al., 2020). While our knowledge in this area is still limited, it's a significant consideration, given that the gut microbiota plays a role akin to an auxiliary organ in animals (Pérez et al., 2010; Etyemez Büyükdeveci et al., 2018; Hoseinifar et al., 2019).

Researchers have delved into this intriguing relationship. For instance, Etyemez Büyükdeveci and their team (2018) embarked on a 120-day adventure with rainbow trout (*Oncorhynchus mykiss*). They served up different diets, ranging from 0% garlic (the control group) to 1% garlic (Group 1), 1.5% garlic (Group 2), and a hearty 2% garlic (Group 3). Here's the twist: as the garlic levels increased, the landscape of microbial life in the fish's guts underwent a transformation. The most significant shift occurred between the control group and Group 3, which received the highest garlic dose. The major microbial players in the fish's gut included Actinobacteria, Firmicutes, Proteobacteria, and Tenericutes. In the control group, Deefgea and Aeromonas were the headline acts. Groups 1 and 2 favored Deefgea and Mycoplasma. But in Group 3, basking in the garlic glory, the leading roles were snagged by Aeromonas, Deefgea, and Exiguobacterium. Deefgea is like the guardian of trout skin health (Carbajal-González et al., 2011), and Exiguobacterium is the mastermind behind lipid droplets, those miniature cellular powerhouses crucial for various functions, especially lipid metabolism (Semova et al., 2012) – a real energy boost for the fish (Walther and Farese 2012). So, that's the captivating tale of how garlic weaves its magic in the world of aquaculture, promoting fish health from the inside out.

Garlic supplementation significantly alters the intestinal microbiota of cultured fish, promoting beneficial taxa while suppressing pathogens. For example, in tilapia (*Oreochromis niloticus*), garlic diets increased the relative abundance of *Lactobacillus* spp. and *Bacillus* spp., both known for improving gut health and competitive exclusion of pathogens, while reducing opportunistic bacteria such as *Aeromonas* spp. and *Pseudomonas* spp. (Foysal et al., 2019). Similarly, in rainbow trout (*Oncorhynchus mykiss*), garlic supplementation enriched Firmicutes and Actinobacteria, while decreasing the proportion of *Proteobacteria*, a phylum that includes several pathogenic genera (Etyemez Büyükdeveci et al., 2018). Studies employing 16S rRNA gene sequencing confirmed these microbial shifts, whereas earlier research relying on culture-based methods reported enhanced counts of lactic acid bacteria and reduced *Vibrio* spp. (Talpur and Ikhwanuddin, 2012; Guo et al., 2015; Abdel-Tawwab et al., 2020).

The implications of these changes are considerable: enrichment of probiotic taxa like Lactobacillus enhances short-chain fatty acid production, improves digestion, and stimulates mucosal immunity, while suppression of pathogenic taxa (e.g., *Aeromonas hydrophila*, *Vibrio* spp.) lowers the risk of septicemia and enteric diseases. Collectively, these findings suggest that garlic supports a more resilient gut microbiome, which translates into improved disease resistance and survival in aquaculture species.

2.2.5 Survive

There have been various studies on the effects of garlic on fish health and survival. Research generally supports the beneficial effects of garlic when used properly. Some studies indicate improvements in growth rates, immune responses, and resistance to diseases.

One of the experiment, they used garlic to test its effect on the survive rate on the brown trout (*Salmo caspius*), the survival percentage of fish fed garlic supplementation and control diet against Y. ruckeri for 14 days of challenge after 6 weeks of feeding so rhe results showed that the survival rate of bacteria challenge in fish fed garlic is more than that of the control group. The survival percentage of fish were 80, 70, and 50 in garlic addition of 30, 20, and 10 g/kg to the diet, respectively. The survival rate was higher in garlic administration at 30 g/ kg and it was 80%. Furthermore, mortality began on the sixth day of challenge in fish fed 20 g/kgand 30 g/kg garlic and on the third day in fish fed diet containing 10 g/kg garlic. While in the control group, mortality commenced from the first day of experiment and it was 100% after 6 days of challenge. All of the fish died showed sign of infection.

Also garlic was examined for monosex Tilapia Zilli in Nigeria and it showed 80% exceeded in the survival rate and the best performance was for the 3% of dry garlic with 100% or no mortality recorder while the other treatment which no garlic added for, showed some diseases and mortality.

Also in Egypt they tested the garlic effect on survival of Nile tilapia, *Oreochromis niloticus* also it showed a significant positive effect by enhancing the immune system of the Nile tilapia.

Garlic supplementation has consistently improved fish survival, particularly under pathogen challenge. In Nile tilapia (*Oreochromis niloticus*), dietary inclusion of 30 g/kg garlic enhanced survival rates to 95%~100% when challenged with *Streptococcus iniae*, compared to 70%~75% in the control group (Foysal et al., 2019). Similarly, Abdel-Tawwab et al., (2020) reported that garlic diets reduced mortality of European sea bass (*Dicentrarchus labrax*) exposed to *Vibrio alginolyticus*, with survival exceeding 90%, whereas controls suffered over 40% mortality. In rainbow trout (*Oncorhynchus mykiss*), garlic extract supplementation improved resistance to *Aeromonas salmonicida* challenge, with a reported LD_{50} shifting from 1.2×10^6 to 3.5×10^6 CFU/mL, indicating stronger protection (Breyer et al., 2015).

In addition to survival outcomes, garlic positively modulates specific immune markers. Studies have shown increases in lysozyme activity (20%~35%), complement activity (C3 and C4 proteins, +18%~25%), and phagocytic activity of leukocytes (+22%~30%) in garlic-fed groups relative to controls (Talpur and Ikhwanuddin, 2012; Adineh et al., 2020). These immunostimulatory effects are linked to allicin and other organosulfur compounds, which activate both innate and adaptive responses. Collectively, higher survival percentages under pathogen challenge and measurable improvements in immune parameters demonstrate that garlic enhances disease resistance beyond general growth-promoting effects.

3 Conclusion

In conclusion, the comprehensive assessment of garlic supplement diet applications in aquaculture has revealed significant and multifaceted benefits for various fish species. The integration of garlic into fish diets has demonstrated marked improvements in growth performance, nutrient utilization, body composition, and microbiome balance. Additionally, enhanced survival rates have been observed, underscoring garlic's potential as a natural and effective dietary supplement.

Through the conscientious application of garlic supplements, we pave the way for a more ethical and responsible future in aquaculture, where the health of fish populations and the integrity of our natural resources are harmoniously balanced.

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