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Shyam Narayan Labh Research Management Cell, Central Department of Zoology Tribhuvan University, Kathmandu, Nepal

Shubha Ratna Shakya

Aquaculture Research Lab, Central Department of Zoology Tribhuvan University, Kathmandu, Nepal

Correspondence: Shubha Ratna Shakya Aquaculture Research Lab, Central Department of Zoology Tribhuvan University, Kathmandu, Nepal. Email:snlabh@gmail.com

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Shubha Ratna Shakya, Shyam Narayan Labh

Abstract

Aquaculture is a source of livelihood for many economically under privileged people in the least developed countries including Nepal. In recent years, with intensification and rapid development of aquaculture enterprises, occurrence of infectious diseases has cause huge economic losses. The main disease causative agents are bacteria, virus and parasites. The excess use of antibiotics and various synthetic chemicals have resulted in drug residue and resistant pathogens in treated fish. Drug residue pollutes the environment and threatens humans consuming them. Antibiotics that accumulate in the environment and fish pose a potential threat to consumers and to the environment. Increased public awareness of the negative effects caused by overexposure to synthetic chemicals has led to search for "green drugs" such as organic and synthetic chemical-free food products. In this regard, garlic (*Allium sativum*) as a natural antibiotic is the earliest known medicinal plant, has shown to be effective for the treatment of many diseases in humans and animals owing to its antimicrobial, antioxidant, anti-cancer, and antihypertensive properties. In aquacultural operations, optimized dose of garlic is strongly recommended. Hence, this review focuses on the application of garlic in on growth performance, flesh quality, antimicrobial activity, as an immunostimulant and antiprotozoal agent in aquaculture.

Keywords: Garlic extract, aquaculture, growth, resistance, challenge, fish.

1. Introduction

Garlic, *Allium sativum* L. has been used for centuries in many societies against parasitic, fungal, bacterial and viral infections ^[1]. Garlic has been proven effective as a hypolipidemic ^[2], antimicrobial ^[3], antihypertensive ^[4], hepatoprotective, and insecticidal ^[5] agent in various human and animal therapies. The use of garlic extracts reduces serum cholesterol levels ^[6] and increases blood coagulation time ^[7]. In aquacultural operations, garlic promotes growth, enhances immunity, stimulates appetite, and strengthens the control of bacterial and fungal pathogens. Many reports have documented that garlic can effectively eliminate principal pathogenic bacteria such as *Pseudomonas fluorescens, Myxococcus piscicola, Vibrio anguillarum, Edwardsiella tarda, Aeromonas punctata, Fibrobacter intestinalis* and *Yersinia ruckeri* in freshwater fish. Garlic improves flesh quality in fishes. These effects of garlic are due to the presence of various organosulphur compounds, including allicin ^[8].

Garlic extracts and most commercial garlic food supplements in the form of tablets and capsules containing garlic powder are based on either the allicin content or the potential to produce allicin ^[9]. The content of allicin and other sulfurous chemicals in garlic varies significantly and depends on several factors. For medicinal applications, higher levels of allicin are favorable ^[10]. The utilization of garlic in aquaculture has developed alongside the application and popularization of Chinese herbs in aquaculture. Most aquatic garlic researches have involved fresh garlic extracts, with experimental subjects either fed a garlic-added feed or treated with a garlic juice immersion. Allicin is the most powerful component present in garlic that actively and directly kills parasites ^[11]. Freshly pressed garlic, liquid garlic products are made for aquarium use, and even most food items containing garlic can be effective. Some people consider garlic to be an immune system booster and compare it to Vitamin C in humans ^[12].

Many scientific studies have shown that allicin can actively kill a wide range of pathogens like fungi, bacteria, and even viruses ^[13]. The medicinal effects of garlic have been known since immemorial time. It is a proven immunostimulant and an anti-infective agent ^[14]. *Allium* species have immune enhancing activities such as promotion of lymphocyte synthesis, release of cytokines, phagocytosis and natural killer cell activity ^[15]. Dietary garlic has been evaluated as an effective immunostimulant in rainbow trout ^[13] and *L. rohita* against *A. hydrophila* infection ^[16].

1.1. Garlinc and Fish Growth

Garlic (*Allium sativum*) has been used to improve the growth and resistance of a number of livestock and fish ^[17]. Significant gain in weight, increase in feed efficiency, protein efficiency ratio (PER) and specific growth rate (SGR) in Nile tilapia (*Oreochromis niloticus*) were observed when fed with diet containing 30 g kg⁻¹ garlic powder diet. ^[18]. However, the best growth performance was observed when *O. niloticus* was fed with diet containing 32 g kg⁻¹ diet of garlic powder ^[19]. Furthermore, Diab *et al.* reported that feed containing 2.5% garlic resulted in the highest growth performance in Nile tilapia (*O. niloticus*). Thus, more detail study is needed to conform the effect of garlic on growth of fish.

Many reports have documented the effect of allicin as a growth promoter. Fo *et al.* mixed a 1% garlic residue premix with the feed of grass carp, *Ctenopharyngodon idellus*, and common carp, *Cyprinus carpio*, in a polyculture system. Weight gain rate, survival rate and feed conversion ratio were increased in common carp when 100 mg kg⁻¹ synthesized allicin and iodized allicin were added to its feed ^[22]. These results are in agreement with those obtained by Khattab *et al.*. In addition, Aly *et al.* and Aly and Mohamed examined the growth rates of Nile tilapia after feeding with garlic (10 and 20 g kg⁻¹ diet fed), and found statistically non-significant increases after 1 or 2 months, but a significant increase was found only after 8 months, indicating that high doses or a long period was needed to enhance the growth rate.

A similar finding was reported by Huang *et al.*, who reported that rice field eel, *Monopterus albus* fed with 800 mg kg⁻¹ composed allicin died within 3 days. Xiang and Liu found that the increased growth rate in red bellied pacu, *Colossoma barchypomum* followed by decreased with increasing amount of allicin. The above results indicate that extremely high concentration of garlic extract or allicin does not improve fish growth; instead, they are harmful to fish health. This may be due to much alkyl sulfide that reaches the intestine, interfering with normal metabolism and suppressing mitosis, resulting in slow growth and even death.

1.2. Garlic and Flesh Quality

At present there is no perfect system for evaluating the flesh quality of cultured fish. The contents of crude protein, crude lipids, amino acids, water loss rate, and folding endurance of muscle may reflect the flesh quality to some extent. Long - term garlic supplemented feeding may lower the lipid and cholesterol content of fish. Moreover, allicin could activate intestinal proteases, which help to convert feed protein into fish protein, increasing the content of palatable amino acids. Aly *et al.* reported that the post-harvest flesh quality and

shelf-life of fish fed a garlic-supplemented diet were improved. Metwally found that the protein content in whole fish increased significantly in the group fed a garliccontaining diet, whereas the total lipid and ash contents decreased significantly in the same group. Xiang and Liu reported that the addition of 25-100 mg/kg garlic to the diet increased the crude protein content and reduced the crude lipid content of *C. barchypomum*. Luo *et al.* found that a compound from *Eucommia ulmoides* and garlic could improve the flesh quality of grass carp, *C. idellus*. These results are in agreement with those obtained by Xiang and Liu, Abdelhamid *et al.*, Khattab *et al.*, Shalaby *et al.*, and El-Dakar *et al.*

1.3. Garlic as Antimicrobial Activity

Garlic has antibacterial effect against Aeromona hydrophila and make tilapia more resistant to infection by A. hydrophila ^[31]. Nya and Austin reported that the use of garlic-supplemented diets for 14 days led to a marked reduction in mortality after challenge with A. hydrophila. Only 4% mortality was recorded in groups fed 0.5 and 1% garlic-mixed feed compared to 88% mortality in the control group. Sahu et al. obtained similar results for controlling A. hydrophila infection in Labeo rohita fingerlings, and they noted that the 0.1 and 0.5% added groups showed the highest level of survival (85%) even compared to the control group (57%). Aly and Mohamed also found that O. niloticus fed a 3% garlic-supplemented feed showed a significantly increased survival rate (85%) even after infection with A. hydrophila. Zhang studied the inhibitory effects of garlic on two isolates of A. hydrophila, AH1 and AH2, in vitro and found that the minimum inhibitory concentrations (MICs) were 15.6 and 1.95 mg mL⁻¹, respectively.

Rahman et al. evaluated the efficacies of antibiotics and medicinal plants on three common bacterial fish pathogens: A. hydrophila, P. fluorescens, and E. tarda. They found that young Thai silver barb, Barbonymus gonionotus fed a diet supplemented with 8 mg mL⁻¹ garlic showed the best recovery rate (90%) during the 10-days experimental period. However, Deresse reported that dilute solutions of garlic completely inhibited the growth of Staphylococcus aureus at concentrations greater than 7.50 mg mL⁻¹ (15.00-60.00 mg mL⁻¹) with an S. aureus inoculum density of 10⁴ CFU/mL. Using the same protocol, garlic had a bactericidal effect at 30 mg mL⁻¹ using a clinical isolate of S. aureus. This is different from the bactericidal concentrations reported by Sivam et al. (160 μ g.mL⁻¹) and Rees *et al.* (0.6-1.3 mg mL⁻¹). These differences may be due to different garlic species in different countries, processing differences, or the inoculum density. Rattanachaikunsopon and Phumkhachorn found that an aqueous extract of A. sativum had an MIC>500 μ g mL⁻¹ in *O. niloticus* infected with Streptococcus agalactiae, a major fish pathogen causing streptococcosis, in contrast to the results of Lee and Musa. Thus, the efficacy of garlic can vary widely due to differences in processing conditions, species, or biological conditions. Garlic had no bactericidal effect on Myxococcus piscicola; however, the combination of garlic and Eucommia ulmoides could kill M. piscicola at a concentration of 25 mg mL⁻¹. Garlic can work synergistically with other medicinal plants; however,

more research is needed before such treatment is put into practice ^[39].

1.4. Garlic as an Immunostimulant

The excess use of antibiotics has resulted in serious health and environmental problems. Consequently, the need of safe and effective alternatives of antibiotics is felt. In this context, immunostimulants have attracted significant attention. Garlic as a natural antibiotic is one of the most natural immunostimulants. Garlic effective has antioxidant properties [40] and also has beneficial effects on the cardiovascular and immune systems ^[41]. Generally, garlic takes effect by facilitates the function of phagocytic cells and increases their bactericidal activities; however, it can also stimulate natural killer cells, complement, lysozyme, and the antibody responses of fish. The activation of these immunological functions is associated with increased protection against infectious disease in fish. Garlic accelerates phagocytosis by macrophages ^[42]. Martins et al. [43] verified that the addition of garlic to fish diets increased the erythrocyte number, hemoglobin content, hematocrit, leukocyte number, and thrombocyte number.

Garlic supplementation induced significant changes in serum total protein and globulin in rainbow trout ^[13]. The increases in the serum total protein, albumin, and globulin contents reflect strong innate immunity ^[44]. These findings are in agreement with the findings of Kyo *et al.*, Iranloye, Ndong and Fall, and Nya and Austin. Aly *et. al.* suggested that garlic improved the immune response of *O. niloticus* via a rapid increase in monocytes, and that over a longer time frame it enhances phagocytic activity, which affords increased protection against an immediate challenge with *A. hydrophila*, illustrating the anti-infection properties of garlic.

1.5. Garlic as an Antiprotozoal Agent

Garlic is effective in treating intestinal parasites has been known for a long time. An extract of garlic was effective against a host of protozoa such as *Opalina ranarum*, *Opalina dimidicita*, *Balantidium entozoon*, *Entamoeba histolytica*, *Trypanosoma*, *Leishmania*, *Leptomonas*, and *Crithidia*^[14]. In addition, it was efficacious at killing wild-type amoebae isolated from the diseased fish, slowing the clinical signs of amoebic gill disease (**AGD**). However, it is necessary to study the toxicity and pathological effect of garlic on Atlantic salmon before using garlic to treat AGD in farmed Atlantic salmon.

2. Conclusion

It has been proved that garlic has immunomodulatory properties and is well capable of enhancing protection against pathogens. Hence, it can be concluded that garlic supplemented diets in fish enhance growth rate and improves the immune response in aquaculture. Garlic can also be used as an alternative to antibiotics or chemotherapeutic agents; however, further research is needed under practical conditions.

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