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THE USE OF GARLIC (*Allium sativum*) AS AN IMMUNOSTIMULANT IN AQUACULTURE

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ABSTRACT

The improvement of fish immunity is a critical factor in successful aquaculture. In recent years, the use of garlic (*Allium sativum*) as an immunostimulant has attracted researchers and farmers' interest in enhancing fish's health and disease resistance. This abstract discusses an overview of the use of garlic as an immunostimulant in aquaculture. Garlic is widely known for its antimicrobial, antioxidant, and immunostimulant properties. Several studies have evaluated the effects of garlic supplementation on various species of cultured fish. The results have shown that the administration of garlic in fish feed can enhance immune responses, such as increased immune enzyme activity and antibody production. Furthermore, active components in garlic, such as allicin and diallyl sulfide, have been proven effective against fish pathogens such as bacteria, viruses, and parasites. However, garlic as an immunostimulant in aquaculture still requires further research. Factors such as the appropriate dosage, interactions with farming conditions, and a deeper understanding of the underlying mechanisms must be considered. To optimise the use of garlic, further research is required to explore optimal dosages, interactions with environmental factors, and a better understanding of its effects on fish immune responses. In conclusion, garlic as an immunostimulant in aquaculture shows promising potential. The oral administration of garlic extract through the fish feed is capable of preventing infections by *Aeromonas* sp. and *Aeromonas hydrophila* in fish species such as Tawes, Catfish, Dumbo catfish, and Nile tilapia, while also enhancing non-specific immunity, such as increased total hematocrit, total haemoglobin, total erythrocytes, and phagocytic activity.

INTRODUCTION

Indonesia has enormous potential for aquaculture. The country is geographically favourable, with a long coastline, abundant rivers, and various aquatic ecosystems, including lakes, swamps and ponds. Several types of fish that are popular for cultivation in Indonesia

include catfish (*Clarias* sp.), Gurami (*Osphronemus* sp.), Milkfish (*Chanos chanos*), Tilapia (*Oreochromis niloticus*) and Catfish (*Pangasianodon hypophthalmus*) (Soegianto, 2019). In addition, many other types of fish have the potential for cultivation in Indonesia, such as carp, pomfret, eel, and saltwater fish such as sea milkfish, grouper and shrimp. The Indonesian government has also encouraged aquaculture development through various programs and policies, including procuring superior seeds, training farmers, and monitoring water quality.

However, paying attention to environmental and sustainability aspects in aquaculture is essential. The use of environmentally friendly cultivation techniques, good management, and monitoring of fish health need to be implemented to ensure the long-term success of aquaculture in Indonesia. Increasing growth, balanced nutritional intake, and disease control in aquaculture are necessary to achieve the desired production goals. One option that can be used to accomplish this is using immunostimulants, which can increase growth and power disease by increasing the fish's immune system (Sihotang et al., 2022).

Immunostimulant is a substance or material that can stimulate an immune response in the fish's body. By enabling the immune system, immunostimulants help increase fish resistance to disease, reducing fish mortality and increasing survival rates. In addition, immunostimulants can also positively affect fish growth, speed up the wound healing process, and increase the efficiency of feed utilisation. Using immunostimulants, aquaculture can optimise its productivity by reducing losses due to disease and improving overall fish growth. This is important to achieve better cultivation performance and improve production yields sustainably (Nuryati, 2006).

Immunostimulants have been an excellent alternative to chemicals or drugs in fish and shrimp farming. The advantage of immunostimulants is that they do not leave residues in the fish's body and are safe for the surrounding environment. In addition, immunostimulants also can increase resistance to infectious diseases in fish and shrimp. Using immunostimulants as a substitute for chemicals or drugs in fish and shrimp farming provides several advantages. First, immunostimulants do not leave residues in the fish's body, so they do not have the potential to contaminate the fishery products they produce. This is crucial in maintaining the quality and food safety of fish humans consume. In addition, the use of immunostimulants is also safe for the surrounding environment. In some cases, using chemicals or drugs can negatively impact the aquatic environment, such as water pollution and ecosystem damage. Immunostimulants can minimise this risk because immunostimulants generally come from natural ingredients that do not hurt the environment (Darwatin et al., 2016).

Immunostimulants have increased non-specific defence mechanisms and disease resistance in farmed fish. One of the known immunostimulants is garlic (*Allium sativum*) (Winarsih, 2002). In the context of aquaculture, the use of garlic has been shown to stimulate growth, boost the immune system, and encourage feeding responses in fish. This effect is associated with the content of organosulfur compounds, especially allicin, which is found in garlic (Fauziah & Wahjuningrum, 2015).

Combining immunostimulants with feed effectively increases non-specific defence systems in fish (Andriani & Hastuti, 2017). Adding garlic extract to the meal can improve the fish's body defence system. The allicin content in garlic can activate non-specific immune responses by increasing cytokine gene expression (Hasrianda & Setiarto, 2022).

Garlic is a good choice because it is easy to obtain and safe to use in aquaculture. Haditomo (2017) reported that using garlic as an antibacterial can increase fish survival, stimulate the immune system, and improve organ function related to blood cell formation. Garlic contains bioactive compounds such as allicin, which enhances the fish's immune system

(Andriani & Hastuti, 2017). In addition, active ingredients in garlic, such as allicin, diallyl sulfide, and saponins, have been shown to have antimicrobial properties that are effective against various fish pathogens, including bacteria, viruses, and parasites. This provides an additional advantage in disease control in aquaculture. Thus, immunostimulants from natural ingredients such as garlic are a practical choice to improve growth performance and control disease in aquaculture. By increasing the immunity of fish, immunostimulants can be an essential strategy to optimise fish production.

RESULTS AND DISCUSSION

Aquaculture in Indonesia does face several problems related to the disease. Some common issues in aquaculture are bacterial, viral, parasitic and fungal infections (Koniyo, 2020). These diseases can cause mass fish mortality, decreased growth, and economic losses for farmers.

One of the factors contributing to the spread of the disease is the high stocking density in ponds or fish cages. Excessive stocking densities can create an unhealthy environment for fish, facilitate the spread of disease, and reduce fish resistance to infection. In addition, using water not of good quality, nutritionally unbalanced feed, and stress due to non-optimal environmental conditions can also exacerbate the situation and trigger disease (Afrianto et al., 2015).

The use of immunostimulants from garlic is an alternative that can be used in controlling diseases in aquaculture. Garlic has been shown to have immunostimulating properties, which can boost the fish's immune system, helping fight disease infections (Nugroho & Nur, 2018). Active compounds such as allicin, diallyl sulfide, and saponins present in garlic have antimicrobial properties that are effective against fish pathogens, including bacteria, viruses, and parasites. In addition, garlic can increase fish non-specific immune responses, such as increasing the number of monocytes and phagocytosis activity. Garlic extract can be added to fish feed or used as a marinade for fish before being transferred to the cultivation environment (Yulihastiana et al., 2021). Using garlic as an immunostimulant is expected to increase fish resistance to disease, reduce mortality, and improve fish growth.

Garlic (*A. sativum*) is a well-known medicinal plant as an immunostimulant (Wahjuningrum et al., 2010). The allicin content in garlic can activate the non-specific immune system by increasing cytokine gene expression, so adding garlic extract to feed can improve the body's defence system (Hismah et al., 2022). Andriani & Hastuti (2017) also stated that the allicin found in garlic could significantly boost the fish’s immune system, making it an effective immunostimulant. Garlic can increase fish's immune system to fight disease and maintain health (Hismah et al., 2022) by increasing the number of monocytes and phagocytic activity. Several studies have used garlic as an immunostimulant, including cultivating catfish seeds. This study revealed that the best concentration of garlic to increase the survival of catfish was 10 g/kg of feed. The same thing was observed by Andriani & Hastuti (2017) in their study regarding the optimal dose of garlic extract in tawes seed feed, where 10 g/kg of feed provided the highest increase in non-specific immune response compared to other doses, thereby increasing survival, life, and grow tawes seeds better.

One option that can be used as an immunostimulant is garlic (*A. sativum*). Garlic can be chosen because of its easy availability and safety in cultivation. Lukistyowati & Kurniasih (2011) reported that garlic has antibacterial properties that can increase fish survival and stimulate the immune system and organ function related to the formation of blood cells.

Garlic contains an active compound called allicin, which boosts the fish's immune system. Based on this information, garlic has the potential as an immunostimulant in catfish culture, which can be used to prevent bacterial infections in fish. Therefore, research is needed to evaluate the effect of using garlic as an immunostimulant in catfish infected with *A. hydrophila* bacteria. This study aimed to understand the impact and determine the optimal dose of garlic extract (*A. sativum*) on the survival and blood profile of catfish infected with *A. hydrophila* bacteria.

To optimise garlic as an immunostimulant in aquaculture, further research is also needed to understand the mechanism of action in more depth. These studies could involve analysing the active components in garlic, such as allicin and diallyl sulfide, and their interactions with the fish's immune system.

Table 1. Use of Garlic (*Allium sativum*) as an immunostimulant in fish

No.	Fish	Methods	Results Improve	Against Disease	References
1.	Tawes (*Puntius java*)	Oral (by mixing garlic extract at 10 g/kg feed)	• Survival rate • Growth rate	*Aeromonas* sp.	Andriani & Hastuti, 2017
2.	Catfish (*Pangasius* sp.)	Oral (by mixing garlic extract at 10 g/kg feed)	• Survival rate • Growth rate • Total hematocrit • Total haemoglobin • Total erythrocytes • Total leukocytes	*Aeromonas hydrophila*	Haditomo, 2017
3.	Catfish (*Clarias gariepenus*)	Oral (by mixing garlic extract at 30 g/kg feed)	• Total hematocrit • Total haemoglobin • Total erythrocytes • Total leukocytes	*Aeromonas hydrophila*	Sulistyaningrum, 2016
4.	Nile Tilapia (*Oreochromis niloticus*)	Oral (by mixing garlic extract at 10 g/kg feed)	• Survival rate • Absolute weight • Total leukocytes • Phagocytosis activity	*Aeromonas hydrophila*	Hismah *et al*., 2022

The use of garlic as an immunostimulant in aquaculture shows exciting potential. The use of garlic extract orally through the fish feed can resist *Aeromonas* sp. and *Aeromonas hydrophila* in Tawes, Catfish, Dumbo catfish and tilapia and increases non-specific immunity such as increasing total hematocrit, total haemoglobin, total erythrocytes and phagocytosis activity (Andriani & Hastuti, 2017; Haditomo, 2017; Sulistyaningrum, 2016; Hismah et al., 2022).

Based on several studies in Table 1, the application of garlic extract has been shown to impact the immune response and growth of fish. The results of this study indicate that garlic extract can increase the immune response in fish fry, such as increasing total leukocytes and

phagocytic activity, which is better than the control group (without garlic extract) and after challenge testing with *A. hydrophila* bacteria. The increase in the total leukocyte count during the rearing period was due to the allin content in garlic. According to Marentek et al. (2013), allin present in garlic significantly increases the reproduction of leukocyte cells.

In addition to affecting the total leukocyte count, garlic contains diallyl disulfide, increasing phagocytic activity in fish. According to Lukistyowati & Kurniasih (2011), diallyl disulfide can boost non-specific immunity by stimulating phagocytic activity and cells involved in the immune response. The results showed that at the end of rearing and after challenge testing with *A. hydrophilla* bacteria, adding garlic extract as much as 10 g/kg of feed gave the highest survival rate for tilapia seeds, 82% and 90%, respectively. At the same time, the control had the highest yield—lowest survival rate (Hismah et al., 2022). In addition to increasing the survival of tilapia fry, garlic extract also significantly increased the absolute growth of fish fry. The high growth rate of tilapia fry-fed feed containing garlic extract, especially at a dose of 10 g/kg of feed, is thought to be due to the allicin content. In addition to having antimicrobial properties, allicin stimulates the movement of the digestive tract, increasing the production of enzymes that help digest food (Pahrurodji, 2019). In addition, garlic can change the aroma and taste of feed, affecting the preference for tilapia for dinner. With good feed consumption, the growth of tilapia will also increase.

After the challenge test, there was an increase in the total leukocyte count outside the normal range. This may be caused by *A. hydrophila* infection, according to the research of Lukistyowati & Syawal (2013), which stated that the condition and health of the fish's body could affect the number of leukocytes. Leukocytes are blood cells that play a role in the immune system, helping to rid the body of pathogens and responding to foreign invasion through the immune system and other immune responses. When fish are sick, leukocyte production increases to fight bacteria and produce antibodies. An increase in leukocytes correlates with increased antibody production and fish recovery from exposure to pathogens or toxic substances.

When given an immunostimulant orally, the fish that received the treatment experienced increased total leukocytes. This response indicates that immunostimulants stimulate fish to boost their immune system by increasing the number of leukocytes which play a role in non-specific defence. Leukocytes are components of blood cells that localise and eliminate pathogens through phagocytosis (Sudiono, 2014)

After infection with *A. hydrophila* bacteria, there was an increase in total erythrocytes. This is caused by garlic as an immunostimulant which enhances the fish's immune system. Haditomo (2017) reported that the protein component in garlic works as an immunostimulator by increasing cell function and activity. However, at 21 days of maintenance after infection, there was a decrease in total erythrocytes. This is presumably because the hemolysin in *A. hydrophila* bacteria causes erythrocyte cell lysis. Pulungan et al. (2022) stated that hemolysin from *A. hydrophila* bacteria can cause lysis of red blood cells. However, at 28 days of rearing, there was an increase in total erythrocytes, indicating that the fish were healthy.

The haemoglobin level on the 14th day of maintenance ranged from 6.40-7.90 G%, within the normal range. After bacterial infection, there is a decrease in haemoglobin levels, which is thought to be caused by impaired binding of oxygen in the blood due to bacterial attack. However, on the 28th day of rearing, the haemoglobin level of catfish increased, indicating that the administration of garlic extract can improve the fish's immune system (Haditomo, 2017).

The hematocrit level on the 14th day of maintenance was within the normal range (22.67-31.67%). After bacterial infection, there was a decrease in hematocrit levels which was thought to be caused by the attack of *A. hydrophila* bacteria on the fish's body. However, at 28 days of maintenance, the hematocrit level increased, indicating that the fish were healthy (Maryani, and Rosdiana, 2020).

Although research shows positive results, garlic as an immunostimulant in aquaculture needs to consider factors such as the correct dose and environmental conditions such as water temperature, salinity, and pH. Inappropriate doses may not be practical, while doses that are too high can have adverse side effects for fish. Therefore, further research is needed to determine the optimal dosage for each fish species. In addition, environmental factors also need to be considered when using garlic as an immunostimulant in aquaculture.

CONCLUSION

The use of garlic as an immunostimulant in aquaculture shows exciting potential. The use of garlic extract orally through the fish feed can resist *Aeromonas* sp. and *Aeromonas hydrophila* on Tawes, Catfish, Dumbo catfish and tilapia and increase non-specific immunity such as increased total hematocrit, total haemoglobin, total erythrocytes and phagocytosis activity. Several studies have proved that garlic supplementation in fish feed can enhance immune response, immune enzyme activity, and resistance to pathogens. The active components in garlic, such as allicin and diallyl sulfide, have antimicrobial properties that are effective against various fish pathogens. However, it is vital to consider the correct dosage and environmental factors that may affect the fish's immune response to garlic. Further research is needed to identify optimal doses and understand interactions with cultivation conditions. Overall, garlic as an immunostimulant in aquaculture offers the potential to improve the health and resistance of fish to disease. However, further research is needed to support the effective use of garlic, including involving various fish species and deepening understanding of its mechanism of action. With better experience, garlic can be a valuable strategy for improving the overall performance of aquaculture.

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