



# Effectiveness of dietary supplementation with *Aloe vera* and garlic (*Alium sativum*) on growth, hematology and immunity of North African catfish (*Clarias gariepinus*)

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**Abstract.** Herbal supplements are starting to be widely used to improve the performance and growth of fisheries commodities because they are safe and do not leave a residue. To increase fisheries productivity, intensive cultivation is carried out but this can cause problems. In intensive cultivation, high stocking densities are applied, resulting in deposits of leftover feed and feces at the bottom of ponds. The accumulation of food waste and settled feces can produce ammonia and nitrite which causes water quality to decrease. Decreased water quality will inhibit fish immunity and growth. To overcome this problem, supplements are needed that act as immunostimulants and growth promoters of the fish immunity and growth rate. The application of herbal supplements is safer to use and does not cause environmental pollution. The aim of this research was to determine the effectiveness of adding *Aloe vera* flour supplements through feed (diet) on the growth and immunity of *Clarias gariepinus* kept for 40 days compared to garlic supplements. The research method was carried out experimentally using a completely randomized design with 3 treatments and 1 control, each with 4 replications. The research results showed that the aloe vera diet and garlic diet could significantly increase growth and non-specific immunity. However, the results show that the ability and effectiveness of *Aloe vera* in increasing the growth and immunity of *C. gariepinus* is higher than that of garlic.

**Key Words:** herbal immunostimulant, fish, growth promoter, immune non-specific.

**Introduction.** Aquaculture is one of the fastest growing food production and procurement sectors in the world. This is related to the increasing global demand for products derived from aquaculture. With the decline in fish catches due to excessive exploitation of fishery resources, aquaculture is the only alternative to meet market demand for consumer needs derived from fish (Ayoola & Ishola 2020). One of the fish that is widely favored by consumers is catfish. This is because catfish are easy to cultivate, have a delicious taste and are relatively cheap. As a result, market and consumer demand increases so that intensive cultivation is then carried out (Fais & Nurohman 2023).

Intensive cultivation can cause problems such as increased organic matter in the water due to piles of feces and leftover feed which can produce ammonia. Ammonia that remains and accumulates at the bottom of the pond can cause toxins that are dangerous to farmed fish. The accumulation of ammonia to toxic levels causes the fish's incapacity to extract energy from feed efficiently, therefore becoming weak, lethargic, comatose and dying (Hargreaves 2004). This is because intensive cultivation uses high stocking densities. High stocking densities cause water quality to decline due to the accumulation of feces so that fish are easily stressed and susceptible to infectious diseases. To overcome this, antibiotics are generally used. However, the use of antibiotics is not recommended because it can cause negative impacts such as resistance, leaving residues in the fish's body so that it is dangerous for consumers. Overcoming this can be done by increasing immunity

through the administration of immunomodulatory compounds that can also increase growth. One of them is by using herbal supplements.

Research on herb supplements, treatment, and prevention has been widely reported but is still relatively new in the field of aquaculture compared to livestock and poultry (Gabriel et al 2019). *Aloe vera* is an herbal plant that is easy to grow and economically cultivated. *Aloe vera* contains many important bioactive compounds such as galactose-mannan, beta-mannan, glycoproteins, lectins, salicylic acid, phenols, and sulfur. The report on the results of research by Kaur & Ansal (2020) stated that *Aloe vera* supplementation can increase fish growth and survival. The increase in growth includes weight gain, a higher specific growth rate, a lower feed conversion ratio, and an increase in the quality of meat at a dose of 1-3%. Furthermore, it was reported by Prasetio et al (2018), that the addition of *Aloe vera* powder supplements through feed at a dose of 40 g kg<sup>-1</sup> feed can produce a high rate of weight growth and immunity to reduce Motil *Aeromonas* Septicemia (MAS), an infection with *A. hydrophila* bacteria in biawan fish. Not many researchers have focused on the benefits and functions of *Aloe vera* despite its importance for the development and strengthening of the fisheries sector. *Aloe vera* reported as stimulating the growth and immunity of fishery commodities. Mahdavi et al (2013) reported that the addition of *Aloe vera* extract can significantly affect the growth of weight and length gain (WG and LG), feed conversion ratio (FCR) and specific growth rate (SGR) compared to controls with a maximum dose of 2.5% of feed in goldfish. Some reports show the ability of *Aloe vera* in boosting immunity, by significantly increasing the immune non-specific response of *Oreochromis niloticus* (Syed et al 2022) in the number of white blood cells (leucocytes). This increment is related to all the differential leucocytes which have an important function to prevent bacterial infection (Prasetio et al 2018).

In addition to focusing on growth, the fish immunity system also needs to be improved because the immunity of fish is relatively low compared to other vertebrates. The use of supplements to improve the immunity of fish began to be widely used, in addition to growth. The addition of *Aloe vera* supplements at doses of 0.2 and 0.5% has a better effect on the parameters of hematological immunity and growth of shirbot *T. grypus* fish (Safari et al 2019). This condition is reinforced by Mahdavi et al (2013), which show that the administration of *Aloe vera* extract can stimulate appetite, is tonic and immunomodulatory and reduces stress so that it has a positive impact on growth (Mahdavi et al 2013).

*Aloe vera* is not yet popular in its use for growth and immunostimulants. Even the herbal garlic (*Allium sativum*) is better known as a noticeable compound in boosting immunity, as reported by Pashaki et al (2018), who added a garlic supplement at a dose 5 g kg<sup>-1</sup> of feed, which could increase immunity in *Cyprinus carpio* fingerlings, but also improve some blood parameters and even the growth. A similar report of Setijaningsih et al (2021) proved that supplemented of garlic through feed improves the growth, increasing the total number of erythrocyte and level hemoglobin in *O. niloticus*. Therefore, it is necessary to know the extent of *Aloe vera*'s ability to improve growth and immunity in fisheries commodities. This study aimed to determine the effectiveness of *Aloe vera* and *Allium sativum* supplements given through feed on *Clarias gariepinus*'s growth and immunity. The effectiveness of *Aloe vera* was compared with the ability of garlic to promote growth and immunity in *C. gariepinus*.

## Material and Method

**Procedures.** This study used *Aloe vera* and garlic (*Allium sativum*) powder as a supplement. *Aloe vera* powder is added to the feed according to the treatment dose and stirred evenly. Feed containing *Aloe vera* and garlic powder separately was given to *C. gariepinus* for 40 days of study. The research method was carried out experimentally using a complete randomized design (RAL) with 3 treatments and 1 control, each with 4 replications.

*Aloe vera* and garlic herbs were separately dried, then powdered. The treatment was in the form of the addition of *Aloe vera* and garlic powder given through feed separately. The dosage of feed given is 3% of the weight of the fish biomass. Feed was

given 2 times a day, in the morning and evening. The treatment of herbs addition of both *Aloe vera* and garlic powder through feed in this study used the same dosage, as follows:

Table 1

Research on herbal supplementation of *Aloe vera* and *Allium sativum* in *Clarias gariepinus*

<i>Treatment</i>	<i>Aloe vera powder</i>	<i>Allium sativum powder</i>
Control	0 g kg <sup>-1</sup> feed	0 g kg <sup>-1</sup> feed
Treatment 1	20 g kg <sup>-1</sup> feed	20 g kg <sup>-1</sup> feed
Treatment 2	30 g kg <sup>-1</sup> feed	30 g kg <sup>-1</sup> feed
Treatment 3	40 g kg <sup>-1</sup> feed	40 g kg <sup>-1</sup> feed

The observed growth parameters include: daily growth rate (SGR), length gain (LG), feed conversion ratio (FCR) and feed efficiency (FE). While the hematology and immunity parameters are in the form of hematocrit percentages and differential leukocytes of lymphocyte of and monocytes.

**Statistical analysis.** The data obtained are expressed in the form of an average value. To find out whether the treatment given has an effect on the parameters, an analysis of variance (ANOVA) was carried out at a confidence level of 95%. Where the data had a significant difference, it was continued with the Duncan Multiple Range Test (DMRT) test at a level of 95%.

**Results and Discussion.** The study was conducted to determine the effectiveness of adding *Aloe vera* powder supplements through feed with different doses on the growth, hematology and immunity of *C. gariepinus* compared to garlic. The observed parameters include the SGR, LG, FCR, FE, hematocrit, and differential leukocytes of lymphocytes and monocytes

**Daily growth rate (SGR) and length gain (LG).** Growth is a change in body shape in length, weight and volume according to changes in time. In fish growth is influenced by internal and external factors. Internal factors include age, genetics and endurance while external factors consist of feed given, stocking density, water quality and environment. The results of the study proved that the addition of either *Aloe vera* or garlic powder supplements through feed can significantly increase the SGR and LG of *C. gariepinus* ( $p < 0.05$ ) (Table 2). This is strongly suspected to be due to the influence of the addition of *Aloe vera* and garlic powder supplements through feed.

Table 2

Daily growth rate (SGR) and length gain (LG) in *Clarias gariepinus* fed with aloe and garlic powder supplements during the 40 days study

<i>Treatment</i> (g kg <sup>-1</sup> feed)	<i>Parameter</i>	<i>Aloe vera powder</i>	<i>Allium sativum powder</i>
Control	SGR	33.87±4.7 <sup>a</sup>	0.07±0.01 <sup>a</sup>
	LG	6.57±0.17 <sup>a</sup>	0.91±0.12 <sup>a</sup>
20	SGR	36.88±5.93 <sup>a</sup>	0.11±0.20 <sup>b</sup>
	LG	7.51±0.40 <sup>b</sup>	1.16±0.13 <sup>ab</sup>
30	SGR	39.74±1.76 <sup>ab</sup>	0.14±0.15 <sup>c</sup>
	LG	8.77±0.22 <sup>c</sup>	1.56±0.28 <sup>bc</sup>
40	SGR	44.59±1.60 <sup>c</sup>	0.15±0.01 <sup>c</sup>
	LG	9.82±0.27 <sup>d</sup>	1.67±0.42 <sup>c</sup>

Values are represented as the means ± SD; different superscript letters in a row show significant difference ( $p < 0.05$ ).

Table 2 shows that supplementation of *Aloe vera* and *Allium sativum* significantly affected SGR compared to the control at a dose of 40 g kg<sup>-1</sup> of *Aloe vera* and all treatment

doses of *Allium sativum*. Likewise, *Aloe vera* and *Allium sativum* supplementation was significant for LG compared to control at all doses for *Aloe vera* and at doses of 30 and 40 g kg<sup>-1</sup> feed for *Allium sativum*. Supplementation of both *Aloe vera* and *Allium sativum* resulted in significant SGR and LG growth compared to the control, but the growth value of SGR and LG was higher with *Aloe vera* supplementation than with *Allium sativum*. This proves that *Aloe vera* supplementation is stronger than *Allium sativum* for the growth of SGR and LG in *C. gariepinus*.

It is clear that *Aloe vera* is the main growth supplement for *C. gariepinus* in this study. This is in accordance with the statement of Prasetio et al (2018), that giving *Aloe vera* powder mixed in feed at the right dosage has a positive effect on increasing appetite and strengthening immunity so that it can stimulate fish growth. A research conducted by Kaur & Ansal (2020) proved that the addition of *Aloe vera* powder to the feed can increase the weight and final length in carp (*Labio rohita* Ham.). Meanwhile, the growth indicators achieved through garlic supplementation are relatively low.

*Aloe vera* supplementation in food can serve as a growth promoter, appetite enhancer and immunostimulant, reducing stress and food loss and protecting fish, while stimulating growth (Mahdavi et al 2013). Further, Syed et al (2022) also proved that the *Aloe vera* diet in tilapia, *O. niloticus*, can increase final growth, SGR and survival as well as improve visceral organ index performance (VSI). The ability of *Aloe vera* is not only shown by morphological and meristic improvements that can be visually observed as an increased growth, but also by an improvement in the performance of visceral organs. As reported by Khanal et al (2021), goldfish fed *Aloe vera* supplements between 0.4%-0.8% during the 90 days study can improve body weight, gastrosomatic index, condition factors, and intestinal histology resulting in better growth performance. So far there have been no reports of garlic supplementation being so effective in promoting growth. The latest report submitted by Samson (2019) stated that the supplementation of 1.5% garlic resulted in a significant survival rate and feed utilization while SGR increased but not significantly.

As reported by Mahdavi et al (2013), the addition of *Aloe vera* extract in the feed was able to increase the SGR significantly compared to controls, with an optimum dose of 2.5%. The addition of *Aloe vera* powder can also increase SGR and weight growth in *O. niloticus*. Supplementation of 20 g of *Aloe vera* into the feed can affect the overall health status of the fish through a laxative effect of *Aloe vera* which can increase water content in the intestines and peristaltic activity (Ayoola & Ishola 2020). The high SGR value indicates that the growth is getting better. Still regarding the effect of *Aloe vera* on SGR, as reported by Sharif Rohani et al (2017), the addition of *Aloe vera* supplements in nanoparticle size to feed may improve the growth performance of Siberian sturgeon fish, such as SGR, weight gain as well as weight and final length. The administration of 0.4-0.8% *Aloe vera* through feed can significantly increase the SGR ( $p < 0.05$ ) in goldfish (Khanal et al 2021).

Based on the results of this study, garlic supplementation increases SGR and LG but to values lower than *Aloe vera* supplements. However, garlic supplements are more important in prevention against diseases. Militz et al (2013) showed how the prevention against infectious diseases in fish can most practically be done by garlic supplementation. This method is declared practical and relatively optimal in particular for the prevention of *Neobenedenia* sp. infection in mariculture.

**Feed conversion rate (FCR) and feed efficiency (FE).** In fish farming, it is necessary to measure the feed conversion value to find out how much feed is needed to increase the fish biomass weight with 1 kg. A low FCR value indicates better feed quality in converting it into biomass weight. In addition to the FCR value, feed efficiency is also measured to determine the level of feed efficiency (%) given to the farmed fish (Table 3).

The administration of *Aloe vera* and garlic supplements through feed separately in this study was also able to significantly increase the FCR ( $p < 0.05$ ) compared to controls. However, when reviewed for its effectiveness, *Aloe vera* supplements are much more effective and optimal than garlic supplements.

Table 3

Feed conversion ratio (FCR) and feed efficiency (FE) in *Clarias gariepinus* fed with aloe and garlic powder supplements during the 40-day study

Treatment (g kg <sup>-1</sup> feed)	Parameter	<i>Aloe vera</i> powder	<i>Allium sativum</i> powder
Control	FCR	3.77±0.65 <sup>a</sup>	15.27±3.50 <sup>a</sup>
	FE	27.17±4.95 <sup>a</sup>	6.90±2.02 <sup>a</sup>
20	FCR	2.06±0.22 <sup>a</sup>	10.90±3.07 <sup>ab</sup>
	FE	49.15±5.71 <sup>b</sup>	9.70±2.57 <sup>a</sup>
30	FCR	1.85±0.36 <sup>a</sup>	7.17±1.65 <sup>b</sup>
	FE	55.79±12.01 <sup>bc</sup>	14.64±4.07 <sup>b</sup>
40	FCR	1.57±0.23 <sup>b</sup>	5.52±0.80 <sup>c</sup>
	FE	64.82±9.33 <sup>c</sup>	18.43±2.89 <sup>b</sup>

Values are represented as the means ± SD; different superscript letters in a row show significant differences ( $p < 0.05$ ).

Several previous studies have reported that adding *Aloe vera* to feed can improve FCR and FE. *Aloe vera* supplementation (both gel and extract) can be added up to 2% (20 g kg<sup>-1</sup> feed), but economically its productivity is more efficient when given only 1%. This situation is due to the fact that the cost of supplementation increases with the doses of *Aloe vera* administered through feed. This means that a dose of 1% will produce a higher efficiency value than a larger dose and automatically the coefficient ratio becomes more optimal (Khan et al 2018). Similarly, Sharif Rohani et al (2017) reported that the FCR values decreased lower than those in Siberian sturgeon fish which were given the addition of *Aloe vera* nanoparticle extract through their feed with an average value of 1.28-0.87. *Aloe vera* supplementation through feed can improve FCR and protein FE significantly (Khan et al 2018).

The high degree of efficiency and the low FCR value can be due to the content of active ingredients found in *Aloe vera*. *Aloe vera* contains nutrients and active ingredients that support growth including fatty acids, amino acids, vitamins and minerals. Amino acids function as ingredients for growth and energy sources, while the vitamin content in *Aloe vera* powder can affect the growth and can increase the appetite. Vitamins are organic compounds that are important for the growth, reproduction and health of fish as well as a booster of metabolism in the body of fish (Prasetio et al 2018). Furthermore, Darmawan et al (2018) stated that a low FCR indicates that the use of energy by fish for life activities is efficient, and the energy lost through excretion (feces and urine) is relatively reduced, so that fish have a large enough excess of energy that can be used for growth. Optimal growth can result in a low feed conversion ratio. On the other hand, the lower the value of FCR, the higher the utilization efficiency of the given feed.

FE significantly increases, in both *Aloe vera* and garlic supplementation. However, as in FCR, the FE value for the *aloe vera* treatment is still superior to the garlic treatment, in this study. FE is an indicator of the success of feeding in the optimization of growth. As explained by Arief et al (2014), feed is of high quality if the feed efficiency value is higher than 50%. Feed efficiency can be seen from several factors, one of which is feed conversion. Still according to Arief et al (2014), the optimal level of FE will be achieved if the conversion value of the feed produced is lower. Sharif Rohani et al (2017) reported that the addition of *Aloe vera* nanoparticle extract to the feed can significantly affect the FE value with an average value of 1.7-2.01, at an optimal dose of 10 g kg<sup>-1</sup> feed. Research on the use of *Aloe vera* for growth performance is more carried out in broilers, while for fish it is still limited. Furthermore, Zayed et al (2020) in their research on broiler chickens reported that giving *Aloe vera* gel through drinking water could significantly increase FE ( $p < 0.05$ ) and at the same time increase weight and improve feed conversion ratios compared to controls.

**Hematocrit.** Immunity parameters include the percentage of hematocrit, lymphocytes and monocytes. The increase in immunity parameters indicates a healthy status of fish, while

the decrease indicates a moderately poor one. *Aloe vera* and garlic supplements are needed to improve the health status of fish so that their immunity remains high or increases. Based on Table 3, the results this study shows that these two supplements (*Aloe vera* and garlic) are able to improve the immune quality of catfish. Extracts of medicinal plants as immunostimulants can increase non-specific defenses against pathogens during periods of stress. Studies show the effect of *Aloe vera* dry extract provides a hematological and immunological response in rainbow trout (*Oncorhynchus mykiss*) (Haghighi et al 2014). In general, *Aloe vera* has the ability to increase immunity and accelerate wound healing (Prasetio et al 2018). Similarly, garlic is also known to be able to increase hematocrit. Farahi et al (2010) reported addition of garlic *Allium sativum* to fish diet can increase percentage of hematocrit and erythrocyte number.

Table 4

Hematocrit (%) and differential leucocyte of lymphocyte (%) and monocyte (%) in *Clarias gariepinus* that were supplemented with aloe powder and garlic during the 40-day study

Treatment (g kg <sup>-1</sup> feed)	Parameter	<i>Aloe vera</i> powder	<i>Allium sativum</i> powder
Control	Hematocrit	30.13±0.07 <sup>a</sup>	11.45±0.01 <sup>a</sup>
	Lymphocyte	54.41±4.01 <sup>a</sup>	62.00±1.41 <sup>a</sup>
	Monocyte	7.12±1.82 <sup>a</sup>	2.48±0.05 <sup>a</sup>
20	Hematocrit	31.93±2.02 <sup>a</sup>	14.59±0.08 <sup>b</sup>
	Lymphocyte	62.24±1.91 <sup>b</sup>	71.50±1.29 <sup>b</sup>
	Monocyte	8.67±3.03 <sup>a</sup>	2.52±0.16 <sup>a</sup>
30	Hematocrit	36.2±2.33 <sup>b</sup>	14.65±0.07 <sup>b</sup>
	Lymphocyte	60.57±1.78 <sup>b</sup>	73.50±1.29 <sup>b</sup>
	Monocyte	7.40±1.84 <sup>a</sup>	2.53±0.07 <sup>a</sup>
40	Hematocrit	39.24±0.72 <sup>c</sup>	16.50±0.06 <sup>c</sup>
	Lymphocyte	64.00±3.79 <sup>b</sup>	76.25±1.25 <sup>c</sup>
	Monocyte	11.64±1.66 <sup>a</sup>	2.47±0.07 <sup>a</sup>

Values are represented as the means ± SE. <sup>abcj</sup>in a row with different superscript letters shows significant difference (p<0.05).

Like garlic, *Aloe vera* can also improve the quality of fish health. *Aloe vera* is useful for preventing the negative influence of environmental disturbances or stress, accelerating wound healing and increasing the body's defenses against bacterial infections (Takril et al 2021). *Aloe vera* supplementation in fish farming is used to improve the non-specific immune system in fish. Respiratory burst activity increased significantly (p<0.05) in fish treated with 1% *Aloe vera* extract. Apart from respiratory burst activity, phagocytic activity of blood leukocytes also increased significantly (p<0.05) in fish given 1% *Aloe vera* supplement (Haghighi et al 2014). The administration of *Aloe vera* powder mixed with feed at a dose of 40 g kg<sup>-1</sup> feed has a positive effect on increasing the broad immune response such as appetite, increase in hematocrit percentage, erythrocyte number and hemoglobin level, including number of leukocytes which are suspected to have a positive effect on weight gain and survival of biawan fish (Prasetio et al 2018). Moghaddam et al (2017) proved that the addition of *Aloe vera* extract to the feed can increase the percentage of hematocrit, red blood cell number and hemoglobin levels: a dose of 1.5% was effective in improving the hematological indicators of Siberian sturgeon fish. Hematocrit can describe the health status of fish: a hematocrit percentage below 22% indicates that the fish has anemia.

The low percentage of hematocrit also indicates the presence of contamination, fish lack of nutrients and stress. Prasetio et al (2018) stated that the hematocrit varies depending on nutritional factors and on the age of the fish. Fish fry with better nutrition have higher hematocrit levels than adult fish or fish fry with poor nutrition. The dietary effect of *Aloe vera* powder supplementation on the hematological indicators of rainbow trout showed a significant increase in hematocrit percentage, hemoglobin levels and red

blood cell number (Mehrabi et al 2019). The application of *Aloe vera* powder mixed with feed has a positive effect by increasing the response to feed, the number of erythrocytes and leukocytes, hematocrit and hemoglobin levels, in addition to also affecting both the weight gain and the survival of biawan fish. The best number of erythrocytes and leukocytes, hematocrit percentage, hemoglobin levels and changes in weight and survival of biawan fish are in the treatment of *Aloe vera* powder in a concentration of 40 g kg<sup>-1</sup> feed (Prasetio et al 2018). The results of a research conducted by Gabriel et al (2019) proved that the *Aloe vera* diet can increase the percentage of hematocrit and hemoglobin levels in catfish. In the current study, the results were the same as in the study conducted by Pashaki et al (2018) on carp (*Cyprinus carpio*) given a garlic supplement diet, which increased the percentage of hematocrit, hemoglobin levels and the number of red blood cells significantly.

**Lymphocyte.** The results of this study showed the same pattern, both *Aloe vera* and garlic supplementation significantly increased the percentage of lymphocytes in catfish. Syed et al (2022) stated that the diet with *Aloe vera* supplements has a positive effect on the non-specific immune response of tilapia *O. niloticus*. Tilapia fed an *Aloe vera* diet gives a positive immune response with an increase in the number of white blood cells (leukocytes). On this *Aloe vera* diet, the increase in white blood cells (leukocytes) includes the increase percentage of lymphocytes, neutrophils and monocytes. Similarly, garlic supplementation increases the percentage of lymphocytes but the value is lower than *Aloe vera* (Table 2). This is as reported by Zare et al (2021): in Eurasian perch fish given garlic supplementation causes white blood cells to increase significantly, as well as the percentage of lymphocytes, although not significantly. Leukocytes are part of the body's immune system or defenses that are non-specific in nature. Leukocytes play an important role in the body's immunity (immunity) to avoid disease (Prasetio et al 2018). This increase in the number of leukocytes on the addition of *Aloe vera* supplements results in good immunity and then promotes optimal growth with an increase in the specific growth rate and increased appetite in the fish. Giving *Aloe vera* powder mixed with feed can increase the immune system, as shown by the increase in the number of leukocytes, and indirectly affect weight gain and the survival of biawan fish (Prasetio et al 2018).

The addition of *Aloe vera* extract at a dose of 5 g kg<sup>-1</sup> feed can function as an immunostimulant so that it can improve the hematological performance of *O. niloticus* (Yunus et al 2021). The increase in hematological performance in rainbow trout fed *Aloe vera* supplements through feed, is caused by the bioactive lipopolysaccharide (LPS) compounds found in *Aloe vera*, which can induce leukocyte-forming cells such as lymphocytes, monocytes and neutrophils so that each of them increases (Mehrabi et al 2019). *Aloe vera* extract mixed in feed given to white snapper causes an increase in the percentage of lymphocyte but not significantly. An increase in the number of lymphocyte cells in white snapper indicates that the fish's immune system is increasing. Lymphocytes are not phagocytic but play an important role in the formation of antibody (Novita et al 2020). *Aloe vera* extract containing phenolic compounds can increase the differential components of leukocytes such as the increase in lymphocytes from 58.6% to 73.70% and neutrophils from 6.09 to 8% (Andayani et al 2020).

**Monocytes.** Similar to the lymphocyte pattern, the results of this study prove that the addition of *Aloe vera* and garlic powder through feed can increase the percentage of monocytes but the increase is not significant. A similar result was reported by Moghaddam et al (2017), the addition of *Aloe vera* extract to the feed did not have a significant effect on increasing the percentage of monocytes. Nevertheless, the percentage of monocytes and neutrophils increased but not significantly. Monocytes are cells that are able to penetrate the walls of capillary blood vessels and enter the tissues and differentiate into macrophage cells. The role of monocytes is very important as the main phagocyte cells for destroying various pathogens that attack on the body of fish. The normal percentage of monocytes in fish ranges from 6.65-29.20% (Muntasiroh et al 2020). However, in garlic supplementation, the increase in the monocyte range between 2.52-2.53 was lower than

that in the given *Aloe vera* (8.67-11.64). This shows *Aloe vera* as an immunostimulant ability higher than garlic.

Novita et al (2020) showed that *Aloe vera* supplementation on white snapper (*Lates calcarifer* Bloch) infected with *Vibrio* sp. bacteria causes the number of white blood cells, the percentage of monocytes and their survival to increase. *Aloe vera* extract containing phenolic compounds can increase the differential of monocyte from 5.70% to 7.72%. This suggests the addition of *Aloe vera* supplements can boost non-specific immunity through an increase in the percentage of monocytes. Therefore, herbs can function and act as an immunostimulant, such as *Aloe vera* (Andayani et al 2020) and garlic (Fazlolahzadeh et al 2011). Diet containing garlic powder (g kg<sup>-1</sup> of feed) in appropriate doses could increase immune non-specific and decrease mortality in rainbow trout (Fazlolahzadeh et al 2011).

**Conclusions.** The effectiveness of diet supplementation containing *Aloe vera* is by far superior to garlic in *C. gariepinus* growth and immunity. In terms of growth, *Aloe vera* and garlic supplementation can significantly improve the specific growth rate, length gain, feed conversion ratio, feed efficiency, but the values are higher in *Aloe vera* than in garlic. In the parameters of immunity, both *Aloe vera* and garlic can increase hematocrit and lymphocytes but their strength is higher in *Aloe vera* supplementation. Whereas in monocytes, both *Aloe vera* and garlic supplementation can increase monocyte slightly but not significantly. To improve immunity and growth in fish farming it is recommended to better use *Aloe vera* powder supplementation than garlic.

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**Conflict of interest.** The authors declare no conflict of interest.

## References

- Andayani S. R. I., Dadiono M. S., Elwira W. T., Setyawan F. H., 2020 Potency of aloe extract as immunostimulant for carp (*Cyprinus carpio*) against *Aeromonas salmonicida*. Biodiversitas 2:860–864.
- Arief M., Fitriani N., Subekti S., 2014 [The effect of giving different probiotics in commercial feed on the growth and feed efficiency of sangkuriang catfish (*Clarias* sp.)]. Jurnal Ilmiah Perikanan Dan Kelautan 6(1):5. [In Indonesian].
- Ayoola S. O., Ishola H. O., 2020 The growth performance and genotoxicity effect of dietary *Aloe vera* on *Oreochromis niloticus* juveniles. Aceh Journal of Animal Science 5(2): 92–97.
- Darmawan J., Tahapari E., Suharyanto, 2018 [Growth performance of siamese catfish (*Pangasianodon hypophthalmus* Sauvage, 1878) selection results in KJA darma kuningan reservoir, West Java]. Limnotek Perairan Darat Tropis Di Indonesia 25(2):88–96. [In Indonesian].
- Farahi A., Kasiri M., Sudagar M., Iraei M. S., Shahkolaei M. D., 2010 Effect of garlic (*Allium sativum*) on growth factors, some hematological parameters and body compositions in rainbow trout (*Oncorhynchus mykiss*). AACL Bioflux 3(4):317–323.
- Fais M. M., Nurohman D., 2023 [Comparative study between catfish farmers with sales systems through middlemen and independent sales]. Manura Ekonomi 10:33–43. [In Indonesian].
- Fazlolahzadeh F., Keramati K., Nazifi S., Shirian S., Seifi S., 2011 Effect of garlic (*Allium sativum*) on hematological parameters and plasma activities of ALT and AST of rainbow trout in temperature stress. Australian Journal of Basic and Applied Sciences 5(9):84–90.



- Gabriel N. N., Wilhelm M. R., Habte-Tsion H. M., Chimwamurombe P., Omoregie E., Iiping L. N., Shimooshili K., 2019 Effect of dietary *Aloe vera* polysaccharides supplementation on growth performance, feed utilization, hemato-biochemical parameters, and survival at low pH in African catfish (*Clarias gariepinus*) fingerlings. *International Aquatic Research* 11(1):57–72.
- Haghighi M., Rohani M. S., Samadi M., Tavoli M., Eslami M., Yusefi R., 2014 Study of effects *Aloe vera* extract supplemented feed on hematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*). *International Journal of Advanced Biological and Biomedical Research* 2(6):2143–2154.
- Hargreaves J. A., Tucker C. S., 2004 Managing ammonia in fish pond. SRAC Publication - Southern Regional Aquaculture Center 4608, 8 p.
- Kaur H., Ansal M. D., 2020 Efficacy of *Aloe vera* as a growth promoting additive in carp (*Labeo rohita* Ham.) grow out feed. *Journal of Entomology and Zoology Studies* 8(2):997–1002.
- Khan I., Dhawan A., Ansal M. D., 2018 Effect of *Aloe vera* supplemented feed on survival and growth of common carp (*Cyprinus carpio* L.). *Indian Journal of Animal Nutrition* 35(2):206.
- Khanal M., Lamichhane S., Bhattarai A., Kayastha B. L., Labh S. N., 2021 Extract of *Aloe vera* (*Aloe barbadensis* Miller) enhances the growth, protein contents, and gastro-somatic index (GaSI) of common carp *Cyprinus carpio*. *Journal of Nutrition and Metabolism*, 14 p.
- Mahdavi M., Hajimoradloo A., Ghorbani R., 2013 Effect of *Aloe vera* extract on growth parameters of common carp (*Cyprinus carpio*). *World Journal of Medical Sciences* 9(1):55–60.
- Mehrabi Z., Firouzbakhsh F., Rahimi-mianji G., Paknejad H., 2019 Immune response, immune gene expression, and experimental challenge with *Saprolegnia parasitica* in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 503(4):330–338.
- Militz T. A., Southgate P. C., Carton A. G., Hutson K. S., 2013 Dietary supplementation of garlic (*Allium sativum*) to prevent monogenean infection in aquaculture. *Aquaculture*, (408–409):95–99.
- Moghaddam B. S., Haghighi M., Rohani S. M., Hamidi M., Ghasemi M., 2017 The effects of different levels of *Aloe vera* extract on some of the hematological and non-specific immune parameters in Siberian sturgeon (*Acipenser baerii*). *Iranian Journal of Fisheries Sciences* 16(4):1234–1247.
- Muntasiroh S., Purbomartono C., Mulia D. S., 2020 [Combination of brown seaweed extract (*Padina* sp.) and vitamin C through feed on non-specific immune system of dumbo catfish (*Clarias gariepinus*)]. *Sainteks* 17(1):7. [In Indonesian].
- Novita, Setyowati D. N., Astriana B. H., 2020 [Blood profile of white snapper infected with *Vibrio* sp. bacteria with aloe vera administration (*Aloe vera*)]. *Jurnal Perikanan Unram* 10(1):55–69. [In Indonesian].
- Pashaki A. K., Zorriehzahra S. M. J., Ghasemi M., Rohani M. S., Hosseini S., 2018 Effects of dietary garlic extract on some blood, immunity and growth parameters of common carp fingerlings (*Cyprinus carpio*). *Iranian Journal of Aquatic Animal Health* 4(2):28–39.
- Prasetio E., Rachimi, Hermawansyah M., 2018 [The use of *Aloe vera* powder in feed as an immunostimulant against hematology of biawan fish (*Helostoma teminckii*) which was challenged with *Aeromonas hydrophila* bacteria]. *Jurnal Ruaya* 6(1):60–73. [In Indonesian].
- Safari M., Chelema Dezfoul Nejad M., Mesbah M., Jangaran Nejad A., 2019 Effects of *Aloe vera* extract on growth and some hematological parameters of shirbot, *Tor grypus* (Heckel, 1843). *Iranian Journal of Fisheries Sciences* 18(3):445–456.
- Samson J. S., 2019 Effect of garlic (*Allium sativum*) supplemented diets on growth, feed utilization and survival of red tilapia (*Oreochromis* sp.). *International Journal of Agricultural Technology* 15(4):637–644.
- Setijaningsih L., Setiadi E., Taufik I., 2021 The effect of garlic *Allium sativum* addition in feed to the growth performance and immune response of tilapia *Oreochromis niloticus*. *IOP Conference Series: Earth and Environmental Science* 744(1).

- Sharif Rohani M., Haghghi M., Bazari Moghaddam S., 2017 Study on nanoparticles of *Aloe vera* extract on growth performance, survival rate and body composition in Siberian sturgeon (*Acipenser baerii*). Iranian Journal of Fisheries Sciences 16(2):457–468.
- Syed R., Masood Z., Ul Hassan H., Khan W., Mushtaq S., Ali A., Gul Y., Jafari H., Habib A., Ishaq Ali Shah M., Gabol K., Gul H., Ullah A., 2022 Growth performance, haematological assessment and chemical composition of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed different levels of *Aloe vera* extract as feed additives in a closed aquaculture system. Saudi Journal of Biological Sciences 29(1):296–303.
- Yunus Y. E., Anshary H., Zainuddin E., 2021 Effect of *Aloe vera* extract on growth and hematological performance in Nile tilapia (*Oreochromis niloticus*). International Journal of Scientific and Research Publications (IJSRP) 11(2):574–580.
- Zare M., Tran H. Q., Prokešová M., Stejskal V., 2021 Effects of garlic *Allium sativum* powder on nutrient digestibility, haematology, and immune and stress responses in eurasian perch *perca fluviatilis* juveniles. Animals 11:(9).
- Zayed R., Abd-Elatieff H., Goda W., El-shall N., Bazh E., Ellakany H., Abou-Rawash A. R., 2020. Effects of aqueous extract of *Aloe vera* leaves on performance, hematological and cecal histological parameters in commercial broiler chickens. Damanhour Journal of Veterinary Sciences 5(1):4–10.

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