Effect of Aloe Vera (*Aloe vera*) Extract on Growth and Hematological Performance in Nile Tilapia (*Oreochromis niloticus*)

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Abstract- Tilapia (*Oreochromis niloticus*) is a freshwater aquaculture commodity that has high economic value. Consistency of increasing fish production has been done through intensive aquaculture. However, disease problem caused by a parasitic infection can reduce the growth rate and even mortality of cultivated tilapia. Control of parasitic infection of fish can be done by administering immunostimulants which has been shown to play a role in activating the fish's non-specific defense system. Immunostimulants can be derived from herbal ingredients of *Aloe vera* extract. The purpose of this study was to evaluate the effect of *Aloe vera* extract mixed into commercial feed on the growth rate and hematological performance of tilapia (*O. niloticus*). Maintenance of fish during the study was carried out in a 40 dm^3 volume aquarium with a size of 40 x 40 x 25 cm. The research design applied was a completely randomized design (CRD) with 4 treatments. The treatments were, differences in the dosage of *A. vera* extract with 3 replications, the doses used were 0, 5, 10 and 15 g / kg of feed. The results showed that differences in the dosage of *A. vera* extract had a significant effect ($P < 0.05$) on the increase in total length and total leucocytes of tilapia (*O. niloticus*). *A. vera* extract at the dosage of 5 g / kg of feed showed the highest length gain of 2.889 cm and the highest average total leucocytes of 50,150 cells / mm^3.

Keywords: Tilapia, *Aloe vera* Extract, Growth Rate, Hematology.

I. INTRODUCTION

Nile Tilapia is one of the most important global-traded fish from freshwater aquaculture. Consistency of increasing fish production has been achieved through aquaculture intensification of this fish species. The wide adoption ofintensive farming technologies of tilapia could be associated with the high biomass yield per unit area that can be produced for this species cultivation. However, disease problem caused by parasitic infections very often occur, which causes a serious problem in fish health and causes economic implications in aquaculture activities (Kaur et. al., 2018). Some ectoparasites can cause a direct impact in the form of death because of their high pathogenicity, there are also cases where parasites are not the direct cause but are associated with a secondary infection (Ghoneim et. al., 2015).

Historically, various antibiotics and chemicals have been the choice for control of this disease because they are easy to obtain and effective to combat against fish diseases. However, because of their carcinogenic effects to human, and detrimental impact in environment, the use of these ingredients is limited (Jorgensen, 2017). An alternative that can be used to overcome this problem is application of immunostimulants that enhance and activating the non-specific defense system of fish. Immunostimulants can be derived from medicinal herbs which can then be used as antiparasitic for controlling parasite infection in fish. Pandey et. al. (2012) reported that the use of herbal ingredients, Indian almond (*Terminalia catappa*) and garlic (*Allium sativum*) have been reported as an alternative herbal ingredient to treat ectoparasite infection of *Trichodina* sp. in juvenile tilapia (*O. niloticus*). Both almonds and Indian garlic have low levels of acute toxicity and can treat trichodiniasis in tilapia seeds. In addition, application of 20 mg / kg dose of neem (*Azadirachta indica)* leaf extract through injection into snakehead fish (*Channa striatus*) infected with the fungi *Aphanomyces invadans*, is able to improve hematological performance as well as immunological parameters such as serum lysozyme activity, phagocytic activity and content of total protein of the fish (Uthayakumar et. al., 2014). Another herbal ingredient that has been shown to have immunostimulant and antiparasitic effect is *Aloe vera* which contains compounds such as alloine, femodine, anthraquinone, isobarbaloine, acetylated mannose (acemannan) (Mehrabi et. al., 2019). Acemannan can increase macrophage activity modulate the entire immune system by stimulating, producing, and releasing antibodies in Rainbow trout (Haghighi et. al.,

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2014). Different studies report that *Aloe barbadensis* supplementation combined with Propolis was able to reduce the intensity of Monogenea parasites *Cichlidogyrus sclerosus*, *C. halli*, *C. thurstonae* and *Scutogyrus longicornis* on tilapia gills compared to the control group (Dotta et. al., 2015). Content of *Aloe vera* is anthraquinone (aloin and emodin), which is a substance with a cathartic and laxative activity that can fight infecting parasites (Dotta et. al., 2015). *A. vera* extract is thought to have effects have as immunostimulant and antiparasitic in tilapia so that it can be used to reduce impact of parasite infection in tilapia culture.

II. MATERIALS AND METHODS

Fish Preparation and Acclimatization

This research was conducted in August to September 2020 at Hatchery Mini, Faculty of Marine and Fisheries Sciences, Hasanuddin University. Hematological performance analysis was conducted at the Laboratory of Fish Parasites and Diseases, Faculty of Marine and Fisheries Sciences, Hasanuddin University. The experimental animal used was tilapia with an average weight of 8.0 ± 0.922 g / fish and total length average of 7.0 ± 0.32 cm. The tilapia fish were purchased from Balai Benih Ikan (BBI) Bontomanai, Gowa Regency. First, tilapia fish were acclimatized to the new rearing environment and feeding with commercial feed for 7 days, then the fish were stocked in a rearing container with a density of 10 fish / aquarium. During the maintenance of tilapia, the test feed was given as much as 3% of the fish biomass with a frequency of 3 times per day.

Feed Preparation

The *Aloe vera* extract used was a 97% *Aloe vera* gel product produced in Cileungsih District, Bogor Regency, Indonesia. The *Aloe vera* gel was freeze dried using freeze dryer following the method of Hastulistiyoso et. al. (2011). The feed used was All Feed-2 commercial feed in the form of floating fish feed produced by PT Central Proteina Prima, Tbk with a protein content of 16%, 6% fat, 6% fiber, and 10% water content which was used as basal feed then added with *Aloe vera* extract according to the treatment dose. Addition of *Aloe vera* extract to the feed followed the method of Alishahi et. al. (2017), briefly first crushing the commercial feed in the form of pellets into flour using a grinder, then weighing the feed and adding *Aloe vera* extract powder according to the treatment dose, then the mixture of commercial feed and *A.vera* extract powder were homogenized for 15 minutes, then adding warm water becomes a paste form, then the mixture was put into a ± 2 mm feed molder to forms a pellet, and then the feed was dried under the sun to reduce the water content.

Eksperimental Design

The experimental fish were acclimatized to the new rearing environment and to commercial basal feed (All Feed-2) for 7 days, then stocking it in a rearing tank with a density of 10 fish / container. The fish were fasted for 24 hours and after that, the fish were feed with test feed with a dose of 3% of the fish biomass per day with a frequency of 3 times, given at 08.00 am, 12.00, and 16.00 pm. Maintenance of fish was carried out for 14 days and on the 15th-day observations were made on the growth rate and hematological performance of the tested fish were analyzed.
This study used a completely randomized design consisting of 4 treatments (dosage of *Aloe vera* extract) with 3 replications. The dosage of *Aloe vera* extract used followed the method of Mehrabi et. al. (2019) 0, 5, 10 and 15 gr / kg of *Aloe vera* extract feed.

Growth Rate

The growth rates observed in tilapia include absolute weight and absolute length. Weight and length was measured on the day 15th-of maintenance.
The Absolute weight, calculated by Effendie formula (1997):

$$Wm = Wt - Wo$$

Note: Wm = growth in absolute weight (grams), Wt = weight of biomass at the end of the study (grams), Wo = Weight of biomass at the start of the study (grams).

The absolute length was calculated using the Effendie formula (1997):

$$Pm = Lt - Lo$$

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Note: Pm = the increasing in absolute length (cm), Lt = the final average length (cm), Lo = the initial average length (cm)

Hematology Performance

The hematological performance observed included total leukocytes, total erythrocytes and percentage of hematocrit. To measure hematology performance fish blood was taken at the base of the tilapia tail using a 1 ml spoit, then the fish blood was placed in an eppendorf tube contained anticoagulant EDTA fluid.

Observation of leukocytes, it begins with sucking the blood of the fish that has been accommodated in the tube using a Thoma Leukocyte pipette up to a scale of 0.5, and then continues to suck the Turk's solution up to a scale of 11, then homogenized by shaking the Thoma pipette like a figure 8. Then drop it on haemacytometer, then observed under a microscope to perform calculations based on the method of Blaxhall and Daisley (1973), namely:

$$\Sigma \text{ Leukocytes} = \Sigma \text{ counted cells} \times 20 / 0{,}4 \text{ mm}^3$$

Total erythrocytes was carried out by taking blood at the base of the fish tail, then accommodating it in a tube. Furthermore, the blood was sucked using a Thoma erythrocyte pipette up to a scale of 1, and continued by sucking Hayem's solution up to a scale of 101, and homogenized. Then drop it on a haemocytometer, and make observations under a microscope to count the number of red blood cells according to the formula of Biaxhall and Daisley (1973), namely:

$$\Sigma \text{ Eritrosit} = \text{Counted cells} \times 50 \times 200$$

For the observation of hematocrit levels, it was done by calculating the percentage of blood clots in a microhematocrit tube after being centrifuged for 5 minutes at a speed of 5,000 rpm.

Statistical Analysis

The data obtained were analyzed using analysis of variance (ANOVA) to obtain differences between treatments. Then a significant difference between mean values was analyzed using W-Tukey's range test at $p \leq 0.05$.

III. RESULTS

Growth Rate

The growth rates including the increase in absolute weight and the increase in the absolute length of tilapia (*Oreochromis niloticus*) fed with the addition of *Aloe vera* extract at different doses were presented in Table 1.

Table 1. Average Growth Rate of Tilapia (*Oreochromis niloticus*) which is fed with different doses of *Aloe vera* extract

Aloe vera Extract Dosage (gr / kg of feed)	Weight Growth Rate (gr)	Length Growth Rate (cm)
0	3,789±0,154 [a]	1,833±0,173[b]
5	3,855±0,474 [a]	2.889±0,356[a]
10	3,822±0,625 [a]	2,689±0,342[ab]
15	3,199±0,665 [a]	2,522±0,555[ab]

Note: Different superscript in the same column indicate a significant difference between treatments at the 95% confidence level (P <0.05).

The increase in the absolute weight of tested fish showed insignificant results (P> 0.05) between treatments. The results of analysis of variance (ANOVA) showed that feeding with different dosage of *Aloe vera* extract had no significant effect on the increasing total weight of tilapia (*Oreochromis niloticus*) (P> 0.05) (Table 2). The highest percentage of the increasing weight was obtained in test fish fed a dose of *Aloe vera* 5 gr / kg of feed with a value of 3.855%, while the lowest percentage was obtained in the fish fed a dose of *A. vera* 15 gr / kg of feed with a value of 3.199%.

The increase in the absolute length of tilapia (*O. niloticus*) showed a significant result (P <0.05) between treatments. The results of analysis of variance (ANOVA) showed that feeding with different doses of *Aloe vera* extract had a

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significant effect on the increase in length of tilapia (*O. niloticus*) (P <0.05) (Table 2). The treatment with the addition of the extract dose of *A. vera* 5 gr / kg showed a difference with the control treatment (without the addition of *A. vera* extract) but did not show any difference with the treatment of 10 and 15 g / kg of A. The highest percentage of the increasing length was obtained in test fish fed with the addition of *A. vera* extract 5 gr / kg of feed with a value of 2.889%, while the lowest was obtained in test fish fed without the addition of *A. vera* extract with 1.833% tilapia.

Hematology Performance

The hematological measured performance included total leukocytes, total erythrocytes and percentage of hematocrit in tilapia (*Oreochromis niloticus*) fed with different doses of *Aloe vera* extract presented in Table 2.

Table 2. Average Value of Total Erythrocytes, Hematocrit and Total Leukocytes of Tilapia (*Oreochromis niloticus*) fed with different doses of *Aloe vera* extract

Aloe vera Extract Dosage (gr / kg of feed)	Total Erythrocytes (Cells/mm^3)	Hematocrit (%)	Total Leukocytes (Cells/mm^3)
0	637.333±868371,656^a	26,029±2,810^a	40.716±2662,861^b
5	721.000±908500,412 ^{a}	21,441±2,496 ^{a}	50.150±3025,309^a
10	2.263.333±90737,717 ^{a}	27,777±3,849 ^{a}	46.533±1421,560 ^{ab}
15	766.333±964520,779^a	23,728±1,250^a	44.766±4023,275^{ab}

Note: Different superscript in the same column indicate significant differences between treatments at the 95% confidence level (P<0.05)

The total erythrocyte of tilapia (*O. niloticus*) showed insignificant results (P> 0.05) between treatments. The results of analysis of variance (ANOVA) showed that feeding with different doses of *A. vera* extract had no significant effect on the total erythrocyte of tilapia. The highest percentage of total erythrocytes was obtained in test fish which were fed with the addition of *A. vera* extract at a dose of 10 gr / kg of feed with a value of 2,263.33 cells / mm^3, while the lowest erythrocyte was obtained in test fish that were fed control (without the addition of *Aloe vera* extract) with a value of 637.33 cells / mm^3.

The percentage of hematocrit of tilapia (*O. niloticus*) showed insignificant results (P> 0.05) between treatments. The results of analysis of variance (ANOVA) showed that feeding with different *A. vera* extract doses had no significant effect on the hematocrit percentage of tilapia. The highest percentage of hematocrit was obtained in test fish fed with the addition of *A. vera* extract 10 gr / kg of feed with a value of 27, 77%. While the lowest percentage of hematocrit was obtained in test fish fed with a dose of *A. vera* extract 5 gr / kg of feed with a value of 21.44%.

Total tilapia (*O. niloticus*) leukocytes showed significant results (P <0.05) between treatments. The results of analysis of variance (ANOVA) showed that feeding with different doses of *Aloe vera* extract had a significant effect on the total leukocytes of tilapia (*O. niloticus*) (P <0.05) (Table 2). Test fish fed with the addition of *A. vera* extract 5 g / kg of feed showed differences with test fish fed control feed (without the addition of *A. vera* extract), but did not differ from other test fish. The highest total leucocytes were obtained in test fish which were fed with the addition of *A. vera* extract at a dose of 5 gr / kg of feed with a value of 50,150 cells / mm^3, while the lowest total leukocytes were obtained in test fish fed without the addition of *A. vera* extract (control) with a value of 40,716 cells / mm^3.

IV. DISCUSSION

Application of *Aloe vera* extract in feed did not affect the weight gain of tilapia. The same result was also reported by Yilmaz et. al. (2019) that no differences in body weight gain of tilapia fed with different doses of *Aloe vera*. In other study conducted by Golestan et. al. (2019), shows that the application of *Aloe vera* extract at a dose of 0.1% and 1% do not differ in the weight gain of Rainbow trout. On the other hand, difference in fish length was onserved in in different dosage applied. The highest average length was obtained at a dose of 5 g / kg of feed which was significantly different (P <0.05) with control without the addition of *Aloe vera* extract. This result is supported by the findings of Manaf et. al. (2016) that addition of *Aloe vera* extract mixed with several other feed supplements was able to increase growth in tilapia significantly compared to control group (without the addition of *Aloe vera* extract and a combination of several other feed supplements). Similarly, a study by Gabriel et. al. (2015) shows that a series of immuno-nutrional ingredients such as proteins, lipids, vitamins, enzymes, minerals, sugars, lignins, saponins and salicylic acids in feed added with *A. vera* can improve the growth performance of fish. Polysaccharide molecules such as acemannan which contained in *A. vera* is believed to have prebiotic properties which are

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indigestible feed ingredients which benefit the host by stimulating the growth or activity of one or several bacterial species that have settled in the intestine so that it can be associated with increased nutrient digestibility, absorption and capacity. Assimilation, through increased digestive enzymes and healthy intestinal microflora. However, different from the present result, Mehrabi et. al. (2019) reported that *Aloe vera* extract at a dose of 5 g / kg of feed had no effect on the growth performance of Rainbow trout (*Oncorhynchus mykiss*) fed for 6 weeks. The researchers attributed the differences in results obtained in previous studies. with various factors such as differences in plant material used, method of manufacture, and plant consumption period (Zanuzzo et al., 2015). Different from reported by Mehrabi et. al. (2019), our result showed a positive influence from the addition of *A. vera* at a dose of 5 gr / kg to feed which could increase the growth of tilapia. The time for consumption and the source of herbal ingredients as reported by (Zanuzzo et. Al., 2015) is the reason that the plant extract are able to increase digestibility or utilization of nutrient efficiency. Therefore our results reveal that the *A. vera* extract at a dose of 5 g / kg added to the feed supports growth performance in tilapia.

Application of *Aloe vera* extract did not show any differences in several hematological parameters. The findings agree with those reported by Gabriel et. al. (2015) that no difference in the number of erythrocytes and hematocrit in tilapia (*Oreochromis niloticus*) in fish which were fed with different concentrations of *Aloe vera* extract. However, significant changes in hematological parameters such as erythrocyte, hematocrit and hemoglobin in fish fed with *A. vera* extract compared without the addition of *A. vera* extract were only noticed after being challenged with bacteria *Streptococcus iniae* indicating that the hematological parameters of the fish given 5% dose of *A. vera* extract feed are different from the fish fed without the addition of *A. vera* extract (Gabriel et. al., 2015). Our result showed that the highest erythrocyte and hematocrit values were obtained in fish fed with *Aloe vera* extract as much as 10 g / kg of feed, while in different line Gabriel et. al. (2015) showed that the highest erythrocyte and hematocrit yields were obtained in tilapia fed with *Aloe vera* extract as much as 0.5% (5 g / kg of feed). Similar results were reported by Alishahi et al. (2010) that supplementation of 0.5% crude extract of *A. vera* in the diet of goldfish (*Cyprinus carpio*) can significantly increase erythrocytes.

The higher erythrocyte and hematocrit increase in fish fed with *A. vera* supplements compared to the control group in this study indicated the ability of *Aloe vera* to stimulate erythropoiesis. Erythropoiesis improves oxygen transport and strengthens defense mechanisms against physiological stress. This can be attributed to the content possessed by *A. vera* such as essential vitamins, riboflavin, thiamine and folic acid, as well as several essential and non-essential amino acids that are essential for the formation of hemoglobin. Besides, the polysaccharides in *A. vera* gel are also associated with increased erythropoiesis (Gabriel et. al., 2015). Meanwhile, thiamine as a vitamin which is responsible for glucose uptake in erythrocytes is known to be one of the main causes of hemopoietic stimulation by the formation of pyruvate dehydrogenase complexes and α-ketoglutarate dehydrogenase in the Krebs cycle (Mehrabi et. al., 2019).

In our study, it was found that the total leukocytes were higher in tilapia fed with *A. vera* extract as much as 5 g / kg of feed and it was different from the control group. Similarly, a study by Mehrabi et. al. (2019) showed that differences in leucocytes in Rainbow trout (*Oncorhynchus mykiss*) fed with the addition of *A. vera* extract compared to fish not given *A. vera* extract in their feed. In the same line, Devi et. al. (2019) showed that *Labeo rohita* fish fed with a dose of 5 mg of Aloe-Emodin extract increased their leukocyte counts at week 6 compared to fish fed a dose of 1 mg, 10 mg or the control group. These results partly correspond with the findings of Gabriel et. al. (2015) who reported that tilapia fed with *A. vera* at a dose of 0.5% (5 g / kg of feed) after being challenged with *Streptococcus iniae* showed higher leukocyte values compared to fish fed with a dose of *A. vera* 1%. , 2%, 4% or control. The findings agree with those Alishahi et. al. (2014) who reported the same thing that supplementation of crude extract *A. vera* at a dose of 0.5% significantly increased goldfish leukocytes compared to other treatment groups. A higher leukocyte response in fish fed with *A. vera* supplementary food than the control group indicated that *A. vera* can stimulate leucopoiesis thereby strengthening the body's ability to remove unwanted foreign objects such as bacteria, fungi, viruses and various other types of pathogens. The acemannan molecules found in *Aloe vera* gel are believed to trigger the fish's body to produce macrophages on white blood cells to fight pathogens (Gabriel et. al., 2015). Meanwhile, the improvement of health indicator parameters such as leucocytes in fish which are influenced by medicinal plants is due to the large number of bioactive compounds contained by *A. vera*. Bioactive compounds affect the intrinsic immune response, further *A. vera* extract shows strong immunostimulating activity due to the content of lipopolysaccharide (LPS) which can induce leukocyte-forming cells, to produce more cells contained in leukocytes, namely lymphocytes, monocytes and neutrophils (Mehrabi and Firouzbakhsh, 2019).

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V. CONCLUSION

As conclusions, addition of *Aloe vera* extract at a dose of 5 g / kg in feed can improve the growth performance in terms of length gain of tilapia (*Oreochromis niloticus*), and furthermore it can act as an immunostimulant by increasing the hematological performance of tilapia (*O. niloticus*).

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