The 1st International Conference on Fisheries and Marine Science

To cite this article: 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **236** 011001

Preface

It is such great a pleasure for me to welcome all of you on behalf of the Faculty of Fisheries and Marine Universitas Airlangga, for the first international conference on fisheries and marine science.
The 1st International Conference on Fisheries and Marine Science (InCoFiMS) is the first initiated international conference held by the Faculty of Fisheries and Marine, which beforehand iwas held in the National Level. This expanded level of this conference with the theme of **"Fisheries and Marine in Supporting Sustainable Development Goals (SDG's) achievement"** is expected to be capable of connecting students, lecturers, researchers, government and professionals from across the world to meet, greet, share and discuss about the potential and best practices in the field of fisheries and marine during the period of focusing on SDG's

The aims of this conference is to develop and improve the goals of Universitas Airlangga to be one of the Top 500 Universities in the world by contribute in improving aquaculture and Fisheries Sustainable sector. And for this conference we also cooperate with Scopus Indexed Publisher In order to assist students, lecturers and researchers in disseminating their findings, to publish selected papers which are expected to help societies to implement the findings in the focus on developing aquaculture and fisheries sustainable.

I strongly hope that all of participants from around the world enjoy the conference in the historical hity of Surabaya, the second biggest city in Indonesia with competitive economic activities for the future of Fisheries and Marine development.

Once again, I am most grateful for your participation and your support. Thank you.

Dr. Woro Hastuti Satyantini
Chief of INCOFIMS

PAPER • **OPEN ACCESS**

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To cite this article: E Setyaningsih *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **236** 012083

The effect of noni fruits (*Morinda citrifolia*) with different ripeness stages against the total erythrocytes and leukocytes of comet goldfish (*Carassius auratus*) infested by *Argulus*

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Abstract. *Morinda citrifolia*, commonly called noni fruit, has some effect when used in the treatment of fish parasites, such as *Argulus*. Noni fruit at different maturity stages have active compounds and therapy effects against parasites. The level of the fish's health can be seen from a hematology observation. The purpose of this study was to determine the effect of noni fruit of different ripeness stages against the erythrocytes and leukocytes total in goldfish (*Carassius auratus*) infested with *Argulus*. The method used in this study was experimental with a completely randomized design (CRD) involving six treatments and three replications. The treatments used were conducted on healthy cometfish as a control (P1), infected fish with *Argulus* (P2), infected fish with *Argulus* soaked in noni fruit in the 1st maturity stage (P3), infected fish with Argulus soaked in noni fruit in the 2nd maturity stage (P4), infected fish with Argulus soaked in noni fruit in the 3rd maturity stage (P5) and infected fish with Argulus soaked in noni fruit in the 4th maturity stage (P5). The results showed that noni fruit in different maturity stages significantly impacts the treatment effectiveness. The total of the erythrocytes and leukocytes reached a normal level in P5 when the color characteristic of the fruit was pale-yellow and the firmness was fairly hard. The total number of erythrocytes and leukocytes in the comet goldfish reached normal values, as follows; 13.86 x 105 cell / mm^3 and 18.76 x 103 cell / mm^3.

1. Introduction

An *Argulus* infestation in the fish's body will cause bleeding, both in the gills and on the surface of the skin. Bleeding in fish causes anemia, inflammation and excessive mucus production in the injured area [1]. Male and female *Argulus japonicus* attacks cause changes in their blood components [2]. If the infestation rate of Argulus japonicusis is higher, then the hematocrit value, the amount of hemoglobin and the number of erythrocytes will decrease and the number of leukocytes will increase. If this condition continues, then this will cause the death of the fish.

Morindacitrifolia, commonly referred to as noni fruit, have several properties related to the treatment of parasites such as *Argulus* [3]. Fruits with different levels of maturity have different active ingredients and properties. Noni fruit with different levels of maturity also have different nutrient and

compound levels [4]. The content of the protein, carbohydrates and vitamin C will increase in number along with the increase in the fruit's maturity. The role of vitamin C is to help in the absorption of iron so then it can be absorbed easily by the body, thereby increasing the number of red blood cells in the body. Noni fruit also contains vitamin A, which plays a role in erythropoiesis. This process interacts with the Fe minerals, thus preventing anemia [5]. Noni fruit also contains scopoletin and alkaloids. Scopoletin produces serotonin, which is a chemical compound that blocks the work of the smooth muscles and nerves and so causes the vasoconstriction of the heart and brain membranes [6]. This will cause the disruption of the Argulus's nutrition intake. Alkaloids and terpenoids in noni fruit have bioactivity that acts as a stimulant agent that helps to maintain the immune system [7].

The level of the health of the fish can be seen from a hematological observation. Blood tests are an important factor in assisting in diagnosis, prognosis and therapy. The blood can be used to find out the health condition of the fish, as any physiological deviations of the fish will cause the blood components to also change [8]. An examination of the number of erythrocytes and leukocytes was a part of the parameters examined in the hematologic observation [9].

2. Material and method

2.1 Material

The materials used in this study consisted of two aquariums that were 100 x 40 x 40 cm and 18 aquariums that were 15 x 15 x 25 cm, aerators, aeration, measuring cups, plastic cups, glass bottles, blenders, a 1ml syringe, an Olympus binocular microscope, a hand tally counter, a hemocytometer (Neubauer), cover glass, a thoma erythrocyte pipette and a thoma leukocyte pipette, pH paper, thermometer and a DO test.

The material used in this study was noni fruit (*Morinda citrifolia*) at different levels of maturity. The sample was made up of 18 comet goldfish (*Carassius auratus auratus*) with a total length of 8-10 cm.

2.2 Method

2.2.1 Treatments

This research used an experimental method with six treatments. The treatments were p0 (control negative), p1 (argulus infected) positive control, p2 (therapy fruit juice made from level 1 noni fruit) with the, characteristic of the rind of the fruit being dark green and the texture very severe. The therapy made of the noni fruit juice at level 2 pieces had the characteristics where the rind of the fruit was a yellowish green; p4 consisted of the level 3 noni fruit juice therapy with the characteristic of having white and yellowish flesh with a severe texture and p5 was the level 4 noni fruit juice therapy with the characteristics of having a white transparent rind with a soft and watery flesh.

2.2.2 Sample collection

The sample of the comet goldfish came from Gunungsari Fish Market in Surabaya. The fish used were healthy fish that were actively swimming and that did not show any abnormal symptoms due to the influence of stress or disease. The noni fruit were obtained from the Mulyosari District, in Surabaya. The fruit was washed, dried, blended and then squeezed. The juice of the noni fruit was stored in a glass bottle. The *Argulus* ectoparasites were obtained from the Blitar Regency.

The comet goldfish were kept in an aquarium (one fish / aquarium) with a volume of water that was as much as three liters. The fish were infected with *Argulus* (five Argulus / fish). The Argulus was placed in a plastic cup for about two hours without the host before being infused with the fish. This was expected to accelerate the attachment of *Argulus* to the body of the comet goldfish. The infested comet goldfish *Argulus* were then returned to the treatment aquarium and maintained for three days. From day 3 to the 9th day of maintenance, the treatment was carried out using the noni fruit juice with a dose that was 3.5% of the volume of water for 15 minutes per day. On the 10th day, the maintenance of the comet goldfish was done by calculating the percentage of *Argulus* release and conducting blood sampling, before calculating the total number of erythrocytes and leukocytes.

2.2.3 Total erythrocyte and leukocyte count

The total erythrocyte counts were based on previous study [10]; blood suction was carried out using a pipette containing a red stirring grain up to a scale of 0.5. Hayem's solution reached a scale of 101. The first drop of blood solution in the pipette was discarded, before the next was then dropped onto a Neubauer-type haemocytometer before being covered with a glass cover. The total number of erythrocytes was calculated with the aid of a microscope with 400x magnification. The total erythrocyte counts were calculated ousing five small boxes of haemocytometer and the total number of the erythrocytes was calculated according to the formula used in a previous study [11].

The calculation of the total leukocytes was done by sucking the blood using a pipette that contained a white stirring grain up to a scale of 0.5. Turk's solution was added to a scale of 11. The first drop of blood solution in the pipette was discarded, and the next was then dropped onto a Neubauer-type haemocytometer before being covered with a glass cover. The calculation of total leukocyte count was conducted with the aid of a microscope with 400x magnification. The total leukocyte count was calculated using four large boxes and the amount was calculated according to the formula used in the previous study. Not only was the blood measured, but so was the water quality

2.2.4 Data analysis

Statistical analysis conducted using ANOVA (Analysis of variance) was the method used to determine the effect of the noni juice extract at different levels of maturity on the total number of comet red blood cells and leukocytes infested by Argulus. Further tests were carried out using Duncan's Multiple Distance Test with a significance level of 5% [12].

3. Results and discussion

3.1 Results

The data of the average calculation of the total number of cometfish erythrocytes and leukocytes could be seen in Table 1. Based on Table 1, the average total number of erythrocytes in the control treatment (P0) or in healthy fish showed as being significantly different from the *Argulus* infestation treatment in P1, P2 and P3 and it was not significantly different from P4 and P5. The treatment of P1 was not significantly different from the treatment of P2 and P3, but it was significantly different from P0, P4 and P5.

Duncan's Multiple Distance Test results for the total leukocytes showed that the P1 treatment, with an average value of 34.8 x cells, was significantly different from P0, P3, P4 and P5 and was not significantly different from P2. The treatment of P0 was significantly different from P1 and P2, but was not significantly different from P3, P4 and P5.

Table 1. The results of the water quality inspection during the study were in the normal range for the maintenance of cometfish, which was a temperature of 28-32.5ºC, a pH 6-8 and 6-8 mg / l.

Treatment	Average ± SD Erytrocyte (10^5 cell/mm^3)	Average ± SD Leucocyte (10^5 cell/mm^3)
P0	14.80± 2.17945	14.53± 3.50297
P1	5.30± 2.42487	34.80± 3.60104
P2	7.70± 3.10000	24.52± 6.91743
P3	6.83± 2.54231	21.73± 3.92248
P4	13.86± 4.64579	18.76± 5.30197
P5	10.63± 2.63122	21.23± 5.73854

3.2 Discussion

Based on the ANOVA test, the results of the research into the total number of cometfish erythrocytes and leukocytes showed that $p < 0.05$. It could thus be concluded that there were significant differences between the treatments. The next step was Duncan's Multiple Distance Test with a significance level of 5%. *Argulus* infestation in the cometfish was shown to affect hematological changes in the comet goldfish. *Argulus japonicus* infestation caused the blood components to change. The blood components such as the total number of erythrocytes, hemoglobin and hematocrit of the fish decreased, but the total amount of fish leukocytes increased [2].

The noni fruit contained several nutrients such as protein, iron, vitamin A and Vitamin C [13]. These nutrients could help to regenerate the red blood cells [5]. According to a previous study [14], vitamin A is needed in several essential processes in the body such as metabolism, blood cell formation, the regulation of cell differentiation and the immune system.

The research data showed that there was a difference between the total number of healthy fish erythrocytes and erythrocytes in the *Argulus* infested fish without treatment. This proves that the five *Argulus* infestations in one fish had an effect on the total number of erythrocytes. According to the previous research [2], the five Argulus infestations in the cometfish decreased the total number of erythrocytes from 2.36 µl to 1.92 µl.

Based on the study, the total number of healthy cometfish erythrocytes ranged from 13.3 to 17.3 x cells $/10^3$. The healthy fish had a total number of erythrocytes ranging from 10.5-30.0 x cells $/10^3$[15]. Giving the fish the noni fruit juice with a maturity level of one, two, three or four did not show a significant difference between treatments. The total number of cometfish erythrocytes which was closest to normal was seen in the P4 treatment using noni fruit with a maturity level of three with a value of 13.86 x 105 cells $/10^3$. This was closest to the average total number of healthy fish erythrocytes recorded in this study. The decreased number of erythrocytes caused the disruption of oxygen transport in the fish's body. Fish that experience anemia for a long time eventually die [16]. According to the previous study [17], the factors that affect the number of erythrocytes include gender, differences in the parent (genetic), nutritional condition, physical activity and age.

The calculation of the total leukocyte count could be indicated as the activity of the immune cell mechanisms in the host [18]. The release of anticoagulant enzymes in the fish's body when blood was sucked had the potential to trigger an immune response because it is considered to be a foreign body. This is in accordance with the function of the white blood cells in the body, namely as a defense tool [17]. According to the previous study [7], the extract of the noni fruit contained xeronine and proxeronin, which functions to normalize the functions of the damaged cells, which prompts the body's resistance to increase. Xeronin also acted to activate the thyroid and thymus glands that function in the immune system. The immune system is a mechanism used by the body to protect and maintain the body's condition from dangers that attack the body. The formation of an immune response is carried out by lymphocyte cells. Macrophages, which are the first defense system, destroy the antigen through the phagocytosis process after antigenic activity which then sends the ‘passwords’ of the pathogen to the lymphocyte cells [19].

Giving noni fruit juice at different levels of maturity showed results that were not significantly different based on the total number of erythrocytes and leukocytes. This was possible because the value of the nutrients contained in the noni fruit had almost the same range of values at each fruit maturity level. According to the previous study [4], the levels of carbohydrate, protein and vitamin C in the noni fruit was close to consistent. The Vitamin C levels in the noni fruit based on fruit maturity level were 116, 140, 156 and 182 mg / 100g respectively.

This was also possible because *Argulus* was still attached to the cometfish. The result of the calculations of the highest percentage of the Argulus released was P4 at 73.33%. This causes the total amount of fish erythrocytes and leukocytes in the P4 treatment to change to near normal because the *Argulus* that sucked the fish’s blood decreased. The percentage of the Argulus released in this study was calculated on the last day of treatment.

Noni fruit at the third maturity level had a higher scopoletin level compared to the level of maturity of the other fruits. Noni fruit at the third maturity level contained scopoletin measuring 57.94 ppm [20]. Skopoletin is a compound that produces serotonin which, functions to inhibit the work of smooth muscles and nerves. This causes the vasoconstriction of the heart and brain membranes [6]. This causes disruption in the nutritional intake of the *Argulus*.

Treatment through soaking was expected to allow it to enter the body of the fish through the gills and skin by diffusion or osmosis. This was in accordance with the physiological processes ongoing in the body of the fish, namely the process of the entry of minerals together with water which diffused into the gills and the skin before spreading throughout the body through the blood to the target organs. Meanwhile, through osmosis, the minerals entered the body through the water directly from the mouth into the intestines, allowing for the absorption of the drug [21]. The behavior of the normal fish without infestation was that *Argulus* looked active while swimming, responded to the food given and showed no signs of injury to the fish's body. The behavior and clinical symptoms of the fish infested by Argulus after three days of maintenance was that some of the fish were seen to be still at the bottom of the aquarium, had a decreased appetite and were bleeding in the area of the *Argulus* bite wounds.

During treatments two and three, the fish were quieter than the fish in treatments four and five. This was because the content of the alkaloids and phenols in the noni fruit of maturity levels of three and four was more than the noni fruit maturity levels of one and two. According to the previous study [3], the alkaloids in the noni fruit at certain concentrations also caused toxic effects for the fish. The condition of the fish after treatment showed a change in the body of the fish, namely bleeding. The bleeding began to stop and some of the fish returned to active swimming.

The water quality was measured through temperature, pH and DO. Water quality played a role in influencing the metabolism of the fish's body. The aquarium temperature ranged from 28 - 32.5ºC. Comet fish grow optimally in a temperature range of 26 - 30ºC[22]. However, comet fish can also survive in a temperature range of more than 30ºC. Comet fish can survive in cold temperatures; in this condition, the comet fish reduce their metabolism and even tend not to eat or move [23].

The pH value in this study ranged from 6 - 8. According to another study [24], comet fish can grow well in a pH range of 6.5 - 8.5. A low pH value causes the fish to experience anorexia, lethargy, and visible blood flow in their fins. A high pH value would also causes harm to the fish, such as the fish experiencing breathing difficulties up to choking and even death [23]. Dissolved oxygen was an important factor that greatly influenced the survival of the fish. The dissolved oxygen value ranged from 6 - 8 mg / l. The measured DO value was still within the safe limits for the maintenance of the comet fish. According to another study [22], the value of the dissolved oxygen was still safe for the maintenance of the comet fish at five mg / l.

4. Conclusion

Giving noni fruit juice of different levels of maturity did not have a significant effect when it came to increasing the total number of erythrocytes and decreasing leukocytes. The total number of erythrocytes and leukocytes was close to normal when the noni fruit juice with a maturity level of three was given. This fruit has a characteristic yellowish-white rind and hard flesh.

5. References

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