



Research Journal of  
**Medicinal  
Plant**

ISSN 1819-3455



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## Research Article

# Effect of Diet Supplemented with Rind Powdered of Mangosteen Fruit (*Garcinia mangostana*) on Haematological Profile of Clown Anemonefish (*Amphiprion percula*)

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## Abstract

**Background and Objective:** Rind of mangosteen (*Garcinia mangostana* L.), is a natural fruit capable of being immunostimulant in humans. This study was aimed to evaluate the effect of dietary effect of Rind Powdered of Mangosteen Fruit (RPFMg) on hematological parameters, growth and survival rate of clown fish (*Amphiprion percula*). **Materials and Methods:** The different dosages (5, 10 and 15%) RPFMg were added in fish feed, while commercial feed as control fish group. Each group has 3 replications. A total of 60 fish (initial weight:  $3.5 \pm 1.1$  g) was kept in 12 aquariums (5 per aquarium) and fed twice a day. They were reared for 45 days. Parameters observed were blood characteristics (total leukocytes, total erythrocytes and hematocrit levels) of fish. **Results:** The RPFMg dietary of fish lead to increasing in the leucocyte count level ( $7.63-11.26 \times 10^4$  cell  $\text{mm}^{-3}$ ) as compared with control group ( $9.86 \times 10^4$  cell  $\text{mm}^{-3}$ ). The dietary RPFMg had been induced a trend decreasing of erythrocytes level ( $1.63-2.73 \times 10^6$  cell  $\text{mm}^{-3}$ ), while in control group was  $2.16 \times 10^6$  cell  $\text{mm}^{-3}$ . Hematocrit levels in fish treatments were 8.35-10.91% while in control group was 9.64%. **Conclusion:** The study indicates that diet of rind powdered of mangosteen is a potential immune stimulant to be used in clown fish culture to control diseases.

**Key words:** Mangosteen, leucocyte, erythrocytes, hematocrit, *Amphiprion percula*, immunostimulant

**Citation:** Asnawih Destiani, Indriyani Nur, Wellem H. Muskita and Agus Kurnia, 2019. Effect of diet supplemented with rind powdered of mangosteen fruit (*Garcinia mangostana*) on haematological profile of clown anemonefish (*Amphiprion percula*). Res. J. Med. Plants, 13: 46-52.

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Plant extracts are known to have an immune stimulant effect<sup>1,2</sup>. One of the immunostimulants that are being developed is mangosteen rind extract. Application of the use of mangosteen peel extract has been widely used in humans, but in aquatic organisms so far not have done many research. Mangosteen peel is a shell/skin that is discarded by consumers or can be called agricultural waste, the waste is very much on the skin of the fruit, about 70% is fruit skin, 20% seeds and 10% is the fruit flesh. Mangosteen peel contains a variety of bioactive substances, i.e., phenolic acids and flavonoids, which have biological and medicinal properties, especially antioxidants<sup>3</sup>. Mangosteen rind contains xanton compounds that are strong enough as antioxidants, antiproliferative, anticancer and antimicrobial<sup>4-6</sup>.

According to Kurniasari *et al.*<sup>7</sup> and Febrina *et al.*<sup>8</sup>, one of the compounds found in mangosteen rind is xanthone, in addition to killing bacteria/virus it can also increase immunity for HIV. Evidenced by the provision of food containing mangosteen peel flour can increase the number of leukocytes in African catfish, but the increase in the number of leukocytes in treated fish is still relatively normal. The increase in the total number of leukocytes has characterized that the administration of mangosteen peel flour can increase non-specific defenses by marked increase in the number of leukocytes, although still in normal numbers<sup>9</sup>.

According to Dahlifa *et al.*<sup>10</sup>, giving mangosteen rind flour to koi fish showed an increase the growth, hematocrit index and survival rate in koi carp. While results of Mardiana and Budi<sup>11</sup> showed an increase in the immune system in tilapia. Effect of xanton extract from mangosteen peel at the right dose will increase respiratory burst and activity phagocytosis in tilapia.

Blood tests are important to establish a diagnostic disease. The blood components will change if there is a physiological disturbance of fish that will determine the health status of fish<sup>12</sup>. Hematology may evaluate normal blood cells variation by intrinsic or extrinsic factors. Indication of changes in fish health conditions either due to infectious factors (microorganisms) or due to non-infectious factors (by the environment, nutrition, genetic). It is important to observe fish blood because it can help to diagnose a disease. In this, if exposed to infectious diseases, blood will experience serious changes. The number of erythrocytes and the number of leukocytes are blood parameters that can show changes in the fish body due to interference<sup>13</sup>.

There is still a lack of information on the use of mangosteen peel flour as an immune stimulant in clown fish, thus research needs to be carried out on the effect of adding

mangosteen peel flour to clown (*A. percula*) fish blood. This study was aimed to determine whether mangosteen peel flour can be used as an immunostimulant. The potential of mangosteen rind flour as an immunostimulant can be known from the blood of leukocytes, erythrocytes and hematocrit levels in clown fish.

## MATERIALS AND METHODS

**Study area:** The study was carried out at Hatchery and Production Laboratory, Fish Feed Nutrition Laboratory and Fish Health Laboratory at the Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari, South East Sulawesi, Indonesia from October, 2017-January, 2018.

**Preparation and proximat test of rind flour:** Mangosteen rind was obtained from Boro-boro village, Ranomeeto district, Konawe Selatan regency, Southeast Sulawesi province. Mangosteen fruit was separated between the fruit skin and fruit flesh. Mangosteen rind was dried in the sun until it was completely dry and then passed through the grinding process into flour. Proximate testing to determine the nutritional content of feed raw materials and feed formulation for each treatment was done by referring to methods AOAC<sup>14</sup>.

**Feed formulation and feed proximat test:** Four types of feed have different dosages of mangosteen peel flour (RPFMg) were used, namely feed A: 0% RPFMg, B: 5% RPFMg, C: 10% RPFMg and D: 15% RPFMg. Mangosteen peel flour, fish flour, soybean meal flour, shrimp head flour, fine bran flour, sago flour, wheat flour, corn flour, corn oil, fish oil, squid oil and top mix were prepared for the production of test fish feed in this study. After that, the ingredients were weighed according to the formulation of the treatment and mix all the raw materials until they are homogeneous, starting from the smallest to the largest number<sup>15</sup>. Then the feed was formed and dried for 3 days. Feed was formed according to the opening of the clown fish mouth.

**Experimental fish and rearing activity:** Test animals used in the study were clown fish (*A. percula*) measuring 4-6 cm with an average initial weight of  $3.5 \pm 1.1$  g. The fish came from the catch in nature by fishermen in Mekar village, Soropia district, Konawe regency, Southeast Sulawesi province, Indonesia and down to the laboratory using transportation.

Test fish were kept for 45 days in the Laboratory of Hatchery and Production, while the manufacture of feed was located in the Laboratory of Fish Nutrition. Blood observation was done in Laboratory of Fish Health, Faculty of Fisheries and

Marine Sciences, Halu Oleo University, Kendari, Indonesia. Test fish were kept using 12 aquarium units measuring 60×50×40 cm, as many as 5 fish per aquarium, each aquarium unit was added tap water 80% of the volume of the aquarium and equipped with aeration installation. Water changes 10-15% of the total water was made every day, namely in the morning before feeding. Feeding was full (*ad libitum*) with frequency of twice a day, in the morning (at 08.00 WITA) and evening (at 16.00 WITA).

**Blood sampling:** Syringes were rinsed with anti-coagulant Na-citrate 3.8%. Fish blood was drawn using a syringe that was inserted into the vertebrae that contains the caudal vein. The blood was allowed to capillary flow, inhaled and slowly pulled. Then the blood samples were collected in eppendorf tubes and put in crushed ice. Blood samples were taken twice, at the beginning and end of the study. Fish blood samples were taken at each treatment, taking 1 test fish in each aquarium. Hematology profiles were examined according to Anderson and Siwicki<sup>16</sup>.

**Research design:** This study used a Completely Randomized Design (CRD), consisting of 4 treatments with 3 replications.

**Observed variables:** The variables observed in this study were blood characteristics (total leukocytes, total erythrocytes and hematocrit levels).

**Data analysis:** Data haemolymph were analyzed using Analysis of Variance (ANOVA) with 95% confidence level, if they showed significantly different results, then Duncan's further tests was done using SPSS for window version 23.0 software.

## RESULTS

**Proximate profile of mangosteen skin flour:** Feed samples may only be considered healthy if they contain a good quantity of macronutrients and/or micronutrients in food. Proximate analysis is a quantitative method used to assess the macronutrient content of food or potential feed. In this process, compounds were divided into five categories in a feed test on the basis of their chemical properties. These include: water, protein, fat, ash and fiber. The results of mangosteen skin flour were obtained by proximate analysis in which 9.4961% water, 3.2151% ash, 5.7850% protein, 1.3091% fat and 47.4025% fiber obtained, while feed formulations and proximate analysis of feed test were presented in Table 1 and 2, respectively.

The formulation of the feed given to experimental fish is shown in Table 1. Feed for treatments contain different doses of mangosteen skin flour, which are 5, 10 and 15%, while control is commercial feed. Due to a different dose of mangosteen skin flour, soybean meal flour adjusts the amount in the formulation. The composition of each ingredient has been adjusted to the nutritional needs of fish, including protein, carbohydrates, fats, vitamin and minerals.

Table 1: Feed formulations for each treatment

Composition (%)	B (5% RPFMg)	C (10% RPFMg)	D (15% RPFMg)
Mangosteen skin flour	5	10	15
Fish flour	21	21	21
Shrimp head flour	20	20	20
Soybean meal flour	25	20	15
Fine bran flour	9	9	9
Sago flour	4	4	4
Wheat flour	4	4	4
Cornstarch	5.5	5.5	5.5
Corn oil	0.5	0.5	0.5
Fish oil	0.5	0.5	0.5
Squid oil	0.5	0.5	0.5
Top mix	5	5	5
Total	100	100	100

B, C and D represents treatments

Table 2: Proximate analysis of feed test

Samples	Parameters				
	Water (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)
B (5% RPFMg)	8.566	10.0802	28.4216	12.1704	6.8849
C (10% RPFMg)	11.2698	10.1604	32.3485	16.4818	7.4033
D (15% RPFMg)	10.5307	8.9447	26.6262	7.8278	9.5833

Table 3: Total leukocytes in clown fish before and after feeding with addition of mangosteen skin fruit flour

Treatments	Mean and SD of total leukocyte ( $\times 10^4$ cell $\text{mm}^{-3}$ )		
	Before feeding	After feeding	Enhancement
A (Commercial feed)	6.88 $\pm$ 0.3351	9.86 $\pm$ 1.9401	2.98 $\pm$ 1.64
B (5% RPFMg)	7.17 $\pm$ 0.3560	8.05 $\pm$ 2.1049	0.88 $\pm$ 2.43
C (10% RPFMg)	6.40 $\pm$ 0.2963	7.63 $\pm$ 2.2015	1.23 $\pm$ 1.92
D (15% RPFMg)	6.63 $\pm$ 0.3019	11.26 $\pm$ 1.1866	4.62 $\pm$ 1.06

Each treatment has 3 replications

Table 4: Total erythrocytes in clown fish before and after feeding with addition of mangosteen fruit skin flour

Treatments	Mean and SD of total erythrocyte ( $\times 10^6$ cell $\text{mm}^{-3}$ )		
	Before feeding	After feeding	Enhancement/decrease
A (Commercial feed)	2.15 $\pm$ 0.97	2.16 $\pm$ 0.30	0.006 $\pm$ 1.03
B (5% RPFMg)	3.27 $\pm$ 0.56	1.63 $\pm$ 0.21	-1.640 $\pm$ 0.39
C (10% RPFMg)	4.29 $\pm$ 2.78	1.80 $\pm$ 0.59	-2.490 $\pm$ 2.81
D (15% RPFMg)	3.30 $\pm$ 0.70	2.73 $\pm$ 0.87	-0.580 $\pm$ 1.57

Each treatment has 3 replications

Table 5: Hematocrit levels in clown before and after feeding by adding mangosteen rind flour

Treatments	Mean and SD of hematocrit (%)		
	Before feeding	After feeding	Decrease
A (Commercial feed)	9.77 $\pm$ 3.71	9.64 $\pm$ 0.69	0.13 $\pm$ 4.06
B (5% RPFMg)	13.59 $\pm$ 3.76	8.35 $\pm$ 3.26	5.23 $\pm$ 5.75
C (10% RPFMg)	12.05 $\pm$ 1.93	8.66 $\pm$ 1.17	3.38 $\pm$ 2.82
D (15% RPFMg)	12.61 $\pm$ 6.75	10.91 $\pm$ 2.58	1.69 $\pm$ 9.29

Each treatment has 3 replications

Table 2 shows the results of the proximate analysis of feed which was formulated as test feed. In accordance with the feed formulation as shown in Table 1, the only difference was mangosteen skin flour as feed additives and soybean meal flour. Nevertheless, the results of the proximate analysis show that there were nutritional differences for all B, C and D as test feed. However, it appeared that all nutritional components were within the optimum range.

### Blood profiles

**Total leukocytes:** The result of total leukocyte on clownfish before and after the addition of mangosteen rind flour is presented in Table 3.

Based on data in Table 3, the highest total leukocyte was in Treatment D (15% RPFMg) followed by Treatment A (commercial feed) then Treatment B. While the lowest total leukocytes was in Treatment C.

**Total erythrocytes:** The results of total erythrocyte on clown fish before and after the addition of mangosteen rind flour are presented in Table 4.

Based on data in Table 4, the total erythrocytes decreased after the introduction of mangosteen rind flour. Treatment C had the highest decrease in total erythrocytes (10% RPFMg) which was  $4.29 \times 10^6$ - $1.80 \times 10^6$  cells  $\text{mm}^{-3}$  followed by

Treatment B and D. While the total erythrocytes in the Treatment A commercial feed increased from  $2.15 \times 10^6$ - $2.16 \times 10^6$  cells  $\text{mm}^{-3}$ .

**Hematocrit levels:** The results of hematocrit levels of clown fish before and after the addition of mangosteen rind flour are presented in Table 5.

The addition of mangosteen rind flour hematocrit levels of clown fish has declined as compared to before feeding. Treatment B was the largest decline in hematocrit levels in clown fish followed by Treatment C and D, while treatment A (commercial feed) was 9.77-9.64%.

### DISCUSSION

Administration of mangosteen rind flour at tested doses might improve the immune system as a result of an increase in total fish leukocytes after testing. However, it did not trigger red blood cell formation, which was characterized by a slight decrease in the percentage of hematocrit and total erythrocyte levels.

The results of the study reported in Table 3 generally ranged from  $6.88$ - $7.17 \times 10^4$  cells  $\text{mm}^{-3}$  prior to the introduction of mangosteen rind flour total leukocytes. Total fish leukocytes were estimated to be in the normal range.

Based on the research of Bastiawan *et al.*<sup>17</sup>, the number of leukocytes in normal catfish ranged is  $20-150 \times 10^3$  cells  $\text{mm}^{-3}$ . Leukocytes are useful for the assessment of the immune system and variations in the proportion of these cells are considered normal<sup>18</sup>. Some errors and lack of standards for analytical methods make the results between authors and species very difficult to compare<sup>19</sup>.

Results indicated by the addition of mangosteen rind flour in feed could increase the total leukocytes in clown fish, ranged from  $7.63-11.26 \times 10^4$  cells  $\text{mm}^{-3}$ . This value indicated that the total leukocytes are in the normal range. The increase in total leukocytes in fish is thought to be influenced by the administration of mangosteen rind flour containing flavonoids such as xhanton which can increase the immune system. According to Perez-Cano and Castell<sup>20</sup>, flavonoid compounds can increase the work of the immune system and the lymphoid system becomes more effective, because it is lipophilic or can damage the microbial membrane, reduce inflammation or bleeding and swelling in the wound<sup>21</sup>.

Lukistyowati and Syatma<sup>22</sup> stated that mangosteen rind contains xhanton compounds, which can kill bacteria and can also increase immunity. Their research showed that feeding with the content of mangosteen rind flour could increase the number of leukocytes African catfish. The increase number of leukocytes was caused by the mangosteen rind containing flavonoids which could increase the ability of the lymph system to produce leukocyte cells. Immunostimulation is the result of an improvement in the immune response of the specimen due to modulation in the immune system. Modulation of response can include the activation, suppression or amplification of the immune response. It is the alteration of the immune system of a specimen by activation or suppression by an agent<sup>23</sup>. Leukocytes components such as; monocyte/macrophages, granulocytes and dendritic cells are trained phagocytes in teleosts<sup>24</sup>. In the body, leukocytes cannot be closely linked to certain organs or tissues, but they work independently that can move freely and communicate and catch foreign particles or invade microorganisms<sup>25</sup>.

Erythrocytes or red blood cells have a very abundant amount in the blood. The abundance of erythrocytes in the blood shows the great oxidative potential by fish<sup>26</sup>. The amount of erythrocytes is influenced by sex, age, environment, nutritional and reproductive status and can vary between different populations of the same species<sup>27</sup>. Variations in normal erythrocyte number values reflect the presence of some functional disturbances, this sanguine parameter being used as a diagnostic<sup>28</sup>.

Based on the data presented in Table 4, the total erythrocytes before the addition of mangosteen rind flour ranged  $2.15-4.30 \times 10^6$  cells  $\text{mm}^{-3}$ . Observation of total clown erythrocytes after the addition of mangosteen rind flour ranged from  $1.63-2.73 \times 10^6$  cells  $\text{mm}^{-3}$ . The total erythrocyte value is thought to be still in the normal range. This is comparable to the statement of Lukistyowati and Syatma<sup>22</sup>, fish feed containing mangosteen rind flour in general can reduce the number of erythrocytes, but the condition can still be tolerated by fish. According to Witeska<sup>27</sup>, the number of normal fish erythrocytes ranged from  $0.5-1.5 \times 10^6$  cells  $\text{mm}^{-3}$  for less active species to  $3.0-4.2 \times 10^6$  cells  $\text{mm}^{-3}$  for more active species. The amount of erythrocytes in fish is influenced by age, sex, temperature, environment and nutrition.

Hematocrit levels examination aimed to see the health condition of fish and to strengthen the diagnosis of a disease. The blood components will change if there are physiological disorders of fish that threaten the fish health status. These changes will occur, both quantitative and qualitative<sup>10</sup>. The results of the study, hematocrit levels of clown fish before mangosteen rind flour administration (Table 5) ranged from 9.77-13.59% in each treatment. This value showed that the hematocrit level is at a low condition. This is comparable to the statement of Radu *et al.*<sup>29</sup>, hematocrit levels are parameters that affect the measurement of erythrocyte volume. The normal hematocrit level of carp fish is 34-40.2%, lower than normal related with anemia.

Hematocrit levels in clown fish showed a decrease after mangosteen rind flour which ranged from 8.35-10.91%. Low levels of hematocrit in fish suspected clown fish in anemic condition. This is also explained by Witeska<sup>28</sup>, low hematocrit levels in fish are indicative of fish having anemia. This condition is caused by the decrease in the number of erythrocytes. In addition, according to Gallagher *et al.*<sup>30</sup>, hematocrit levels <22% indicate that fish are in anemic condition and allow disease infection. High or low hematological parameters levels describe changes in fish health conditions, one of them can be seen from the volume of erythrocytes<sup>31</sup>. Furthermore, according to Bastiawan *et al.*<sup>17</sup>, high levels of hematocrit indicated that fish contaminants and triggers experience stress, in contrast if hematocrit levels become low, it describes fish lacking vitamins, proteins or fish are experiencing infections, where fish that have anemia have hematocrit value <10%. This has an impact on stunted fish growth due to the low amount of erythrocytes which causes food supply to cells, tissues and organs to be reduced, so that the metabolic process of fish will be inhibited. Meanwhile, based on research by Lukistyowati and Syatma<sup>22</sup>, feeding containing mangosteen rind does not affect the hematocrit levels of African catfish.

According to Dahlifa *et al.*<sup>10</sup>, low hematocrit levels, due to the ability of fish to absorb carotenoids in accordance with the nutritional composition of the food eaten. Decrease in hematocrit levels of clown fish after the addition of mangosteen rind flour, indicated that feeding containing mangosteen rind flour did not indicate the presence of red blood formation. This can be seen from the decrease in the value of erythrocytes and hematocrit at the time of measurement. Similar results were also reported in the study of Adrial *et al.*<sup>32</sup>, feeding containing mangosteen rind flour to comet fish showed a decrease in hematocrit levels which before treatment was around 20.01-22.85 to 10.64-12.87%. This showed that the mangosteen rind flour at the dose tested does not have a good influence on the formation of comet fish red blood cells. However, Ningsi *et al.*<sup>33</sup> concluded that clown fish fed with supplemented dietary dosage of mangosteen peel flour 5, 10 and 15% increased absolute growth ranged from 0.61-1.4 g. The findings of this blood parameter study showed that clown fish were better treated in feed formulation with 15% mangosteen peel flour. Further studies are needed on the inexpensive extraction methods for commercial applications of mangosteen peel flour extract, administration of oral or immersion methods, their concentrations and the time of fish exposure to these extracts.

### CONCLUSION

It can be concluded that the administration of mangosteen rind flour mixed in feed at the tested dose can improve the immune system analyzed descriptively seen from the increase in total leukocytes of clown fish after giving test feed. However, it does not trigger red blood cell formation which is characterized by a decrease in the percentage of hematocrit levels and total erythrocyte values.

### SIGNIFICANCE STATEMENT

The research discovered the possible use of mangosteen rind flour for blood profile improvement of clown fish which can be beneficial to prevent fish disease in the culture. The research discovered the possible use of mangosteen rind flour at a sufficient dose mixed in feed that may be beneficial to fish immunostimulation, thereby increasing their survival rate. This study will help the researchers to uncover the critical areas of fish health that many researchers were not able to explore. Thus a new theory on the use of phytopharmaca in feed formulation may be arrived at.

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