Original Research Article

Enhancement of Color Brightness on Clown Fish (*Amphiprion percula)* With Addition of Tomato Flour Extract

Abstract

The prospect of clown fish cultivation business is still being expanded to make profitable business activities and increasingly attract ornamental fish lovers. However, it is difficult to get clown fish with a good level of color brightness. So it can reduce the level of sales production. This study aims to determine the effect of the addition of tomato flour to increase the brightness of the color clown fish at the Ambon Marine Aquaculture Fisheries Center. The research was conducted in March to June 2020 at the Ambon Marine Aquaculture Fisheries Center. This study used Completely Randomized Design (RAL) with 4 treatments and 3 replications. The treatment is to mix tomato flour in the feed with a concentration of 0 mg / gr (Treatment A), concentration 20 mg / gr (Treatment B), concentration 40 mg / gr (Treatment C) and concentration 60 mg / gr (Treatment D). The fish used is a clown fish with a size of 1 cm with a spread of 4 fish with a circulatory maintenance system. The observed parameters include the brightness ~~level~~ of the clown fish color, which is measured using the colour grab camera app, the growth of which is the average length and weight average seeds measured using a ruler with a precision rate of 1 cm and a digital scale with a precision rate of 0.01gr and survival rate. The results showed that feeding with the addition of flour tomato did not give significant effect to increase the brightness of the color on the clown fish. However, it affects the growth (length and weight) and survival rate of clown fish seeds.

Keywords: Clown Fish, Color, Tomato Flour

1. Introduction

Clown fish is one of the leading commodity of ornamental fish water sea, life in the waters of the coral reef symbiosis with anemones and has a high economic value. Data Center for Statistics and Information Secretariat General of the Ministry of Marine Affairs and Fisheries, the export volume of seawater ornamental fish in 2007 - 2011 increased by 0.26%. The purpose of marketing nemo fish among them are Australia, Japan, Germany and France that the ornamental fish nemo has been identified in the world as much as 34 species [14]. Meanwhile, according to [2] clown fish (Amphiprion) has 28 species of amphiprion that have been identified that are found in shallow to deep waters, on a shallow base.

The species is known by its common English name which includes orange clown fish, clown anemone fish, percula clown fish, anemone percula fish, orange anemone fish, true

Comment [OC1]: The manuscript needs major improvement.
Information to reproduce the study is lacking.
What is informed the choice of the dose of tomatoe powder?
The authors did not conduct toxicity studies to evaluate the toxicity of the tomato powder inclusion.
Presentation in Figures is very poor and needs major improvement.
The results are misleading in some case.
The discussion of the study lack depth and needs major improvement.

Comment [OC2]: Do the authors mean "tomato powder" instead of "tomato flour"?!

Comment [OC3]: … clown fish culture …

Comment [OC4]: Poor sentence structure

Comment [OC5]:

Comment [OC6]: Is this needed in the Abstract? More over, it is a repetition of the previous sentence

Comment [OC7]: ???

Comment [OC8]: ???

Comment [OC9]: Ambiguous

Comment [OC10]: Ambiguous

Comment [OC11]: ???

Comment [OC12]: ??

Comment [OC13]: This is at variance with the conclusion in the discussion regarding survival

Comment [OC14]:

Comment [OC15]: The Introduction section of the manuscript has to be rewritten. It is difficult to understand. The authors need improve the English. In addition, the authors need to provide more information on the clown fish, and the justification for addition/enhancing the brightness of the colors and information on related studies. This will give the readers better background for the study.

percussion clown fish, black fin clown fish, eastern clown fish, eastern clown anemone fish, and orange clown anemone fish. Common names in other languages include bantay bot-bot (Cebuano); orangegul klovnfisk (Denmark); pata (Davawenyo); maumanu ni masao (Gela); clownfisch (Jerman); samok-samok (Kagayanen); paja-paja (Makasar); badut biak fish, gelang roay (Melayu); amfiprion (Polandia); baro-baro (Visayan); dan bantay-kibot (Waray-waray). List of common names that is complete can be found on the Fishbase website [9].

Clown fish have an attractive shape and color pattern that is orange (orange), white stripes on the head, body and base of the tail, and the presence of a black silhouette on the top of the body, and it suitable for fish-only aquarium fillers and reef aquariums [15]. This nemo fish lives in groups and always side by side with sea anemones where other fish are unable to survive in the anemone space. The specific symbiosis made this Amphiprion ornamental fish earned the nickname *Anemonfish* or *Badutfish* [2]. In this symbiosis the fish gets protection and feeds on nonmetabolic materials released by anemones. On the other hand, anemones are cleaned and protected from predators [2].

The difficulty of obtaining seeds with quality that meets export criteria with bright orange seed color and thick black strips resulted in decreased production sales rate. Decreased color brightness can be caused by strees due to the environment, lack of sunlight, disease, lack of feed, especially lack of color components in feed. Adding carotene substances can increase color brightness in clown fish. Carotene substances are found in fruits and vegetables, one of which is papaya fruit and carrots. The red orange fruit contains betakaroten (provitamin A) which is an ingredient in the formation of vitamin A. The results of [13] study on the addition of carrot pollen to increase the color of clown fish with a dose of 40 ppm beta carotene had a real influence. While the research results of [8] that papaya extract conducted by spraying methods on clown fish seed feed did not see significant changes.

2. Materials and Methods

2.1 Time and Location

This research was conducted for 4 months at the Ambon Marine Aquaculture Fisheries Center.

2.2 Tools and Materials

Tools and materials used are aquarium size 40x40x40 cm, colour grab camera as color identification, clown fish seed as much as 48 heads and tomatoes for the manufacture of tomato flour.

2.3 Methods

The research method used Completely Randomized Design (RAL) with 4 treatments and 3 replicates:

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Comment [OC17]: This needs major improvements:
1. What informed the dosage regimen of the tomato powder?
2. What is the condition in the aquarium the clown fish were maintained?
3. What kind of aquarium was used?
4. Fish were sampled once a week, what is the time of the day when they were sampled?
5. How were the fish fed?
6. What is the composition of the feed?

Comment [OC18]: Fish condition:
What gender, age, size and condition of fish were used in the study?

Comment [OC19]: Which months?

Comment [OC20]: Where?

Comment [OC21]: Single sentence paragraphs are not the best.

Comment [OC22]: Make, model identification

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Comment [OC26]: How many fish in each replicate?

- Addition of tomato flour with a concentration of 0 mg/g (control)
- Addition of tomato flour with a concentration of 20 mg/g
- Addition of tomato flour with a concentration of 40 mg /g
- Addition of tomato flour with a concentration of 60 mg/g

Tomato flour is made by drying first in the oven at temperature of 65^0C and blended until smooth. Tomato flour mixed on the feed is formed according to the mouth openings of clown fish seeds. Sampling is conducted by direct observation in the field of research conducted. Sampling is done once a week by measuring the color brightness of clown fish seeds using the colour grab camera application, growth includes absolute length and weight using a ruler with a precision of 1 cm and a digital scale with a precision of 0.01 gr. Water quality parameters include temperature, pH, DO and salinity. The administration of tomato flour to increase the brightness of clown fish seed color is analyzed using non-parametric statistical methods with wallis kruskal test on spss 22.

The basis of wallis kruskal test decision is as follows:

• If the value of Asymp.sig > 0.05, then there is no difference or H0 is accepted and Ha is rejected

• If the value of Asymp.sig < 0.05, then there is a difference or H0 is rejected and Ha accepted.

3. Results And Discussion

The results showed that in treatment A (control), B (20 mg / gr), C (40 mg/gr) and D (60 mg/gr) occur fluctuations in color brightness every week. Decrease the brightness of the color on the clown fish resulted in fish is pale yellow. This is allegedly because some of the fish did not respond to feed properly so that the fish shortage of feed and nutrients. According to the [10] that decline the brightness of the color on the clown fish is change the color of the morphology, the shortage of the amount of pigment in chromatophore changes caused by the stress due to the environment are less suitable, less sunlight and lack of color components in the feed. While according to [12], less feeding will result in decreased fish color and slow growth.

In increasing the brightness of the color of clown fish need to add color components in the form of betakaroten contained in fruit or vegetables that are red. According to [11] all parts of tomatoes contain betakarotein but the most dominant is found in tomato meat. The number of carotene in tomatoes is 56.90 ppm consisting of lycopene, a-carotene, and b-carotene. In the management of feed, tomatoes are processed into flour and mixed with fish feed. In drying tomatoes using a temperature of 65^0C. According to [11], drying tomato can use temperature 60 – 70^0C so do not use high temperature because it can damage the nutrients contained in the fruit of tomato.

To see the effectiveness of the flour tomatoes mixed in feed the fish measured based on the HSB (*Hue, Saturation and Brightness*). The following average results HSB on clown fish can be seen in figure 1, 2 and 3.

Comment [OC27]: Do the authors mean 0 – 60 mg/g body weight?

Comment [OC28]: This is not the appropriate symbol/sign

Comment [OC29]: This is tomato powder!!

Comment [OC30]: ???

Comment [OC31]: ???

Comment [OC32]: Establish acronym first

Comment [OC33]: English

Comment [OC34]: Rewrite

Comment [OC35]: Figures should have a legend accompanying it, which describes components of the Figure. The Figures should have the control (Kontrol) with the values expressed as other to maintain uniformity

Comment [OC36]: Variations in the color brightness are bound to be observed. This may be due to changes in the lighting, feed availability, competition, environment and time of sampling etc. How did the authors control for these?

Comment [OC37]: ?

Comment [OC38]: ?

Comment [OC39]: ??

Comment [OC40]: Authors should be more scientific!

Comment [OC41]: ?

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Comment [OC44]: Authors should describe what these mean, and how they are measured

Comment [OC45]: Establish the acronym first

Comment [OC46]: Figure

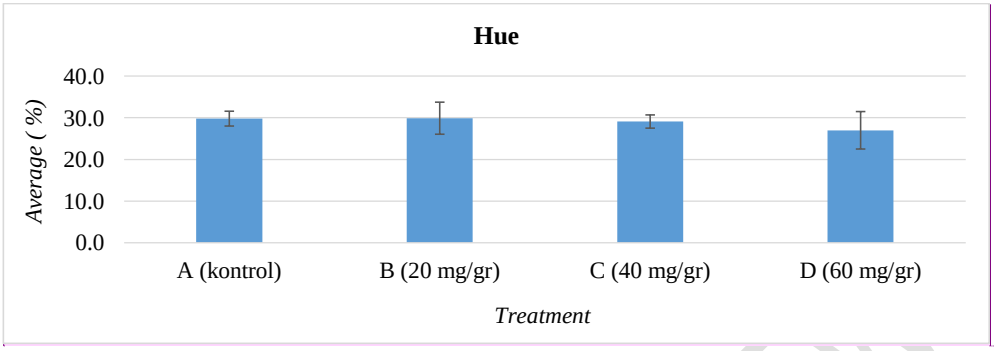

Fig. 1. Average hue value

Based on Figure 1, the average brightness level of the fish's color with attention to the *hue* result. *Hue* values in treatment A (control) 29.8 ± 1.78 %, B (20 mg/gr) 29.9 ± 3.85 %, C (40 mg/gr) 29.01 ± 1.59 % and D (60 mg/gr) 27 ±4.50 %. So it can be concluded that on treatment D has a low hue value. The lower the value of hue, it will produce a red color. Hue values range from 0-360° which means the value of 0° is red and continues to increase until the value of 90° is interpreted as yellow (17]. Here's Figure 2 of the average saturation result as follows:

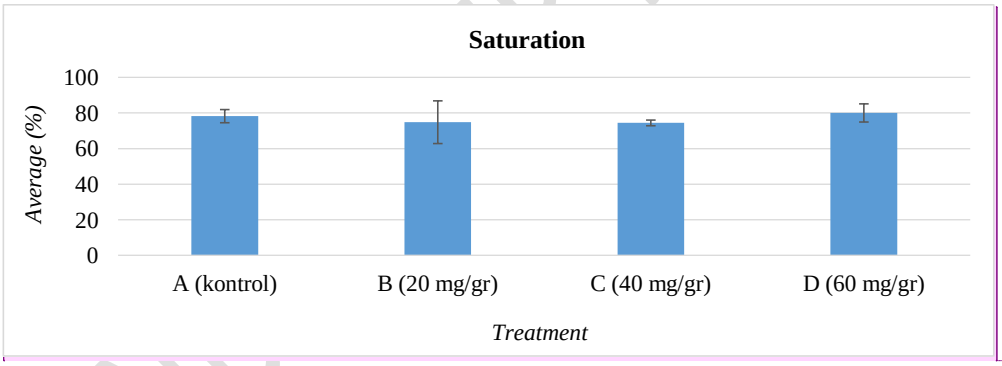

Fig. 2. Average saturation value

Based on the result of Figure 2, the saturation value in treatment A (control) 78.2 ± 3.7 %, B (20 mg/gr) 74.8 ± 12 %, C (40 mg/gr) 74.4 ± 1.6 % and D (60 mg/gr) 80 ±5.1 %. So it can be concluded that the highest saturation value in the treatment D. The higher value the saturation is owned, then the color appear brighter [5]. Here in figure 3 can be seen the average value of the brightness is as follows:

Comment [OC47]: Kontrol???

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Comment [OC50]: Going by the error bars only, it appears there is no significant difference between the groups. Concluding that group D has lower hue may be incorrect.

Comment [OC51]: The discussion here adds no value

Comment [OC52]: ??

Comment [OC53]: ??

Comment [OC54]: The Figure does not have a legend.

Comment [OC55]: ???

Comment [OC56]: Are the values here significantly different going by the error bars??

Comment [OC57]: This conclusion may be incorrect

Comment [OC58]: The discussion did not value to the results interpretation, comparison with information in literature. It needs to be improved.

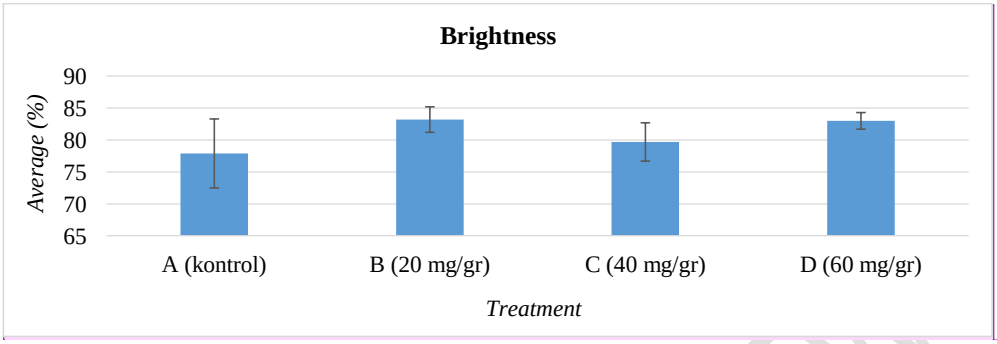
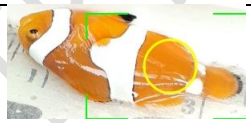
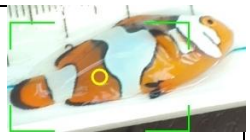


Fig. 3. Average brightness value

Based on Figure 3, the brightness value in treatment A (control) 77.9 ± 5.4 %, B (20 mg/gr) 83.2 ± 2 %, C (40 mg/gr) 79.7 ± 3 % and D (60 mg/gr) 83 1.3 %. So it can be concluded that in treatment D has a high brightness value. The higher the value of the brightness that is owned, then the colors appear brighter while the lower the value of brightness then the dark [5].

Table 1. Final sampling of clown fish color brightness levels

			
A (kontrol)	B (20 mg/gr)	C (40 mg/gr)	D (60 mg/gr
Hue: 29,8 %	*Hue*: 29,89 %	*Hue*: 29.01 %	*Hue*: 27 %
Saturation : 78,2 %	*Saturation* : 74,8%	*Saturation* : 74,4 %	*Saturation* : 80 %
Brightness : 77,9 %	*Brightness* : 83,2%	*Brightness* : 79,07%	*Brightness* : 83 %

Based on Table 1 in treatment A (control) there is no mixing of flour tomato on the feed, so the result from the hue is low and the saturation and the brightness is not too high so as to produce the orange color. In treatment A,B,C and D the value of the hue and low saturation and brightness is high enough so that it produces a reddish-orange color. When viewed from the average result of HSB treatment D there is little change compared to the treatment A, B and C, but the classification of colors remains the same that is reddish orange. It is suspected that the dose for treatment D has not been able to meet the needs of karetonoids in the body of fish. According to [19] in determineing the dose to be used need to be considered, if too much can decrease the endurance of the fish while if the deficiency results in color on the body of the fish is not maximal.

Comment [OC59]: Kontrol???

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Comment [OC62]: Comma (,) or period (.) in the values.

Comment [OC63]: The error values should be included. As presented here it is misleading.

Comment [OC64]: This is incorrect. The fish may already have adequate carotenoids and supplementation with tomato powder is of no value.

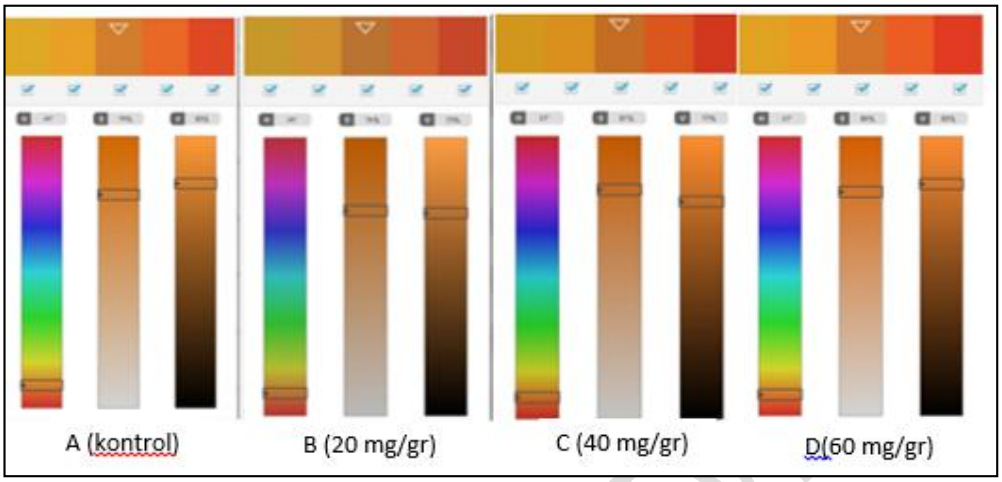

Fig. 4. Image of color identification pattern in *color grab camera* app

The lower the value of the hue it produces a bright red color as well as diimbangin with saturation and high brightness. According to [18] that the color of clown fish is reddish orange or blackish orange with a high level of brightness. According to [11] changes in the intensity of color brightness in fish can be increased by adding carotenoid sources to fish feed and the appropriate environment so that fish can develop well. The color performance of hybrid clown fish is better than pure breed clown fish with individual body parts of hybrid and pure offspring showing significant color variations between each other [1]. Water quality during observations such as temperature, salinity, DO and pH is still within tolerance limits. It can be seen from the parameters of the temperature during the observations ranges between 27-29^{0}C. This is in accordance with [6] that, the effect of water temperature on the environment can stimulate growth and appetite, because the digestive process of food at low temperatures will be very slow and vice versa will be faster in warmer waters. Optimal and ideal temperature in the maintenance of survival reaches a range between 25-30^{0}C. The result of pH measurement during the study was 7, indicating a value that is still within the tolerance limit. This is in accordance with the statement [7], that, clown fish can live well on the level of acidity ranged from 6-9. The results of measurements DO is 4-6,14 mg/L the content of oxygen that is better for the purposes of cultivation is 5-9 mg/L.

Comment [OC65]: ?

To further confirm the results obtained were analyzed with the kruskal wallis. Based on the kruskal wallis showed that feeding with the addition of flour tomato did not give significant effect to increase the brightness of the color clown fish with the results of the kruskal wallis $P < 0.05$. In terms of growth, the average length increase can be seen in Figure 5 as follows:

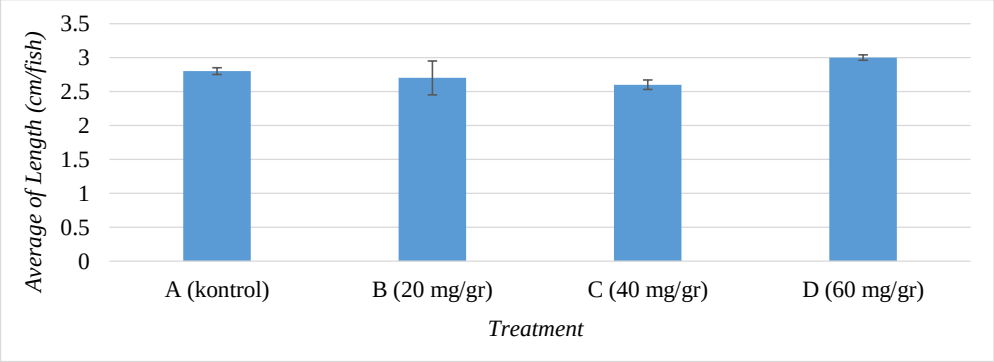

Fig. 5. Average value of observations of length and weight on the seed clown fish

Based on figure 5, it can be concluded that the increase of the length of the average in each of the treatment is not too much different result. In treatment A (control) 2.8 ± 0.05 cm, B (20 mg/gr) 2.7 ± 0.25 cm, C (40 mg/gr) 2.8 ± 0.07 cm, and D (60 mg/gr) 3 ± 0.04 cm. So that the highest average length increase is found in treatment D followed by treatment A, B and C. Average length of high on treatment D is suspected because the fish respond feed properly so that the fish consume feed and absorb the nutrients contained in the feed and used as energy so that the fish can grow well. This is in accordance with [3] that if the fish has the level of feed consumption, the intake of nutrients that will be accepted more and will be used for growth. In treatment A has a value not too far with the value of treatment D. It is suspected because fish respond to feed well but the nutrients obtained only nutrients from feed due to the treatment A the absence of additional tomato flour. According to [4] that the essential nutrient in growth is vitamins. The tomatoes contains a variety of vitamins: vitamins A, C, D, E, K, D and B. In the treatment of B and C fish are suspected to have a low appetite because each fish has a different level of feed consumption and optimal so if the feeding has passed the optimal level of feed consumption then the feed is not used for growth but discarded in the form of feces.

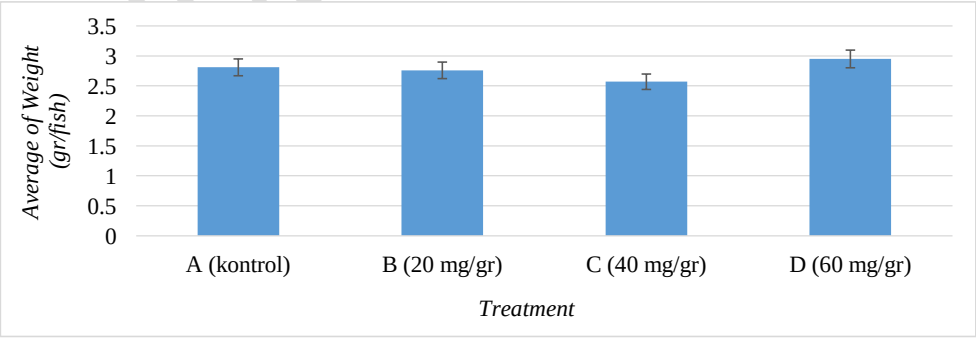

Fig. 6. Average weight of clown fish seeds

Comment [OC66]: What length (standard, forked, or total)?

Comment [OC67]: This is ambiguous. The values presented in Fig. 5 is at variance with what the authors have here. Treatment/group C in the Fig. 5 is lower than treatment/group B.

Comment [OC68]: Treatment/group D may be significantly higher than the A and C in Fig.5 and the values presented here.

Comment [OC69]: So why are the shorter than the control?

Based on figure 6, the increase in the average weight in each treatment is not too much different result. In treatment A (control) 2,81±0.94 gr, B (20 mg/gr) 2.76 ± 00.92 gr, C (40 mg/gr) 2.57 ± 0.86 gr and D (60 mg/gr) 2.95 ± 0.98 gr. So that the highest average weight gain is found in treatment D, followed by treatment A, B and C. The highest average weight gain on treatment D. The mixture of food pellets and flour tomatoes give a red color to the blood so that the fish are more interested to eat it. In accordance with the statement [8] that in addition to the protein factor of food eaten, food attractiveness factor in the form of color and availability of food is also a determining factor of fish growth. Good growth is also suspected because clown fish seeds can absorb the nutrient content in tomatoes so as to get new energy in doing their activities. This is in accordance with [3] one of the nutrients that are important for growth is vitamins. Vitamins are a role in metabolism. In particular tomatoes are very important in the formation of collagen, to produce fish that grow normally.

In treatment A has a value that is not too far in the treatment D is suspected good growth in terms of length and weight indicates that the food given and eaten by the fish and can meet the need for maintenance of life. This is in accordance with [3] which states that energy is used by fish for basic metabolism, movement, production of sexual organs, treatment of parts of the body as well as the replacement of cells that have been damaged and for growth. While in treatment B and C have a low appetite. According to [12] less feeding will result in slow growth. So it can be concluded that feeding with the addition of tomato flour can increase the growth of clown fish.

The survival of clown fish from the beginning of maintenance to the end of maintenance of the absence of dead fish indicates that the survival of clown fish during the study is 100% suspected due to the low dense spread and nutrition of feed provided to meet the needs of clown fish and water quality is still limited to normal. According to [15] high survival is suspected because feed nutrients (protein, fat, carbohydrates, vitamins and minerals) have met the needs of clown fish as well as water quality such as temperature, salinity, and pH are still within tolerance limits. So it can be concluded that feeding with the addition of tomato flour can improve the survival of clown fish.

4. Conclusion

Feeding with the addition of flour tomato did not give significant effect to increase the brightness of the colors on the fish clown with the results of the kruskal wallis $P < 0.05$. But it affects the growth (length and weight) and survival of clown fish seeds.

Comment [OC70]: Not in alignment with 100% survival reported by the authors.

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