

THE EFFECT OF ADDITION OF TURMERIC FLOUR (*Curcuma longa* Linn) INFEED ON THE PERFORMANCE OF THE GROWTH AND IMMUNE SYSTEM OF FISH (*Oreochromis niloticus*)

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ABSTRACT

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This study aims to determine tilapia's growth and immune system (*Oreochromis niloticus*) through feeding with added turmeric flour. This study used a completely randomized design with five treatments, namely K- (feed without adding turmeric flour and injected with physiological solution), K+ (feed without adding turmeric flour and injected with *A. hydrophila* bacteria), P1 (addition of turmeric flour 1 % / kg of feed + bacterial infection of *A. hydrophila*), P2 (Addition of 2% turmeric flour/kg of feed + bacterial infection of *A. hydrophila*), and P3 (Addition of 3% turmeric flour/kg of feed + bacterial infection of *A. hydrophila*) each treatment had three replicates for a total of 15 trials. The results showed that there was a significant effect ($p > 0.05$) on growth but no significant effect ($p < 0.05$) on the survival of tilapia, so further tests were carried out to determine the best treatment in producing tilapia growth, it is known that P2 (Addition of 2% turmeric flour/kg of feed + bacterial infection of *A. hydrophila*) is the best treatment in this study.

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a fishery commodity that has good prospects. This fish is one of the essential commodities of freshwater aquaculture in Indonesia. Fish farming is preferred because it is easy to maintain, has a fast rate of growth and reproduction, and is resistant to pests and diseases. Judging from its eating habits, this fish is all-eating (omnivorous), so it is easy to be given additional feed.

The feed given to fish is considered good not only from the components that make up the feed but also from the size of the components contained in it. Feed is one component that supports aquaculture business activities, so the available feed must be adequate and meet fish needs. Feed availability is also very influential on the growth and survival of farmed fish.

The feed used in general is a commercial feed which spends around 60-70% of the total production costs incurred (Hamdani et al., 2018). The feed quality strongly influences the feed utilization by fish in terms of nutrient content or the level of digestibility of the feed itself. Besides acting as the primary energy source, quality feed is also expected to increase the digestibility of fish so that growth becomes optimum.

One way to increase the growth of tilapia is to optimize the physiological function of the fish's organs, namely the digestive tract. A vital organ that plays a role in the digestive tract is the intestine because it is closely related to the activity of digestive enzymes in the fish's body (Rojtinnakorn *et al.* , 2012; Putri *et al.* , 2016). Digestive enzymes have an essential role in the process of digesting feed nutrients. The availability of digestive enzymes will affect the effectiveness of enzymes in digesting the feed given and, in turn, affect growth. One way to optimize digestive enzymes is by giving turmeric (*Curcuma longa* Linn .) natural ingredients (feed additive). According to Handayani, 2006 in Putri, *et al.* , 2016).

Turmeric (*Curcuma longa* Linn .) contains curcumin and essential oils. Curcumin is a polyphenol compound found in turmeric ranging from 3-6%. According to (Sinurat *et al.* , 2009), turmeric flour contains 9.61% curcumin and 3.18% essential oil. Curcumin has a function that can stimulate the walls of the gallbladder to secrete bile into the small intestine to improve the digestion of fats, proteins, and carbohydrates so that the activity of absorption of food substances increases, and the essential oil in turmeric prevents excessive release of stomach acid so that gastric conditions do not require too acidic and facilitate the absorption of nutrients by the small intestine.

Turmeric is also in the pharmaceutical industry and has excellent potential for pharmacological activity (Joe *et al.* , 2004). The secondary metabolites of turmeric plants include antibiotics, alkaloids, steroids, essential oils, resins, phenols, tannins, and others. In many studies, turmeric is an anti-bacterial and anti-inflammatory because it contains curcumin compounds and essential oils (Hudayani, 2008). This study confirms that curcumin is relatively safe and can be used as an immunomodulator for the immune system (Berto, 2010 in Pangestika *et al.* , 2012). So it is necessary to research the use of turmeric in tilapia feed to determine the performance of adding turmeric powder to feed on the growth and immune system of tilapia (*Oreochromis niloticus*).

METHODOLOGY

This research was conducted at the Private Institution of the City of Mataram, NTB, from September 6 to November 27. The test animals used were Tilapia obtained from cultivators in Lingsar Village, with a total of 150 fish weighing 9 - 12 grams, each treatment containing ten fish.

This study used a one-factor, Completely Randomized Design (CRD) method with five treatments, and each treatment had three replications. The level of treatment in this study are:

K- = Addition of 0% turmeric flour/kg feed + physiological solution infection

K- = Addition of 0% turmeric flour/kg feed + *S. iniae* bacterial infection

P1 = Addition of 1% turmeric flour/kg feed + *S. iniae* bacterial infection

P2 = Addition of 2% turmeric flour/kg feed + *S. iniae* bacterial infection

P3 = Addition of 3% turmeric flour/kg feed + *S. iniae* bacterial infection

Data analysis

The parameters observed in this study were the growth rate of specific weight and length = $((\ln W_t - \ln W_o)/t \times 100)$, $((\ln L_t - \ln L_o)/t \times 100)$; Feed conversion = $(F/100 \times (W_t - W_o)/W_o)$; Feed efficiency = $(W_t - W_o)/(F \times 100)$; survival = $((N_i - N_o) \times 100)$. Red blood cells = $N = n \times 10^4$; white blood cells = $N = n \times 50$. W_o = initial weight; W_t = final weight; t = maintenance time; L_o = initial length; L_t = final length; N_o = initial number of live fish; N_i = final number of live fish; F = total amount of feed given; n = number of blood cells; N = number of blood cells

in 1 mm³ of blood. Whether or not the effect of the treatment is determined by ANOVA inspection of variance. If there is a difference between the treatments, proceed with the BNT, BNJ, and Duncan follow-up tests. To determine the use of the follow-up test, it is seen based on the uniformity coefficient value.

RESULT

Based on the research that has been done, the data obtained are specific growth, feed conversion, feed efficiency, survival, red blood cells, white blood cells, bacterial counts in the intestine, and water quality.

Specific Growth Rate

The specific growth rate is closely related to the increased body weight, and length of the fish from the feed consumed. Observing the specific growth of tilapia for 30 days of rearing showed differences in the feed given turmeric flour and feed without the addition of turmeric flour. Can be seen in Figure 4.

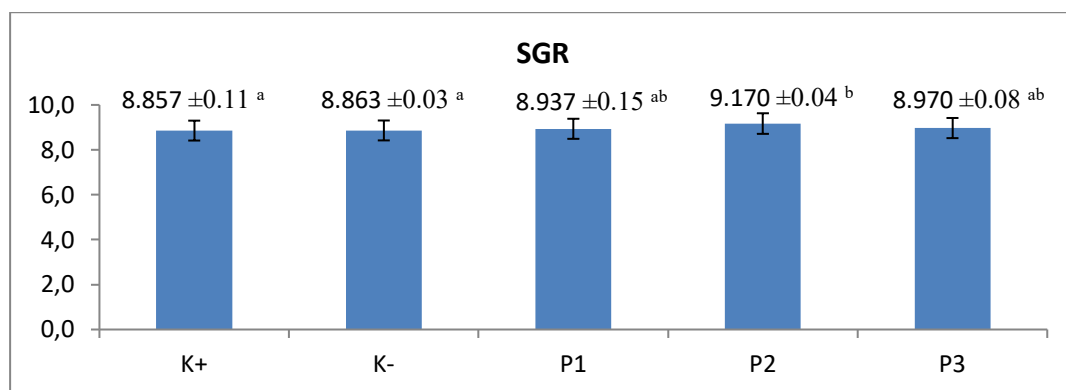


Figure 1. Tilapia specific growth rate

Figure 1. shows that the highest specific weight of tilapia was found in treatment P2 at 9.170%, followed by P3 at 8.970%, P1 at 8.937%, and K- 8.863%, while K+ produced the lowest average specific weight of tilapia of 8.857 %. Based on the results of the analysis of variance (ANOVA) test, the addition of turmeric powder to the feed had a significant effect ($P > 0.05$) on the specific weight of tilapia.

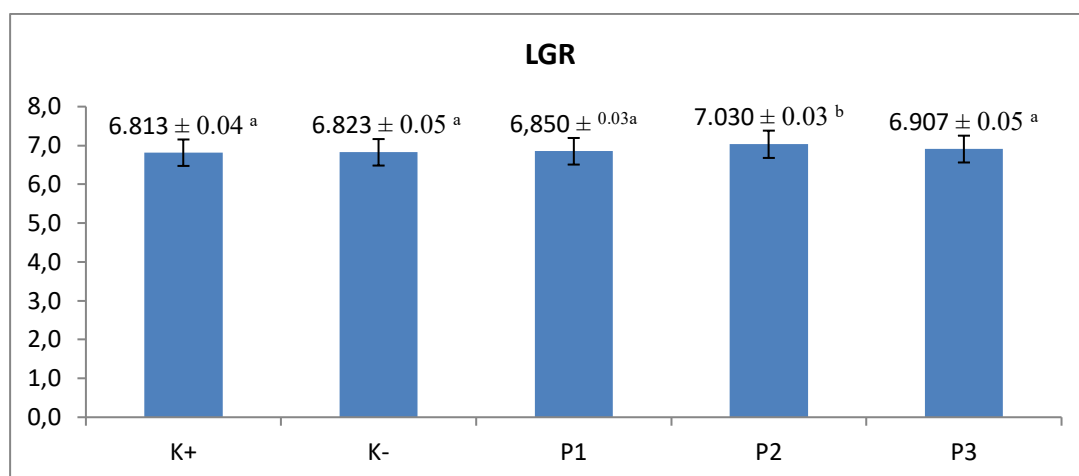


Figure 2. The length growth rate of tilapia

Figure 2. shows that the highest specific length growth of tilapia was in treatment P2 of 7.030%, followed by P3 of 6.907%, P1 of 6.850%, K- of 6.823%, and, while K+ produced an average specific weight of fish The lowest value is 6.813%. Based on the results of the analysis of variance (ANOVA) test, the addition of turmeric powder to the feed had a significant effect ($P > 0.05$) on the specific length of tilapia. Figures 4 and 5 show that the specific growth rate pattern is the same between the weight and length of tilapia.

Tilapia Survival

The survival rate is the number of fish that live at the end of rearing from the number of fish stocked at the start and is presented as a percentage (Faziel *et al.* , 2017). The survival value of Tilapia can be seen in Figure 3.

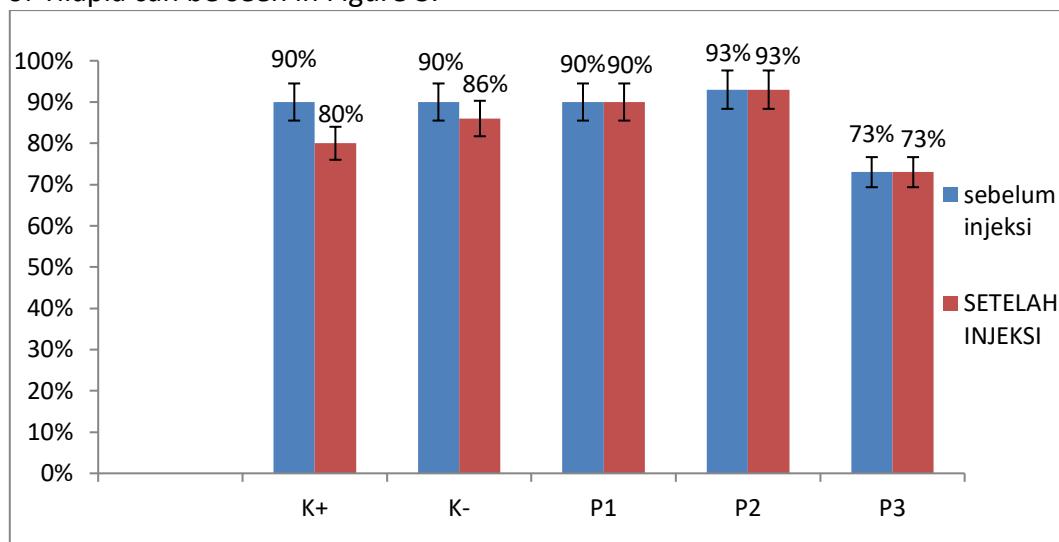


Figure 3. Survival before and after injection

Based on Figure 3. above, the survival rate for tilapia was obtained, which ranged from 93.33–73.33%; the highest survival rate for tilapia was found in treatment P2 of 93.33%, while the lowest survival rate was found in treatment P3 with a value passability of 73.33%. Based on the results of the analysis of variance test (ANOVA), the addition of turmeric powder to the feed had an insignificant effect ($P > 0.05$) on the survival of tilapia; namely, there was no significant difference in each treatment for the survival of tilapia.

Feed Conversion Ratio

Feed conversion is the amount of feed given during rearing to produce fish weight gain at the end of rearing; body weight produced by the amount of feed can be determined by looking at the initial weight of the fish and the final initial weight Amirkolaie *et al.* , (2005) in Garcia *et al.* , (2012). A smaller FCR value indicates that the feed consumed by fish is more efficiently used for growth. In contrast, a more excellent FCR value indicates that the feed consumed is less efficient (low growth utilization). The results of tilapia feed conversion observations during the study can be seen in Figure 4.

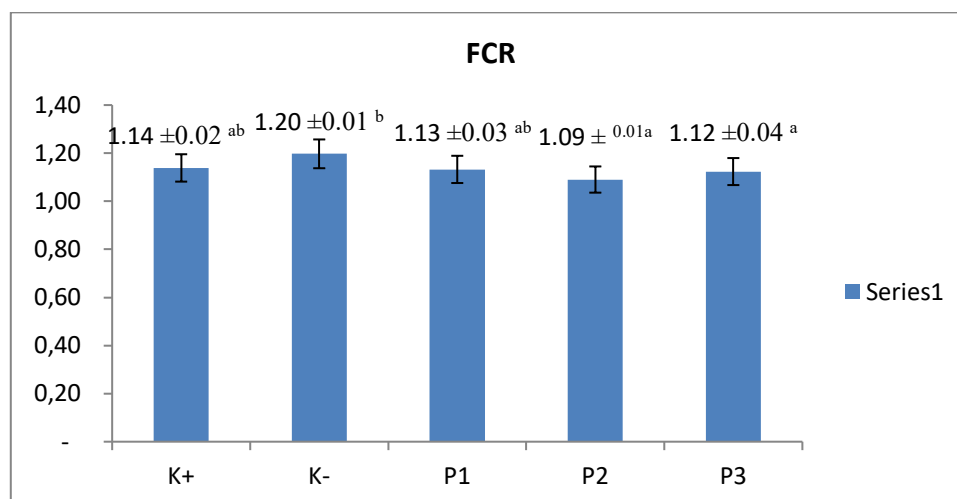


Figure 4. Tilapia feed conversion

Based on Figure 4 above, it can be seen that the treatment with the lowest feed conversion value was P2 of 1.09%, then P3 of 1.12%, P1 of 1.13%, then K+ of 1.14%, and the highest feed conversion value, namely in the K- treatment of 1.20%. Based on the results of the analysis of variance (ANOVA) test, the addition of turmeric powder to the feed had a significant effect ($P > 0.05$) on tilapia feed conversion.

Red Blood Cells

Red blood cells (Erythrocytes) are the most numerous blood cells. The results of the calculation of red blood cells from the five treatments can be seen in the table.

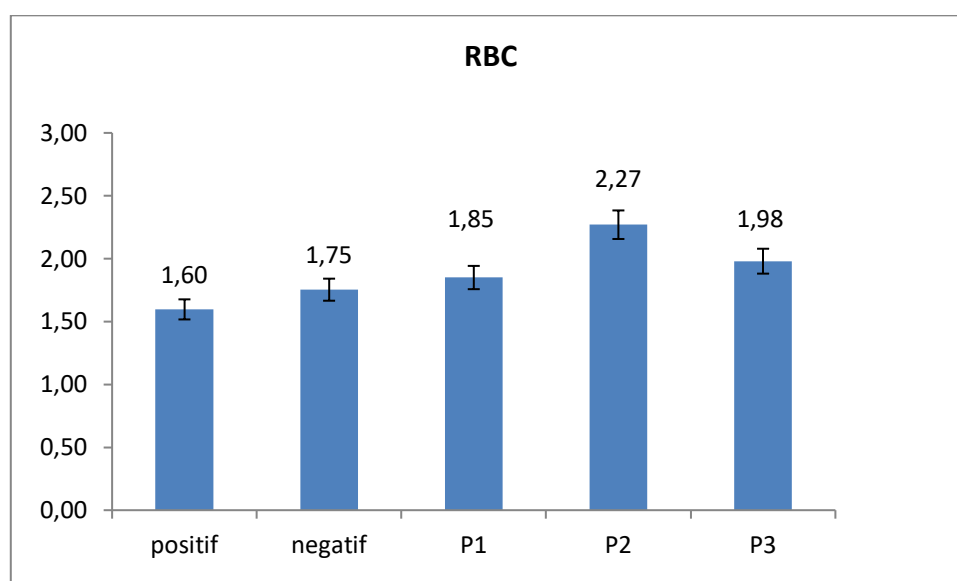


Figure 5. Red Blood Cells

Mathematically the highest red blood cell value was in treatment P3 (feed + 3% turmeric flour) of 1.98×10^6 cells/mm³, followed by P2 (feed + 2% turmeric flour) of 1.95×10^6 cells/mm³, then P1 (feed + 1% turmeric flour) of 1.83×10^6 cells/mm³, for negative and positive controls respectively and 1.73×10^6 cells/mm³ and 1.60×10^6 cells/mm³.

White blood cell

White blood cells (leukocytes) are a part of the blood that produces antibodies. More leukocytes are produced when the body is sick. The increase in leukocytes also indicates an increase in the number of antibodies. The results of the white blood cell count of the five treatments can be seen in the table.

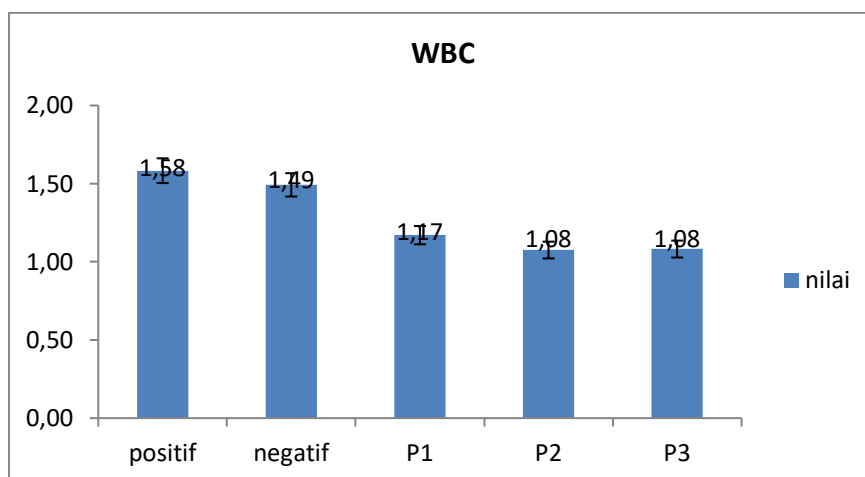


Figure 6. White Blood Cells

Mathematically the highest white blood cell value was in the K+ treatment of 1.58×10^5 cells/mm³, followed by a K- of 1.49×10^5 cells/mm³, then P1 of 1.17×10^5 cells/mm³, for treatment P2 and P3 that is equal to 1.08×10^5 cells/mm³ and 10^5 cells/mm³. Based on the test results, it was found that adding turmeric powder to the feed significantly increased the number of leukocytes produced ($P < 0.05$).

Total bacteria

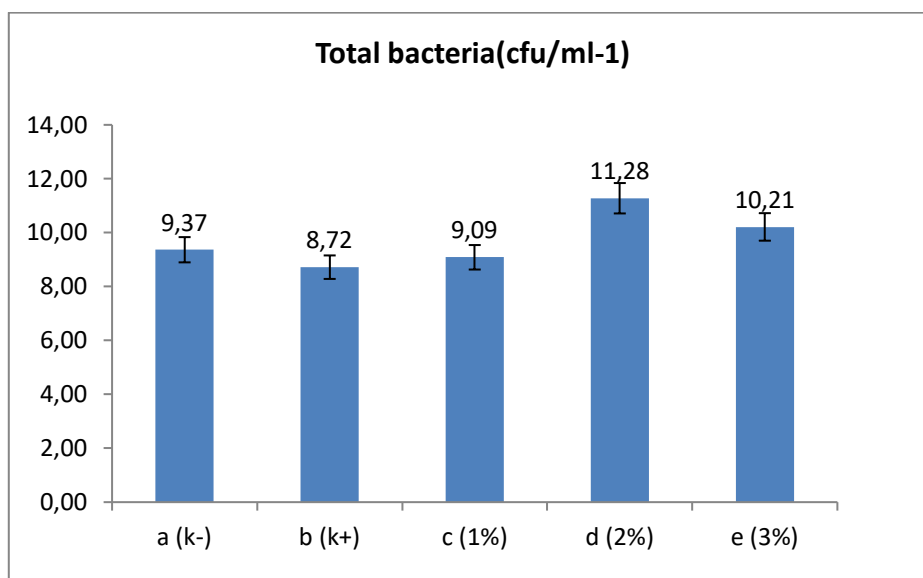


Figure 7. Total bacteria

Based on the test results, it was found that the addition of turmeric powder to feed had a significant ($P < 0.05$) effect on the bacteria in the tilapia intestine. This is because the feed contains turmeric. Mathematically, the lowest bacterial value in the gut of tilapia was in the

k+ treatment of 8.72×10^6 cfu/ml, followed by P1 of 9.09×10^6 cfu/ml, then K- of 9.37×10^6 cfu/ml, then for P3 of 10.21×10^6 cfu/ml and the highest bacterial value was in the P2 treatment of 11.28×10^6 cfu/ml.

Water quality

Water quality is one of the determining factors for success in fish farming, water quality that is not optimum will be a source of disease development so that it can infect cultivated fish. Water as a growing medium must meet the requirements and must pay attention to the quality of the water Kordi, (2010) in Andesra, (2019)

Table 3. Data on water quality values during the study

Parameter	Unit	Results	Eligibility Library
Ph	-	6.7 - 8.3	6 - 9 (Muchdar, 2014)
Temperature	$^{\circ}\text{C}$	$25^{\circ}\text{C} - 27^{\circ}\text{C}$	$25^{\circ}\text{C} - 30^{\circ}\text{C}$ (Suyanto, 1994 <i>in</i> . Muchdar 2014)
DO	mg/l	4 - 6,2	min. 3 mg/l (SNI, 2012)

DISCUSSION

The growth rate of specific weight is closely related to the increase in body weight of the fish from the feed consumed. The difference in the value of the specific growth rate in each treatment is likely due to the effect of adding turmeric powder to the feed; the fish can utilize the feed appropriately provided because the content of turmeric in the feed supports it. The feed given during the tilapia study was enriched with turmeric curcumin content. In the opinion of Anonymous (2015) in Malau (2016), curcumin content in the feed, besides being an antibacterial agent, antioxidant, and preventing damage to tissues, curcumin also functions as an appetite enhancer. The content of this substance is thought to affect fish body weight gain. This statement is related to the statement of Prabowo *et al.* (2017) in Andesra (2019), namely, curcumin can increase the appetite of fish by stimulating the bile wall to secrete fluid and stimulate the release of the pancreas, which will improve the digestion of carbohydrate, fat, and protein feed ingredients so that the absorption of nutrients be better and will increase the growth of fish.

The treatment with the highest specific weight and length, namely the treatment with a concentration of turmeric flour of 2% (P2), showed the highest growth rate in weight and length; this was thought to be due to the content of curcumin and essential oils which have the potential to stimulate digestive enzymes for growth, as well as the concentration of Turmeric flour, is given optimally so that the content contained in turmeric works well. While the treatment in producing the lowest weight and length growth was found in (K+), which had as much as 0% turmeric flour because it served as a control, this was allegedly due to much competition in getting food.

In this study, the survival rate of the tilapia was still relatively good because it was above 50%. This is the same as Husen's statement (1985) in Simangunsong (2017) in Afdola (2018), saying that a survival rate of $\geq 50\%$ is considered good, 30-50% is moderate, and for survival less than 30% is said to be not good.

Adding turmeric flour to feed can affect the survival of tilapia because turmeric contains curcuminoids which can neutralize free radicals. This is related to the opinion of Ratnasari (2012), namely the high survival of tilapia at the rearing due to the optimal concentration of turmeric flour, which is more able to neutralize free radicals due to disease compared to feed with too high a concentration of turmeric flour. It contains tannins and alkaloids, which give it

an astringent taste and are toxic to fish, as well as curcumin which changes its function from antioxidant to pro-oxidant. Concentrations that are too high contain curcuminoids, tannins, and alkaloids.

Then after being injected with the bacteria, the survival of the tilapia was also not too different from the previous one. Tilapia mortality after injection occurred in only a few treatments. However, the highest fish mortality was in the favorable control treatment, namely treatment without adding turmeric flour but injecting bacteria. This shows that injecting bacteria into tilapia gave death to the favorable control treatment but not too much of a threat to tilapia, given other turmeric powder in their feed because it contains curcumin and essential oils. Giving turmeric flour to feed can provide an immune system in fish so that bacteria entering the fish's body does not pose a threat. According to Goenarwo *et al.* (2009) in Faith *et al.* (2016), curcumin in turmeric has anti-inflammatory, antioxidant, antibacterial, and immunostimulating effects. Then according to Chattopadhyay *et al.* (2004) in Napirah (2013), The essential oil content of the turmeric plant is also known to have antibacterial activity so that it helps increase the body's resistance to attack by pathogenic bacteria.

The addition of natural ingredients to feed is a precaution so that fish are protected from pathogen attack; this is the same as Wahjuningrum's statement (2010) in Malau (2016), which states that the provision of feed enriched with natural ingredients is better than treatment of fish that have been infected with bacteria. This is because preventing the immune response in the fish's body has been formed before there are virulence factors from the *Aeromonas hydrophila* bacteria.

Fish mortality during the study was thought to have been caused when the weight and length measurements stressed the fish. In this study, the characteristics of fish experiencing stress were not responding to the feed given, swimming to the surface, and slow movements. Subandiyono and Astuti (2010) in Noviana *et al.* (2014) said that fish that experience physiological disorders (stress) will result in a drastic decrease in fish appetite, and it will be difficult for fish to do activities such as swimming and breathing. Furthermore, Noviana *et al.* (2014) said that the internal and external factors of the fish could influence the survival rate. However, considering that not all kept tilapia will die, it is inevitable that the tolerance capacity of each fish varies in each treatment container.

This study found that the administration of turmeric flour in the feed gave a better feed conversion value compared to the feed that was not given turmeric flour. In treatment, P2 with a dose of 2% is the most optimal dose for tilapia in utilizing feed to increase growth because P2 is the lowest value of all treatments. According to Putri *et al.* (2012), the feed conversion value is closely related to the quality of the feed given, where the lower the conversion value, the better the feed quality. The low feed conversion value is also likely due to the presence of curcumin in the feed, which has a distinctive odor, bitter. It smells unpleasant, thereby increasing the fish's appetite. Meanwhile, the highest feed conversion value was in the control treatment because turmeric flour was not added, so the fish's appetite was not as good as the treatment given additional turmeric flour.

According to Widyastuti *et al.* (2010) in Listiowati and Pramono (2014), the smaller the FCR value means, the better quality feed; this indicates that the amount of feed consumed is greater than the amount of feed remaining. The FCR value is still considered efficient if it is less than 3. All treatments have an FCR value below three, so it is still said to be efficient

According to Ihsanudin *et al.* (2014), the smallest FCR value can be interpreted as having the best FCR value because the use of feed for growth is very efficient; this is due to the relatively large pattern of fish appetite, so the need for feed used for growth is fulfilled. The

Food Conversion Ratio (FCR) is quite good, around 0.81.6.

Based on the test results, it was found that the addition of turmeric powder to the feed had a significant effect on increasing the number of erythrocytes produced ($P < 0.05$). This is because feed containing turmeric can increase the body's resistance to fish; in this case, it can increase the total number of erythrocytes. In the opinion of Cunningham (2002) in Islamic (2018), the increase in the number of erythrocytes is due to the presence of a compound contained in turmeric, namely curcumin, which can increase the work of the digestive organs so that fish appetite increases. The increase in erythrocytes indicates that feeding turmeric-containing feed can play a role in increasing fish growth and also improving fish health conditions. In addition, the number of erythrocytes is influenced by several factors, including age, gender, nutritional state, blood volume, ambient temperature, hemoglobin levels, hematocrit values, and other blood contents.

The number of erythrocytes was observed on day 37 during the study, namely, the first infected with *Aeromonas* bacteria on the 31st day. Then, the fish were observed up to the seventh day to see their activity; after that, they were observed under a microscope. In observing total red blood cells, it was found that all treatments showed an increase after being infected with *Aeromonas* bacteria however did not show a significantly different effect between each treatment with added turmeric powder. According to Ary (2007), in Dopingtanung (2008), fish infected with the disease should experience a decrease in the number of red blood cells (erythrocytes). It was caused by kidney and lymph function disruption, which will produce red blood cells (erythrocytes). Another opinion was expressed by Matofani *et al.* (2013) in Susanto (2014), namely erythrocytes have decreased allegedly due to phagocytosis of incoming bacteria; this process requires oxygen resulting in a decrease in erythrocytes.

The results of this study indicate that the infection with *Aeromonas* bacteria and the addition of turmeric powder shows the number of erythrocytes after being analyzed, and the calculation shows the range of erythrocytes with a total number of 1.60×10^6 - 1.98×10^6 cells/mm³. However, there was an increase; this study's total number of erythrocytes was still within the normal range. This is under Hartika's statement (2014), which says that, generally, red blood cells in the fish range from 20,000 - 3,000,000 cells/mm³.

F factors affect the number of erythrocytes, namely species, feed nutrition, size, and age. This statement is the same as the opinion of Dallman and Brown (1989) in Emu (2010) in Susanto (2014), namely the factors that affect the number of erythrocytes are species, parental differences, feed nutrition, size, physical activity, and age. In addition, factors that affect the number of erythrocytes are physiological factors and environmental conditions.

According to Andesra (2019), an increase in total leukocytes occurs because when there is infection from antigens or foreign bodies, curcumin will work by activating the lymph organs and kidneys to increase the production of leukocyte cells so that they can ward off antigens or foreign bodies that enter the fish's body.

Fish. An infection can also cause an increase in leukocytes in fish; this statement is the same as Anderson and Siwicki (1993) in Susandi (2017), explaining that an increase in the number of leukocytes in fish is due to fish experiencing stress or an infection which indicates an increase in the number of leukocytes in the blood. The same statement was also explained by Ary (2007) in Susandi (2017) that an increase in the total leukocyte count occurs due to the response of the fish body to environmental conditions, stress factors, and disease infections.

Volk and Wheeler (1993) stated that the growth phase of bacteria could be divided into four phases: the lag phase, the logarithmic (exponential) phase, the stationary phase, and the

death phase.

According to the 1974 Fisheries Research Institute, the principle of determining the Total Plate Count (TPC) is to determine the size of the bacterial population present in the digestive tract of fish because bacteria are the main factor causing ongoing spoilage. The working procedure consists of four interconnected stages: preparation, inoculation, incubation, and counting.

The growth of microorganisms is influenced by several factors, namely the availability of nutrients, water, temperature, pH, oxygen, oxidation-reduction potential, the initial population size, the presence of inhibitory substances, and other microorganisms (Fardiaz, 1992).

The lowest temperature during the research was 25 °C, and the highest temperature was 27 °C, while for the pH during the research, the lowest value was 6.7, and the highest was 8.3. Then for DO, the lowest value is four, and the highest is 6.2. Based on the water quality results, it shows good conditions and is still suitable for tilapia cultivation.

Based on the results obtained, the temperature of the maintenance medium ranged from 2.5 – 2.7 °C. Temperature affects fish's metabolism; usually, at low temperatures, the metabolism of fish will decrease, and vice versa if high temperatures accelerate metabolism. (Khairuman and Amri, 2011) States that the optimum temperature for tilapia to breed and grow optimally is at a temperature of 25-30°C

The pH value during the study ranged from 6.7 to 8.3. The pH level is critical because fish or aquatic biota live in a specific pH range, and if the pH does not meet the needs of the fish, growth will be hampered. A pH value < 7 is considered acidic, a pH = 7 is stated as neutral, and a pH > 7 is stated as alkaline. If the pH in the continuous rearing medium is too acidic or too alkaline, it will inhibit the growth of fish. The optimal pH for the growth of tilapia is in the range of 6-8 (Arifin, 2016).

Dissolved oxygen is the amount of oxygen present in water which is used as a parameter to determine whether or not water or water is suitable for fish farming. According to Arifin (2013), it is normal for tilapia to live in waters with a dissolved oxygen content of 3-6 mg/liter. The water quality data obtained showed that the highest DO was 6.2 mg /liter, and the lowest was 4 mg/liter. Several factors, including water turbidity from leftover feed and fish metabolism, could cause variations in oxygen levels during a power outage (Fahrizal, 2017).

CONCLUSION

Adding turmeric powder to the feed significantly affected the growth of tilapia, namely at a specific weight of 8.970 g, a specific length of 7.030%, and a feed conversion of 1.09%, but had no significant effect on the survival rate of tilapia fry.

The addition of turmeric flour to the feed also had a significant effect on the tilapia immune system, namely on red blood cells by 2.27%, on white blood cells by 1.08%, and on the calculation of bacteria in the intestine by 11.28%.

The optimum dose for providing the best growth in tilapia seeds is the P2 treatment with 2% turmeric powder/kg feed.

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