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Utilization of Telang Leaf Meal as Antioxidant Source in Feed of Tilapia (*Oreochromis niloticus*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author YA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MDE and Junianto managed the analyses of the study. Author Rosidah managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study aimed to determine the effective percentage of telang leaf meal on feed to increase antioxidant levels in tilapia liver feed.
Study Design: A total of 12 aquariums with sizes 60 x 40 x 40 $cm^3$and volume 96 L prepared and filled with 30 L water. Heater was installed and the temperature was raised slowly until it reached 34℃. Feed in the form of a mixture of telang leaf flour and commercial feed was given 3 times a day according to treatment and with the provisions of 3% of body weight of fish per day. Analysis of antioxidant levels was analyzed from the content of the superoxide dismutase enzyme as an antioxidant bioindicator in the body of the fish.
Place and Duration of Study: The cultivation was carried out at Ciparanje Hatchery and Fisheries Area, Faculty of Fisheries and Marine Science, Universitas Padjadjaran and Dismutase Superoxide Level Analysis was carried out at the Central Laboratory of Universitas Padjadjaran in June and July 2019.
Methodology: This research was carried out by giving high temperatures as a source of stress, for 40 days. Tilapia were given a mixture of telang leaf meal as a source of anti-oxidants. At the end of

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research, Test liver fish taken superoxide dismutase levels were observed and analyzed descriptively. Daily growth and survival rate of fish were analysed using F test at 5% level, and if there were significant differences, Duncan's test was performed.
Results: By giving heat stress continuously for 40 days the result is obtained the highest average superoxide dismutase level in treatment B (telang leaf meal 5%) as much -0.92 inhibition and lowest in treatment D (telang leaf meal 15%). A decrease in SOD levels identifies a decrease in antioxidant activity in the body of Tilapia. Daily growth rate obtained showed no significant difference. The fish will continue to grow but not as rapidly, because tilapia utilize protein up to 40% of commercial feed used to repair cells damaged during stressful periods and as a source of energy. Survival of fish decreases with increasing concentration of telang leaf flour, where the feed given has been mixed and resulted in the fish's response to the feed decreases, consequently not only the fish adapted to the environment, fish also adapt to new types of feed.
Conclusion: Based on the results of research that has been done then conclusions can be drawn that the addition of telang leaf mealup to 15% in commercial feed by giving heat stress since the beginning of the culture period does not provide an effect of increasing antioxidant levels.

Keywords: Antioxidants; tilapia; stress; telang leaf meal.

1. INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a species of freshwater fish that is widely cultivated and consumed by the community [1], Because tilapia has the ability to grow fast and high adaptability [2]. Global tilapia production reached more than 5.88 million tons (with 4.13 million tons from aquaculture and 0.837 million tons from capture) in 2017 [3]. Tilapia also has high market demand, with the production volume of tilapia reaching 695 thousand tons in 2012 [4] and increased to 1,280 thousand tons in 2017 [5]. Tilapia production volume increased by 84% for 5 years. This high production can be increased by intensive or super-intensive cultivation.

In intensive aquaculture of tilapia, one challenge faced by farmers is stressful fish. According to [6], stress makes fish more susceptible to diseases. So a better method is needed to prevent stress-related illnesses [7].

Prevention of diseases caused by stress can be done with the role of artificial feed given since fry, especially functional feed. Continuity of feed procurement is a very important factor in intensive aquaculture efforts, because the cost of feed is expensive, and takes up to more than 50 percent of total production costs [8].

Besides having high nutritional value, functional feed also has the ability to maintain health and control disease, as well as other positive effects on animal nutrition [9]. The use of synthetic antioxidants in feed can be bad; that is, if consumed by humans, it can cause impaired liver function, intestinal mucosa, and lungs, where the allowed dose should not exceed≥ 0,1% [10,11]. So we need a source of antioxidants from natural ingredients, examples of natural ingredients that can be used as additives, to give a functional effect is the telang plant.

Telang plant (*Clitoria ternatea*) is a plant that has a lot of well-documented pharmacological potential, and can be added as supplement in a feed, because it contains anthocyanin compounds which have high antioxidant activity [12]. Telang plants contain a number of phenols and flavonoids in the leaves and flowers, which plays a role in inhibiting and fighting free radicalsas DPPH, hydroxyl radicals, and hydrogen [13]. The content of antioxidant activity can increase the body's ability to ward off disease and minimize stress.

Increased levels of antioxidants in counteracting free radicals can be measured by looking at the enzyme superoxide dismutase, which is widely found in the liver. The superoxide dismutase enzyme converts superoxide free radicals into oxygen and hydrogen peroxide.

Testing of antioxidant activity in vivo needs to be done, bearing in mind that there is still little research into antioxidant activity in vivo especially in fish. Testing of antioxidant activity in vivo was carried out by the method Marklund dan Marklund [14] because the material is easier to obtain, and can analysis can be done quickly; with cheap instrument, and simple operating protocol [15].

The general objective of this study was to determine the effect of adding telang leaf meal in the feed to increase antioxidant levels in the fish's body. Otherwise, the specific objectives of this study are to find out the percentage of effective dosage of telang leaf meal to increase levels of antioxidants, to find out the relationship of survival to the increase in antioxidant levels from feeding the telang leaf meal mixture and to find out the relationship of feed efficiency with levels of antioxidants in fish given feeds added withtelang leaf meal mixture.

2. MATERIALS AND METHODS

This research was conducted from June to July 2019. Fish cultivation was carried out at the Ciparanje Hatchery and Fisheries Area, Faculty of Fisheries and Marine Science, Universitas Padjadjaran. Antioxidant analysis on tilapias' liver was done in Central Laboratory of Universitas Padjadjaran.

The materials used in this study included: Tilapia, Telang Leaves, Commercial Feed PF 1000, CMC, Pyrogalol. This research was conducted in 40 days by giving heat stress from the beginning. Data on superoxide dismutase levels at the end of maintenance were compared between treatments.

2.1 Research Implementation

First, the telang leaves were picked and washed clean. Telang leaves that are taken must be overstated due to the shrinkage of fresh telang leaf weights. Drying was done by aerating the leaves for 3 days. After dried, then the leaves were grounded into meal.

Feed was made by mixing commercial feed with telang leaf meal (TLF) according to the treatment. Commercial feed is weighed according to division; feed A (control) with 100% commercial feed, feed B with 95% commercial feed and 5% TLF, feed C with 90% commercial feed and 10% TLF, feed D with 85% commercial feed and 15% TLF. In the mixing process, CMC were added as much as 1% of total feed. Feed mixture was stirred evenly to form a paste. Dough that has been shaped were then air dried for 1 day.

Tilapia is first given a dowry for 1 day, after that a sampling is done to determine the initial fish weight and determine the dose of feed to be given. Density is aboutten tilapia fish per aquarium. Furthermore, feed was given 3 times a day of 3% of fish body weight. Heater was installed as a source of heat stress with initial temperature 26℃ at 09.00 WIB, the temperature was slowly raised until it reaches the temperature of 34℃ at 13.00 WIB, the fish were left in heat stress for 4 hours, after 17.00 WIB the temperature was normalized again. Calculation of the number of individuals, calculation of dead fish, and checking of water quality were done every ten days. As much as 1/3 water change was done every two days and about 2/3 times every week.

2.2 Superoxide Dismutase Analysis

The examination of antioxidant levels was carried out at the end of the study by taking samples of the liver of tilapia fish. Tilapia liver organ is taken by composite namely by mixing three livers samples from each test but still in one treatment. Intake of the liver by dissecting the abdomen. The liver is put into a coolbox and immediately taken to the laboratory to be stored in a cooler at ± 4℃, then testing the levels of antioxidants. Testing levels of antioxidants is done by the method Marklund and Marklund [14], by measuring SOD activity by utilizing the auto-oxidation process of pyrogalol, This SOD enzyme will be a reference for damage caused by oxidative stress. The value obtained will show the effect of antioxidants in reducing oxidative stress that occurs.

Tilapia liver is taken as much as 1,15 gram and mixed with 13 mL extraction solution containing 0,15 M KCL 0,79 M EDTA (pH 7,4). The solution that has been made is homogenized using a manual homogenizer. Then homogenate is centrifuged at 9000 rpm using Beckman's centrifuge J2-21 with rotors JA 10 at temperature 4℃ during 10 minutes. The supernatant is then used to test the activity of the enzyme superoxide dismutase.

Analysis of SOD levels was carried out with the help of pyrogalol solution as free radicals. Pirogalol was taken 75 µl 10 mM mixed with 2850 µl buffer fosfat 50 mM (pH 8,2) then added 75 µl sample. The degree of auto-oxidation of pyrogalol when added to the sample is compared with the standard (with 75 µl extraction solution) by measuring the increase in absorbance value at λ 340 nm during 2 minutes. One unit of enzyme SOD expressed as the number of enzymes needed to inhibit pyrogalol autoxidation reaction by 50% [14].

2.3 Observation Parameters

Analysis of superoxide dismutase levels in tilapia liver using the method [14].

SOD Activity (%) = [A-B/A] x 100%

With:

- A = Reduction of absorbance of 180 minutes with absorbance of 0 minutes of control solution (%) and
- B = Reduction of absorbance of 180 minutes with absorbance of 0 minutes of sample solution (%).

The survival rate of the test fish is carried out using a formula [16]:

$$SR = \frac{Nt}{No} \times 100\%$$

With:

- SR = Survival Rate
- Nt = The number of fish at the end of the research
- N_0 = The number of fish at the beginning of the research

Efficiency of feed in test fish is calculated by formula [17]:

$$EP = \frac{Wt+D-W0}{F} \times 100\%$$

With:

- EP = Feed efficiency (%)
- Wt = Fish weight at the end of the research (gram)
- D = Weight of dead fish during research (gram)
- W0 = Fish weight at the beginning of the research (gram)
- F = Amount of feed consumed during research (gram)

Data on survival and efficiency of tilapia feed were analyzed using F test at 5%, and if there are real differences then carried out further tests. Test for superoxide dismutase content and water quality were analyzed descriptively.

3. RESULTS AND DISCUSSION

3.1 Superoxide Dismutase Levels

This study analyzes the antioxidant activity of SOD found in tilapia liver which has been given treatment in the form of feeding containing antioxidants and heat stress as a source of oxidative stress. The value of antioxidant activity is indicated by the percentage of inhibition carried out by the SOD enzyme against free radicals formed during oxidative stress, % of the resistance obtained (Fig. 1).

Antioxidant activity obtained is negative, this shows no antioxidant activity in the liver of tilapia, rather it shows antagonistic activity against pyrogalol, or no inhibition process occurs at the end of aquaculture [18]. Oxidative stress due to ambient temperature can cause an increase in free radicals which results in the erosion of antioxidants in the body [19].

Negative value of treatment B (5% TLF) and C (10% TLF) obtained still shows a better result than the control feed. These results are in accordance with the proposed hypothesis. But the increasing concentration of giving TLFshow decreased levels of antioxidants where there is the influence of tannins in telang plants which give a bitter taste to the feed [20]. Same is the case with research results [21] that the provision of jaloh leaf meal which has antioxidant levels in tilapia with hear stress will result in decreased antioxidant activity as an increase in the concentration of jaloh leaf meal. This is also reinforced by opinions [22] that the tannin content has a bitter taste so that the fish don't like it and causes food inhibition in test animals.

Research on the effects of telang plant in fish is still lacking, especially if it is also accompanied by stress, in the form of heat stress. In this case, sources of plants that have antioxidant levels are found to have an effect on increasing antioxidants in the form of SOD. One of the results of research [23] on the supplementation of Moringa leaf flour on the stress index of tilapia showed that the addition as much as 1.5% of moringa leaf flour can increase the enzyme superoxide dismutase as an indicator of antioxidants in tilapia and effectively reduce stress due to hunger.

Research conducted [24] on the effects of the antioxidant content of *Clitoria ternatea* on day injuries in mice, showed that significant administration of 200 mg / Kg of *Clitoria ternatea* leaves would reduce levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin significantly. This activity is called the hepatoprotective effect associated with strong antioxidant activity on *Clitoria ternate* for healing the liver.

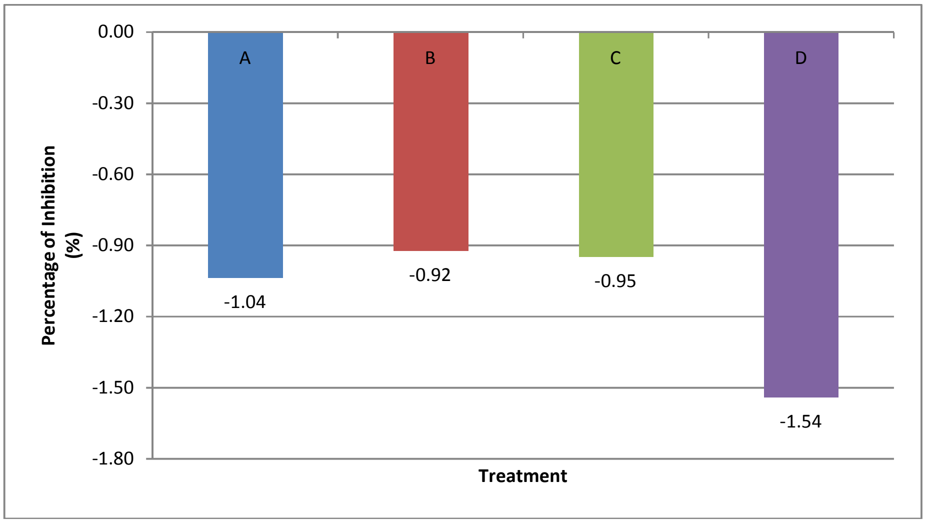

Fig. 1. Percentage graph of dismutase superoxide enzyme inhibition to free radicals

Decline in the value of SOD levels in addition caused by the use of TLFis less effective due to tannin content also caused by giving heat stress as a source of oxidative stress, this is in accordance with research conducted by [25] with the result that the SOD enzyme is the most active enzyme in responding to environmental stress conditions. Where the enzyme superoxide dismutase decreases at high temperatures between 30℃ - 35℃, however SOD will increase along with a temperature drop of up to 10℃ - 15℃.

The results of the treatment, where the fish were fed with telang leaves meal shows the same results as the control, because heat stress was given since the beginning of the study, which results in the erosion of existing antioxidants derived from TLF in each feeding. In this case it is advisable not to give heat treatment since the beginning of the experiment, so the fish can increase antioxidant levels in the body first, before the arrival of free radicals from outside which will erode antioxidant levels.

3.2 Survival Rate

The percentage of survival of tilapia was showed in Fig. 2. The survival rate of tilapia that is kept has a value above 60% and shows a positive response to all feeds given, in accordance with [26] that the minimum survival rate of tilapia for nursery is 60%. This shows a high survival value obtained by feeding fish with a duration of one day for each treatment.

The commercial feed used contains 40% protein, the protein content is used to maintain life especially more absorbed when the ambient temperature is not optimal, metabolism and afterwards used for growth.

Table 1. Average survival of tilapia during maintenance

Treatment	Survival Rate (%)
A (Commercial Feed)	93 ± 0.06^{b}
B (5% TLF)	83 ± 0.06^{ab}
C (10% TLF)	77 ± 0.06^{a}
D (15% TLF)	70 ± 0.01^{a}

With: Values followed by the same lowercase indicate no significant difference based on Duncan's Multiple Range Test at a 95% confidence level

The results of analysis of variability in the survival of tilapia have a significantly different effect on the survival rate of tilapia because the calculated F value is greater than the F table (Table 1). The survival rate of tilapia is significantly different from the best treatment, treatment A and B, where this shows that the additional TLF feed gives a negative response to the survival of tilapia during aquaculture.

The survival of tilapia has decreased (Fig. 2) where the fish in treatment A has the highest survival rate compared to other treatments. High survival in treatment A (control) due to tilapia utilizes the feed to adapt well in extreme environmental conditions and tilapia that are kept do not need to adjust to the type of feed provided, because the food used during cultivation is the same as the food used when the fish were still in the farm.

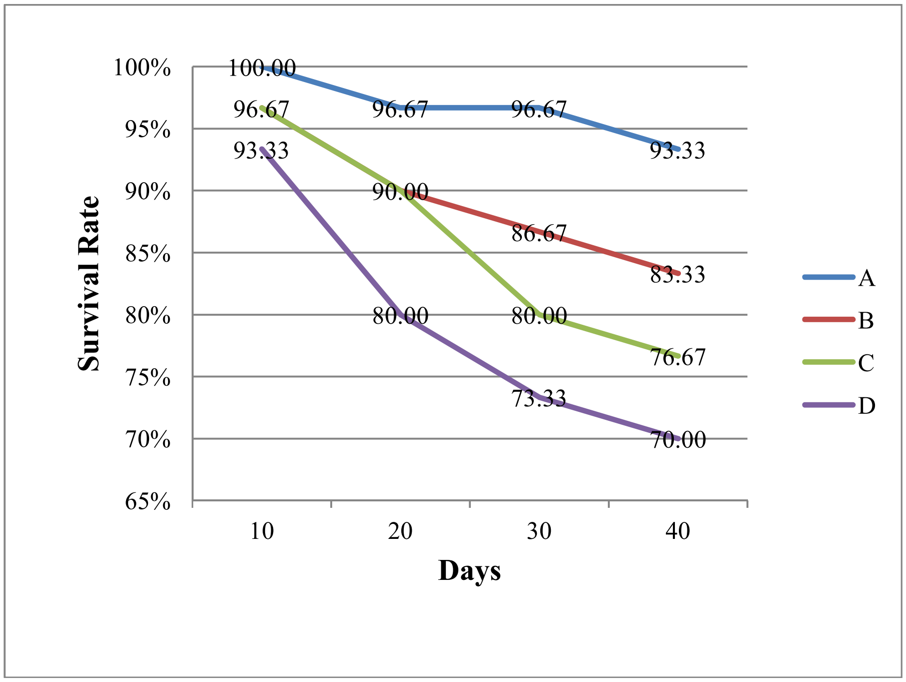

Fig. 2. Graph of survival rate of tilapia

In treatments B and C, the survival of tilapia decreases, because the feed given has been mixed with telang plants so that it affects the response of fish to feed. This can be seen in preliminary research conducted to determine the response of fish to feed that has a different TLF mixture. The result is that with increasing TLF mixture, the fish's response to feed is slower.

Treatment D is the treatment with the lowest survival. This is consistent with the preliminary research described above where treatment D feed had the highest TLF dose, so the fish has a low response. As a result, in addition to adapting to the environment, the fish must also adapt to new types of feed so that the stress that occurs is higher.

From some of the explanations above, it is found that the effect of stress comes from the high ambient temperature, so the fish must utilize every feed given to survive. As well as the increasing concentration of TLF in feed, the response of fish is lower because it has to go through the adaptation of new feed that is done since the beginning of aquaculture. This explains why TLF administration of up to 15% is not significantly different. This is supported by research [27] about the effect of high temperatures on the survival of tilapia the result is that temperatures higher than 32℃ will reduce survival to some extent. This is what explains that the higher the TLF concentration content, the smaller the survival rate of fish.

Less optimal use of similar feed was also obtained from research results [28] about the survival of tilapia which is fed with supplementation with jaloh leaf meal, where the survival of the highest test fish is in the treatment B. The more the concentration of jaloh leaf meal, the lower the survival rate. The results of studies with TLF are different from the results of jaloh leaf meal. Although both have high tannin content. In studies with TLF, ambient temperatures have been made high from beginning. Consequently, it will affect the ability of fish to adapt to new feed, resulting in lower survival.

Decreased survival as the temperature increases is found in the research [29] where at a temperature of 30℃ and 32℃ uniform seed size, but the longer the cultivation time the size of tilapia will be different and affect the high mortality rate through the formation of a food hierarchy, and the survival rate at these temperatures is around 79% to 85%.

3.3 Feed Efficiency

The low level of feeding efficiency indicates that the feed given is not fully utilized by tilapia for its growth, but is used to defend the body from high ambient temperatures, where temperatures affect the digestibility of fish [28].

Table 2. Average feeding efficiency during aquaculture

Treatment	Feed efficiency (%)
A (Commercial Feed)	43.1±1.40[a]
B (5% TLF)	41.7±1.77[a]
C (10% TLF)	42.5±1.12[a]
D (15% TLF)	42.9±1.62[a]

With: Values followed by the same lowercase indicate no significant difference based on Duncan's Multiple Range Test at a 95% confidence level

Based on the results of analysis of variance (Table 2) the value of the obtained feed efficiency was not significantly different between treatments. This shows that feed with the addition of telang leaf meal does not provide a positive response to the efficiency of feeding. Efficiency of feed is closely related to digestibility which illustrates the percentage of nutrients that enter the fish's body and absorbed by the digestive tract of fish. Feed efficiency is influenced by stress caused by the environment which is in accordance with the results of research [30] which shows stress in fish farming will disrupt eating behavior, especially reducing food intake and negative effects on feed conversion efficiency.

Eficiency of tilapia feed at high temperature maintenance it was found that water temperature 32℃ significantly reduced feed efficiency, this is due to increasing temperatures and makes the process of eating fish slow [27]. Higher temperatures will accelerate the rate of food that is digested through the intestinal tract, thereby reducing the digestion and assimilation of nutrients.

Research [23] showed that by adding 1.5% Moringa leaf flour to tilapia feed, it can increase body mass gain and feed conversion ratio under stressful conditions due to hunger.

3.4 Water Quality

Water quality is a very important factor in aquaculture, the water used must meet the optimal needs of the fish. The measured water quality parameter is temperature, *Dissolved Oxygen* (DO), and pH. Water quality measurements carried out 5 times with a span of 10 days for 40 days of cultivation. The results of measurement of water quality parameters can be seen in Table 3.

Temperature is a very important factor in fish farming that determines the success of tilapia culture, because fish are cold-blooded animals [31]. Temperature during aquaculture affects fish growth and survival. Optimum temperature for cultivation is around 26℃ - 33℃. Increased water temperature up to 33℃ can cause heat stress as a source of oxidative stress. This condition results in low feed efficiency which indicates that the nutrients absorbed by the body are used to adjust to the ambient temperature. In heat stress treatment, the temperature is increased to the maximum limit, during the increase in temperature to the optimal limit that is 30℃, the fish will take food that makes the process of digestion and metabolism also increases.

Dissolved oxygen (DO) is a water quality parameter that shows the solubility of oxygen in a body of water, where dissolved oxygen is influenced by temperature [32]. Dissolved oxygen levels during aquaculture range between 3.9 – 7.4 mg/L which is in a bad condition, where it is < 5 mg/L. This is caused by an increase in temperature, which exceeded the optimal temperature of aquaculture, which is 30℃. According to [33], oxygen levels will increase as the temperature lowers, and vice versa.

The results of measurement of the pH range during tilapia aquaculture are around 6.87 – 8.25. According to SNI, this optimum pH is between 6.5 – 8.5. The pH value that can interfere with fish life is a pH that is too low (acidic) and pH that is too high (base), for the most part fish can adapt to the aquatic environment with a pH around 5 – 9 [34].

Table 3. Water quality parameters during aquaculture

Treatment	Max temprature (℃)	Min temprature (℃)	DO (mg/L)	pH
A (Commercial Feed)	32 – 33	26 – 27	4.3 – 7.4	7.03 – 8.17
B (5% TLF)	33	26 – 27	4.0 – 7.3	7.01 – 8.20
C (10% TLF)	32 – 33	26	3.8 – 7.1	6.87 – 8.25
D (15% TLF)	33	26 - 27	3.9 – 7.1	7.01 – 8.25
SNI Standard	30	25	Min 5	6.5 – 8.5

4. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the addition of up to 15% of telang leaf meal to commercial feed by giving heat stress since the beginning of the aquaculture period does not have the effect of increasing antioxidant levels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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