The Effect of Vitamin B Complex on Survival, Growth and Stress Resistance of Nile Tilapia (*Oreochromis niloticus*) Larvae

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Abstract- Nile tilapia (*Oreochromis niloticus*) is one of the important economic value fishery commodities and has been cultivated commercially. The main problem experienced is the low seed production through hatchery units due to the low survival rate, especially in the larval stage. This research was conducted at CV. Aquaculture Lestari, Makassar aims to evaluate the effect of vitamin B complex on the survival, growth, and stress resistance of nile tilapia (*O. niloticus*) larvae. The study used a container in the form of a black plastic basin with a capacity of 40 L which would be filled with 30 L of media water. The test animals used were 3-day old nile tilapia larvae which were kept for 30 days. The feed used is artificial feed in the form of powder and pellets. The study was designed using a completely randomized design consisting of 4 doses of vitamin B complex treatment with 3 replications each. The four doses are 0, 50, 100, and 150 mg/L. The analysis of variance showed that the supplementation of vitamin B complex had a very significant effect ($p < 0.01$) on the survival, growth, and level of stress resistance (CSI) of nile tilapia larvae. The highest survival rate and growth rate, and the lowest CSI of nile tilapia larvae were produced at a dose of 50 mg/L i.e., 75.44%; and 12.26%/day; and 66.00, respectively, while the lowest survival rate and growth rate as well as the highest CSI were obtained at a dose of 0 mg/L, i.e., 48.11%; and 8.68%/day; and 90.67, respectively.

Index Terms- nile tilapia, growth, stress resistance, survival rate, vitamin B complex

I. Introduction

Nile tilapia is one type of fishery commodity with important economic value. This species of fish is preferred by the public because of its delicious taste and high nutritional value (Ansah *et al*., 2014; Mapenzi and Mmochi, 2016; Barosso *et al*., 2019). Nile tilapia has several advantages, including: its fast growth rate and can reach a much larger body weight with a fairly high level of productivity. From the aspect of productivity, nile tilapia is very potential and productive if it is cultivated in various fields, not only in ponds but also in highland ponds (Watanabe *et al*., 2002; Tran *et al*., 2021).

The main problem in the development of nile tilapia is the availability of seeds continuously and of good quality. Currently, efforts are being made to produce nile tilapia seeds through hatcheries. However, the main problem faced in hatchery efforts is the low survival rate, especially in the larval phase (Pandit *et al.*, 2017). The cause of the low survival rate in larvae is low feed quality and a less than optimal rearing environment. According to Suleman and Fotedar (2017) the larval phase is the most critical phase in the fish life cycle. After hatching, the life of the larvae is completely dependent on the source of food or energy reserves that have been prepared by the mother. The quality of energy reserves is very influential on the life and development of larvae. Poor feed quality causes disturbances in larval development, larvae are easily stressed and can even cause death (Das *et al*., 2012).

One of the efforts to overcome the problem of low survival in nile tilapia larvae is through improving feed nutrition. One of the nutrients needed by fish larvae is vitamins. Vitamins are organic nutrients that have various essential functions in metabolic processes (Lall and Kaushik, 2021). Although it is needed in small quantities, it is no less important to meet the nutritional needs of fish (Byrd *et al*., 2020). Vitamin B complex has a function as a coenzyme and precursor in metabolic processes. Vitamin B complex plays a role in good metabolic processes in cultivars so that the energy possessed can be used in a good growth process and will have an impact on survival within a certain time (Hansen *et al*., 2015). Vitamin B complex is also thought to play a role in the rate of daily weight gain of fish. The absence of vitamin input can affect the reduced feed intake by fish, thereby reducing the intake of the amount of nutrients needed for maximum fish body growth (Li *et al*., 2016). Research on the use of vitamin B complex has been carried out on juvenile Blunt Snout Breammun (Li *et al.*, 2016), milkfish larvae (Salsabila *et al*., 2019). The results of these studies prove that the administration of vitamin B complex can increase the survival and growth of some fish larvae. However, the role of vitamin B complex in nile tilapia larvae is not known for certain.

This study was aimed to evaluate the effect of vitamin B complex on survival, growth rate, and stress resistance level of nile tilapia (*O. niloticus*) larvae.

II. RESEARCH METHOD

This research was carried out by CV. Aquaculture Lestari, Makassar. The research uses a container in the form of a black plastic basin with a capacity of 40 L which will be filled with 30 L of media water. These containers are equipped with aeration equipment. The media water used is freshwater sourced from the Local Water Company (aka PDAM).

The test animals used were 3 day old nila tilapia larvae which were reared for 30 days. The larvae were obtained from CV Aquaculture Lestari, Makassar. Prior to stocking, the larvae were weighed using a digital scale with an accuracy of 0.01 g.

The feed used is artificial feed in the form of powder and pellets. Feeding started on days 1 to 14 using MS Prima feed PF 0, and entering the 15th day until the end of the maintenance was given MS Prima Feed PF 500. The dose of feed given was 5% with a frequency of feeding 3 times a day at 08.00 morning, 1:00 p.m. and 5:00 p.m.

The administration of vitamin B complex is done once a day according to the treatment dose. Giving is done in the morning after the water change. Water changes are carried out every day as much as 20% of the volume of media water.

At the end of the study, harvesting was carried out by counting the number of live nile tilapia larvae and re-weighing the weight of the larvae. Weighing was done using a digital scale with an accuracy of 0.01 g.

The study was designed using a completely randomized design consisting of 4 treatments with 3 replications each, so that in this study there were 12 experimental units. The four doses of vitamin B-complex were 0, 50, 100, and 150 mg/L. Placement of research containers was done randomly.

The parameters observed in this study were as follows:

a. Survival rate

Tilapia larvae survival was calculated using the following formula:

$$S = (N_t/N_o) \times 100$$

Where: S is nile tilapia larvae survival (%), N_t is number of live nile tilapia larvae at the end of the study, N_o is Number of nile tilapia larvae at the beginning of the study.

b. Growth rate

The growth rate of nile tilapia larvae was calculated using the formula

$$SGR = 100 \times (\ln W_t/W_o)/t$$

Where: SGR is daily specific growth rate (%/day), W_t is average weight of nile tilapia larvae at the end of the study (mg), W_o is average weight of nile tilapia larvae at the beginning of the study (mg)

c. Stress Resistance

Stress resistance test was conducted to see the physiological condition of nile tilapia larvae after being given Vitamin B-complex. Stress measurement was carried out at the end of the study by taking 10 tilapia larvae randomly from the rearing medium and put into a 1 L volume glass beaker filled with water with high salinity (25 ppt). The number of stressed white snapper larvae was observed at every 5-minute interval during a 1-hour period.

Assessment of nile tilapia larvae resistance was carried out qualitatively based on the behavioral response or abnormal movement of tested tilapia larvae to death during the stress experiment. The indications of nile tilapia larvae experiencing stress in the form of abnormal (up and down movement) and spinning until they die.

Evaluation of larval stress resistance was calculated using the Cumulative Stress Index (CSI) formula from the Ress *et al.* (1994) as follows:

$$CSI = D5 + D10 + D15 + \ldots + D60$$

Where: CSI is cumulative stress index, D is number of stressed nile tilapia larvae at a certain minute.

The higher the CSI value, the lower the larval resistance level, and vice versa the lower the CSI value the higher the larval resistance level.

d. Water Quality Parameters

During the experiment, several water quality parameters were measured for the rearing media for nile fish larvae. The parameters measured were temperature, pH, dissolved oxygen, and ammonia. Temperature was measured using a thermometer, dissolved oxygen with a DO meter, pH with a pH meter and ammonia using a spectrophotometer. Measurements of temperature, pH, and dissolved oxygen were carried out twice a day at 6 am and 5 pm. Ammonia was measured three times during the study, namely at the beginning, middle, and end of the study.

The data obtained in the form of survival rate, growth rate, and stress resistance were analyzed using analysis of variance (ANOVA). If there is a significant effect, it is continued with the W-Tuckey test. As a tool for carrying out the statistical test, the SPSS version 22.0 computer software package was used. The water quality parameters were analyzed descriptively based on the viability of nile tilapia larvae.

III. RESULTS AND DISCUSSION

a. Results

Survival, Growth, and Stress Resistance of Nile Tilapia Larvae

The average survival rate, growth rate, and stress resistance level (CSI) of nile tilapia larvae given various doses of vitamin B complex are presented in Table 1.

Table 1. Average survival, growth rate, and stress resistance of nile tilapia larvae in various doses of vitamin B complex

Dose of Vitamin B-Complex (mg/L)	Survival rate (%)	Growth rate (%/hari)	CSI
0	48.11 ± 1.58^{d}	8.68 ± 0.07^{c}	90.67 ± 4.16^{a}
50	75.44 ± 0.51^{a}	12.26 ± 0.53^{a}	66.00 ± 3.60^{c}
100	62.56 ± 0.84^{b}	9.80 ± 0.12^{b}	75.67 ± 2.08^{b}
150	54.11 ± 5.03^{c}	9.05 ± 0.16^{c}	79.33 ± 0.58^{b}

Note: different letters in the same column indicate significant differences among treatments at the 5% level ($p < 0.05$)

The results of the analysis of variance showed that the administration of B-complex vitamins had a very significant effect ($p < 0.01$) on survival, growth rate, and stress resistance (CSI) of nile tilapia larvae. Based on Table 1, the high survival rate and growth rate of nile tilapia larvae were produced at a dose of 50 mg/L and the lowest at 0 mg/L. Meanwhile, the lowest CSI value was obtained at a dose of 50 mg/L and the highest at 0 mg/L.

The relationship between vitamin B-complex dose and survival rate (Y_1), growth rate (Y_2), and CSI (Y_3) of nile tilapia larvae with quadratic pattern with the equation $Y1 = 50.293 + 0.5426x - 0.0035x2$ ($R^2 = 0.74$); $Y2 = 9.0637 + 0.0623x - 0.0004x2$ ($R2 = 6.00$), and $Y3 = 88.650 + 0.4737x - 0.0028x2$ ($R^2 = 0.69$) (Figures 1, 2 and 3).

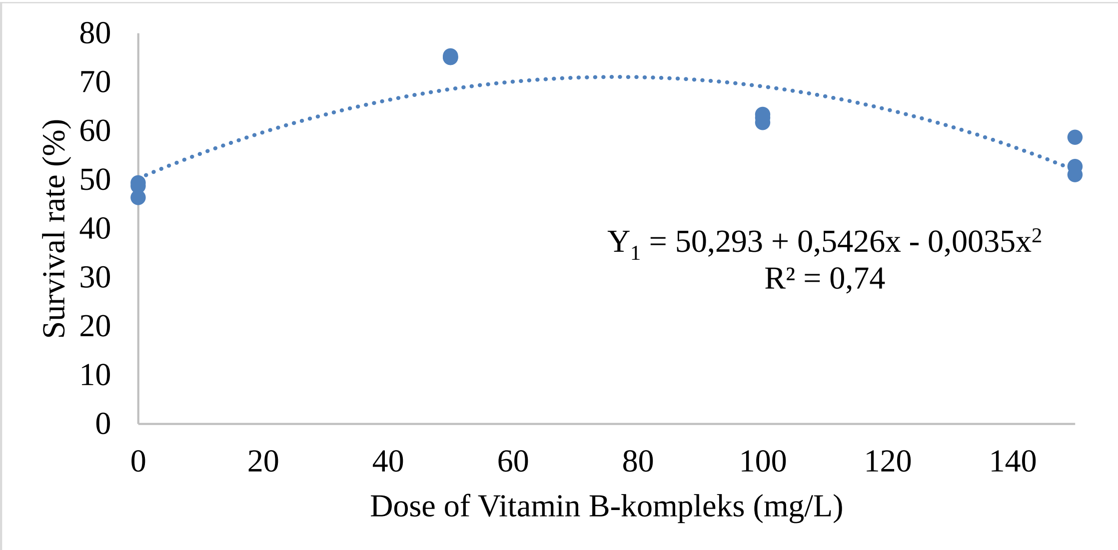
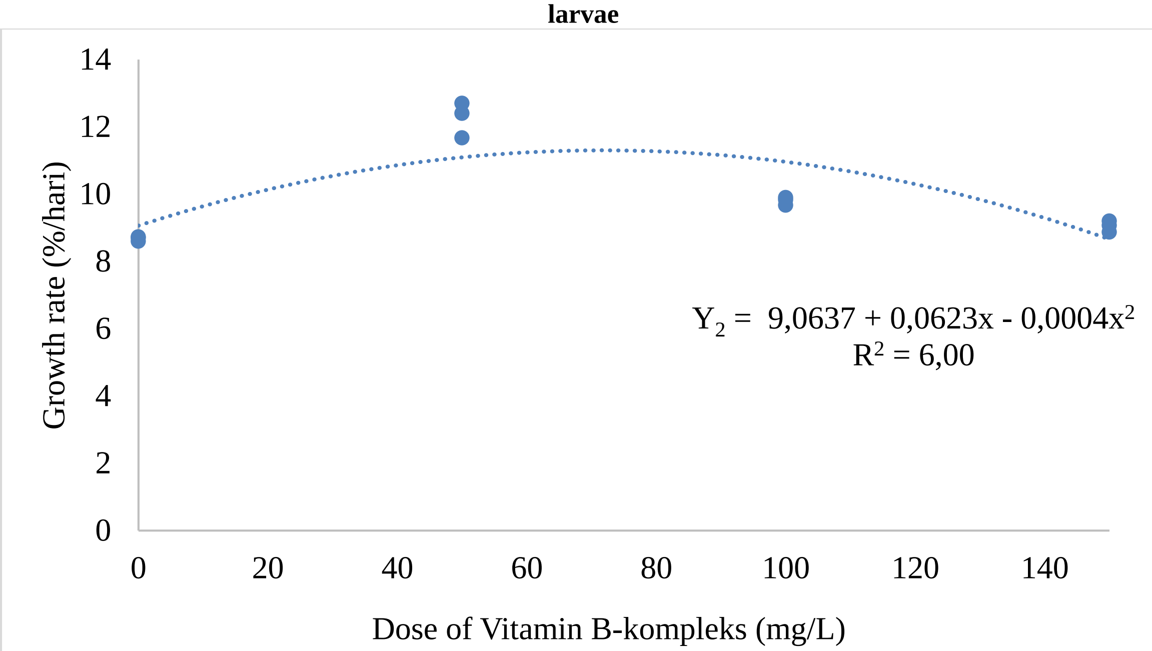


Figure 1. Relationship between B-complex vitamin dose and survival of nile tilapia larvae



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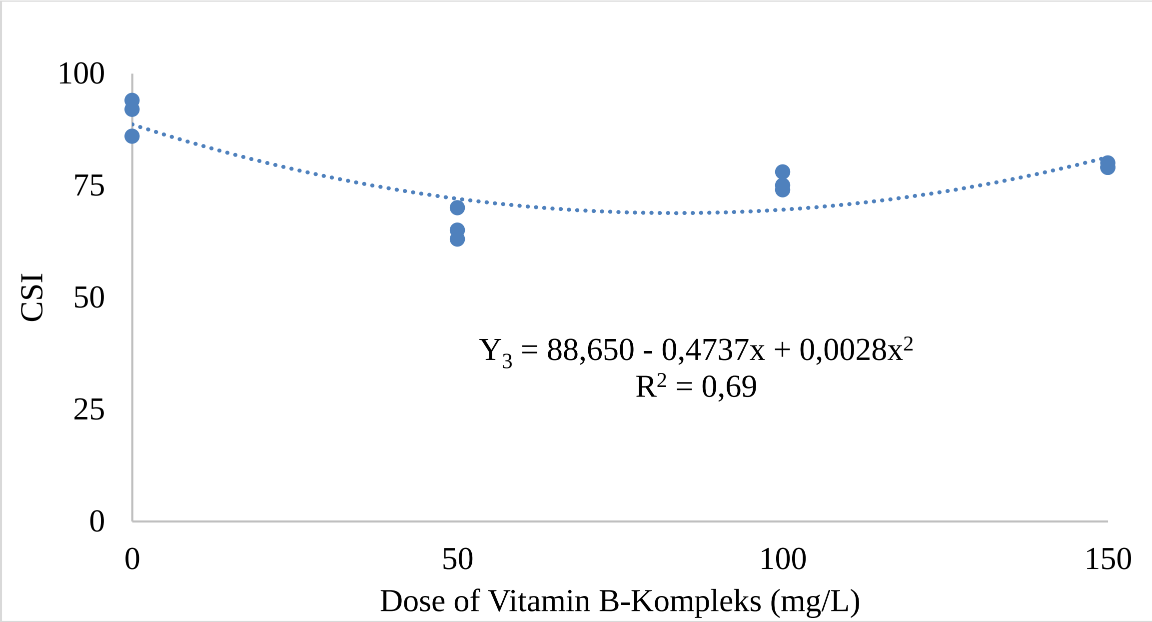

Figure 3. Relationship between B-complex vitamin dose and CSI of nile tilapia Larvae

Based on the regression equation above, it can be predicted that the optimum (op) dose for survival (Y_{op1}), growth rate (Y_{op2}) and CSI ((Y_{op3}) of tilapia larvae can be achieved at doses of 77.43 (Yo_{p1}), 77.87 (Y_{op2}), respectively. , and 84.59 mg/L (Y_{op3}).

Water Quality Parameters

During the research, measurements of several water quality parameters of tilapia larvae rearing media were carried out including: temperature, pH, dissolved oxygen, and ammonia. The range of parameter values is presented in Table 2.

Table 2. Range of water quality parameter values for tilapia larvae rearing media

Parameters	Range Value
Temperature	28-30
pH	7.1-7.6
Dissolved Oxygen (ppm)	4.2-5.1
Ammonia (ppm)	0.02-0.09

b. Discussion

Based on Table 1, the survival rate, growth rate and the highest level of stress resistance (lowest CSI) of nile tilapia larvae were produced at a dose of 50 mg/L while the lowest (highest CSI) was at 0 mg/L. Nile tilapia larvae that were given vitamin B-complex at a dose of 50 mg/L had a high resistance to stress so that the larvae could exist to survive and grow. If the larvae experience stress, the adrenal glands will produce high levels of the hormone cortisol, which is a stress-causing hormone (Sadoul and Giffroy, 2019). In these conditions, when given vitamin B complex, especially vitamin B5, which plays a role in improving the function of the adrenal glands that manage stress (Singh, 2016). If stress can be overcome, the body's work system in fish larvae will return to normal and larvae can exist to live and grow (Rehman *et al*., 2017). Vitamin B complex has an important role in energy metabolism. According to Sherwood (2014), the reshuffle of carbohydrates into glucose will produce energy and the energy produced can suppress the release of the ACTH hormone, which is a stress-causing hormone. With the availability of energy, in addition to increasing larval resistance to stress, it also increases survival and growth.

The low survival rate, growth rate, and stress resistance level of nile tilapia larvae at a dose of 0 mg/L were due to the absence of absorption of B-complex vitamins from the media. In general, the B-complex vitamins (thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate, and cyanocobalamin) have unique coenzyme functions in cellular metabolism, and play an important role in energy-producing metabolic pathways for carbohydrates, fats and proteins and in maintaining nervous system function (Dai and Koh, 2015; NRC, 2011). This was stated by several researchers about the use of B vitamins such as McLean and Hannan (2007); Swart *et al.* (2013); Clarke *et al.* (2014). Thiamine (vitamin B1) in its active form is an important cofactor for enzymes involved in carbohydrate, lipid and amino acid metabolism, and in the synthesis of neurotransmitters. It is also important for a number of enzymatic processes in energy production, including decarboxylation and transketolase reactions that occur in the cell cytoplasm (Dai and Koh, 2015; Halver and Hardy, 2002; NRC, 2011). Pyridoxin (vitamin B6) in animal tissues is converted to the active form pyridoxal phosphate, involved as a cofactor in enzymatic reactions involving amino acids, such as transamination, decarboxylation and dehydration. It is also involved in the synthesis of collagen, nucleic acids, porphyrins, neurotransmitters, glycogen catabolism, fat metabolism (especially essential fats). acid), messenger RNA synthesis and in immune system function (Dai and Koh, 2015; Halver and Hardy, 2002; NRC, 2011). Vitamin B6 acts as a cofactor for lysyl oxidase which is essential for the enzymatic action of lysyl oxidase in collagen crosslinking. In several studies the effects of additional vitamins (thiamine, pyridoxine and cyanocobalamin) both singly and in combination were tested on premium commercial micro feeds, which was not expected to be vitamin B restriction. vitamin B1), pyridoxine (vitamin B6) and 5-fold cyanocobalamin (vitamin B12) can provide additional benefits to the performance of bone growth and development (Pinto *et al*., 2018). Previous studies on various fish species tended to show an increase in growth with an increase in dietary B vitamins. This was the case for vitamin B1 in juvenile Schizothorax, a species of stingray found in China (Xiang *et al*., 2016), in which the increase in growth was almost unchanged with levels above 21.5 mg/kg feed. Similarly, administration of vitamin B6 more than 5 mg/kg and vitamin B12 more than 0.051 mg/kg did not result in increased performance in juvenile carp (He *et al.*, 2019) and fingerling bream bream (Li *et al*., 2016).

The addition of vitamin B complex exceeding 50 mg/L will reduce the survival, growth, and stress resistance of nile tilapia larvae. This is thought to have exceeded the needs of tilapia larvae. According to Elango *et al.* (2015) although vitamins are needed by humans to meet nutritional needs, excess vitamins can have a negative effect. The accumulation of this vitamin under certain circumstances can lead to toxic or toxic conditions (hypervitaminosis).

The results of measurements of several water quality parameters of the maintenance media showed that the temperature values of the maintenance media were 28-30oC, pH 7.1-7.6, dissolved oxygen 4.2-5.1 ppm, and ammonia 0.02-0.09 ppm. The range of water quality parameter values is still in a reasonable range for tilapia larvae. According to Alfia *et al.* (2013) and Siniwoko (2013) that a good temperature for tilapia ranges from 25-30oC, pH 7.0-7.5 (Amri and Khairuman, 2013; Siniwoko, 2013), dissolved oxygen ranges from 4-7 ppm, and ammonia < 0.1 ppm water (Amri and Khairuman, 2013).

IV. CONCLUSION

Based on the results of this study, it can be concluded that the administration of vitamin B-complex at a dose of 50 mg/L resulted in better survival, growth rate, and stress resistance in nile tilapia larvae.

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