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| RESEARCH ARTICLE

Effect of RNA/DNA in Tilapia Fish Larvae with Administering Daphnia sp. enriched with Amino Acid

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| ABSTRACT
The quality and availability of seeds are the main problems in tilapia cultivation. One of the parameters that can be used to evaluate the quality of tilapia fish seeds is the RNA/DNA ratio. This research analyzes the influence of feeding Daphnia sp. enriched with amino acids on RNA/DNA performance in tilapia fish larvae. This research was carried out at the Nutrition Laboratory of the Aquaculture Study Program, Bosowa University, Makassar. RNA/DNA ratio analysis was carried out in October 2023 at the Takalar Brackish Water Aquaculture Fisheries Center Test Laboratory. This research used a Completely Randomized Design (CRD) with four treatments and three replications as a quantitative experimental method. The treatments are : A (Daphnia sp. + amino acids 1 mL/liter); B (Daphnia sp. + amino acids 3 mL/liter); C (Daphnia sp. + amino acids 5 mL/liter) and D (Daphnia sp). The results showed that providing Daphnia sp. enriched with 3 mL/liter amino acids can increase tilapia larvae's RNA/DNA ratio, which amounted to 0.866. This research helps increase the availability of quality tilapia seeds.

| KEYWORDS

Daphnia sp.; Amino Acid; Oreochromis niloticus; RNA/DNA ratio

| ARTICLE INFORMATION

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1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the freshwater commodities most in demand by Indonesian and foreign people (Richards, 2022). The original habitat of this species is the Nile River and the surrounding waters (Lind *et al*., 2019). Tilapia fish has high nutritional value, contains monounsaturated and polyunsaturated fatty acids (Tonial *et al.,* 2014) and easy to spawn, thus supporting the development of cultivation businesses in the community (Debnath *et al.,* 2023). One of the supporting factors in the success of fish farming is the availability of feed (Pulkkinen *et al*., 2022). Two types of feed can be given to fish: natural and artificial. Natural feed is feed that is available in nature, while artificial feed is feed that is mixed from several kinds of ingredients which are then processed into a special shape according to the mouth openings of the fish being farmed (Mulyani, 2023). The main problem in tilapia cultivation is the high mortality of the larval stage, indicated by low fish survival. The growth ffrate and survival rate of fish are influenced by the availability of food as a source of energy for growth and development (Révész and Biró, 2019).

Daphnia sp. is one of the natural feeds used in freshwater fish hatcheries. *Daphnia* sp. is small and lives in fresh waters; it is often referred to as water fleas. The advantages of *Daphnia* sp. as natural food are that fish larvae easily digest it, does not reduce water quality, contains sufficient nutritional content, and is easy to cultivate. (Cremer *et al*., 2022; Ebert, 2022; Hartnett, 2019). The nutritional content includes 4.58% protein, 0.41% fat, 1.06% carbohydrates and 0.05% ash, the quality of *Daphnia* sp. affects its physiology (Aminah and Sofarini, 2023; Taipale *et al*., 2014).

Giving *Daphnia* sp. as a natural feed enriched with amino acids, has become an effort to improve the quality of nutrition for fish larvae (Samat *et al.,* 2020). *Daphnia* sp. as small zooplankton organisms are rich in nutrients, such as essential amino acids that are important for the growth of tilapia fish larvae (Navarro *et al*., 2021). Amino acids contained in *Daphnia* sp. have the potential to influence gene expression and protein synthesis in tilapia larvae through their influence on RNA/DNA performance (Silva *et al*., 2019). Previous research shows that sufficient and balanced amino acids in feed can improve physiological processes, growth, survival and health of fish (Candebat *et al*., 2023; Deborde *et al*., 2021; Rita, Teodósio, 2022; Wang *et al*., 2022). However, research on administering *Daphnia* sp. enrichment with amino acids has not been conducted extensively. The RNA/DNA ratio is one of the parameters used to evaluate the nutrition of natural feed given to Tilapia fish larvae (Roessler *et al.,* 2020), this is because RNA and DNA are very important for cell metabolism in tilapia fish larvae. DNA stores genetic data in protein synthesis, and the amount of RNA in cells will increase with protein synthesis. Therefore, the RNA/DNA ratio can be used as a measure of protein synthesis activity which causes weight gain (Mulyani *et al.,* 2023).

This research analyzes the influence of giving *Daphnia* sp. enriched with amino acids on RNA/DNA rasio performance in tilapia fish larvae

2. Materials and Methods

2.1 Material

This research uses tilapia (*O. niloticus*) obtained from the Parangtambung Fish Seed Center Makassar, South Sulawesi, by holding the larvae in oxygenated plastic and placing them in styroform with ice cubes and taking them to the research location by car with a length of ± 1 cm and a weight of ± 0.5 grams, *Daphnia* sp. as feed natural tilapia fish.

2.2 Method

This research was carried out at the Nutrition Laboratory of the Aquaculture Study Program, Bosowa University, Makassar and testing for the RNA/DNA Ratio was carried out at the test laboratory of the Brackish Water Aquaculture Fisheries Center (BPBAP) Takalar. Tilapia fish larvae were placed in 9 container jerry cans with a volume of 20 liters equipped with an aeration system. Each container has 15 tilapia fish larvae and 18 liters of fresh water. Tilapia fish larvae were reared for 30 days by feeding *Daphnia* sp., who were given amino acid booster liquid enrichment at different doses according to the treatment. The feeding technique was carried out ad libitum (Sarker Pallab *et al.,* 2020), with a frequency of twice a day (Salger *et al.,* 2020). Disposal of remaining feed and replacement of media water by 30 - 50% is carried out every day (Shaw *et al*., 2022).

2.2.1 Experimental design

This research is experimental dan uses a quantitative approach. This research used a Completely Randomized Design (CRD) with three replications. The food provided is *Daphnia* sp., which has a protein content of 50% and fat of 20-27% dry weight. *Daphnia* sp. enrichment process with amino acids was carried out in a 3-liter container. then treated according to the specified concentration. Enrichment of *Daphnia* sp. was done for 4 hours (Alfian *et al.,* 2022). Table 2 shows the treatment doses used in this research.

2.2.2 RNA/DNA Rasio

Before the RNA/DNA ratio analysis was carried out, live tilapia larvae were used as test samples to ensure that the test sample tissue was not damaged (Xiao *et al.,* 2022).

The RNA/DNA extraction process was carried out using the silica extraction method using the Silica-Extraction Kit. DNA extraction using lysis buffer and RNA extraction using RNA extraction IQ2000. 20 mg sample of tilapia larval muscle tissue was put into a 1.5 mL eppendorf tube and added to 900 µL GT Buffer solution. Tilapia larvae were crushed and centrifuged for three minutes at 12,000 rpm. After that 600 µL of the supernatant was put into a new Eppendorf tube, 40 µL of silica was added. The mixture was centrifuged at 12,000 rpm for 15 seconds. With 500 µL of GT buffer, the supernatant was removed and cleaned. After that, the mixture was centrifuged for 15 seconds at a speed of 12,000 rpm. The supernatant was removed and the silica was cleaned with one milliliter of 70% ethanol. The mixture was centrifuged for 15 seconds at 12,000 rpm. Diethyl pyrocarbnate (DEPC) 1000 µL solution was added after ddH2O removed the ethanol. The mixture was incubated for ten minutes at 55°C. After that, the mixture was vortexed and centrifuged for two minutes at a speed of 12.00 rpm. The NanoDrop method was used to transfer 500 µL supernatant into a new eppendorf tube to measure the RNA-DNA ratio of test samples. The purity of DNA/RNA was measured with a wavelength of 260/280 = 1.8 - 2 (Moruf and Adekoya, 2021). RNA and DNA ratio measurements were calculated by dropping 1-2 µL of each test sample from each extracted genome on a UV-Vis Spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific) directly connected to a computer. Using the appicartion will read the nucleic acid concentration in ng/µL will be read (Moruf and Adekoya, 2021). The absorbance was read at a wave 260 nm (Parenrengi *et al.*, 2016), (Sarnecka *et al.,* 2019). The following formula is used to calculate the DNA/RNA ratio (Gusmiaty *et al.,* 2016).

$$[\text{DNA}] = \text{Å}260 \text{ x } 50 \text{ x Dilution factor}$$

$$[\text{RNA}] = Å260 \text{ x } 40 \text{ x Dilution factor}$$

notes :

Å260 = optical density at 260 nm;

50 = a solution with an optical density value of 1.0 which is equivalent to 50 ug/mL double-stranded DNA (dsDNA);

40 = a solution with an optical density value of 1.0 is equivalent to ssRNA (40 ug/mL).

2.3 Analysis Data

To analyze the data, Microsoft Excel was used to tabulate the data, and the effect of treatment on the RNA/DNA ratio was analyzed by analyzes of variance (ANOVA) and the W-Tucket test (Rouder *et al.,* 2016) using the SPSS version 26.0 program.

3. Results and Discussion

The quality of tilapia larvae can be analyzed through the RNA/DNA ratio (Bhat *et al.,* 2022). The results of the analysis showed that giving natural Daphnia sp. feed enriched with amino acids on the RNA/DNA ratio in tilapia larvae had a significant effect ($p<0.05$). Tukey test results showed that treatment B differed from treatments A, C, and D with a p-value <0.05, but no significant difference existed between treatments A, C, and D.

The results of measuring the RNA/DNA ratio were derived from the administration of Daphnia sp., which were enriched with amino acids at different doses in tilapia fish larvae, as seen in Figure 1. Treatment B (*Daphnia* sp. **+** amino acids 3 ml/liter) has the highest RNA/DNA rasio result of 0.866. Apart off that, the result of treatment D (*Daphnia* sp.) was 0.794, treatment A (*Daphnia* sp. **+** amino acids 1 mL/liter) was 0.788 and the lowest was treatment C (*Daphnia* sp. **+** amino acids 5 mL/ liter) of 0.778. The high ratio of RNA/DNA in treatment B (*Daphnia* sp. **+** amino acids 3 mL/liter) proves that the enrichment of *Daphnia* sp. Using 3 mL/liter of amino acids is the most optimal for the growth of tilapia larvae. The high RNA/DNA ratio indicates protein synthesis activity is occurring. expression of amino acid requirements in optimal natural food for *Daphnia* sp. This indicates that amino acids can complement each other's nutrition as natural food for tilapia larvae. Enriching 3 mL/liter of amino acids in *Daphnia* sp. can provide maximum nutritional intake.

Treatment B (*Daphnia* sp. **+** Amino Acids 3 mL/liter) had the highest RNA/DNA ratio due to the expression of amino acid requirements in *Daphnia* sp. as natural food is appropriate. The statement of the value of amino acid requirements in percent protein is based on the assumption that amino acid requirements depend on the protein content of the feed. Treatment A (*Daphnia* sp. **+** amino acids 1 mL/liter) requires amino acids that are too low due to unbalanced amino acid intake which can have a negative impact on the growth, survival and physiological status of the fish, which can cause problems such as liver disease and intestinal damage (Wang *et al.,* 2022). Treatment C (Amino acids *Daphnia* sp. 5 mL/liter) expressed the need for amino acids too high because the protein content of the feed was high, more amino acids were broken down as an energy source (Giri *et al.,* 2009). The efficiency of utilization of amino acids in feed decreased along with increasing the protein content of feed

One parameter that is widely used in fish and crustacean research is RNA/DNA ratio analysis. Various aspects of fish and crustacean growth are influenced by external factors, such as the environment, and internal factors, such as the feed provided and the biosynthesis. RNA/DNA functions as a quality indicator. The amount of RNA in fish basically indicates the expression level of genes responsible for growth in response to environmental conditions. The amount of DNA also shows the number of tissue cells in the fish's body (Bradley *et al*., 2022; Chícharo and Chícharo, 2008). Therefore, the RNA/DNA ratio can be used to measure the growth potential, based primarily on the organism's ability to biosynthesize proteins (Chang *et al*., 2021; Kou *et al*., 2022). To evaluate seed quality, RNA/DNA characters were used in African catfish by Roro *et al*. (2017), Their results showed that a higher RNA/DNA ratio was associated with better growth in African catfish. The study conducted by Mulyani *et al.* (2023) found that a single feed of *Phronima* sp. or *Artemia salina* 100% can increase crabs' RNA/DNA ratio. Apart from that, research on seed quality based on the RNA/DNA ratio has also been carried out on crustaceans, such as tiger shrimp (Parenrengi *et al*., 2016), *Scylla serrata* (Misbah, 2018), crab (Jamal, 2019) and Coral (Miyata *et al.,* 2022)

4. Conclusion

The results showed that *Daphnia* sp. enriched with 3 mL/liter amino acids could increase the RNA/DNA ratio of Tilapia larvae. To improve the growth and health of tilapia larvae, natural food *Daphnia* sp. enriched with amino acids can be given regularly. This can be part of a more efficient cultivation method.

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Authors' Contributions

All authors prepared the final manuscript. The contributions of the respective authors are as follows: PJC collected the data; SM and AA wrote the manuscript; and SM, AA and IC made the main conceptual ideas and critical revisions of the article. All authors discussed the findings and made their contributions to the final manuscript.

Conflict of Interest

The authors declare they have no competing interests.

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Highlight Research

1. The quality of tilapia larvae can be analyzed through the RNA/DNA ratio
2. The extraction process ratio RNA/DNA was carried out using the silica extraction method using the Silica-Extraction Kit
3. The RNA/DNA ratio of tilapia larvae can be improved with natural feed *Daphnia* sp. enriched with amino acids
4. Research on the RNA/DNA ratio can encourage increased production of quality tilapia seeds

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Appendixes

Table 1. Research Treatment Dosages

Treatment	Doses
A	*Daphnia* sp. + amino acid 1 mL/liter
B	*Daphnia* sp. + amino acid 3 mL/liter
C	*Daphnia* sp. + amino acid 5 mL/liter
D	*Daphnia* sp.

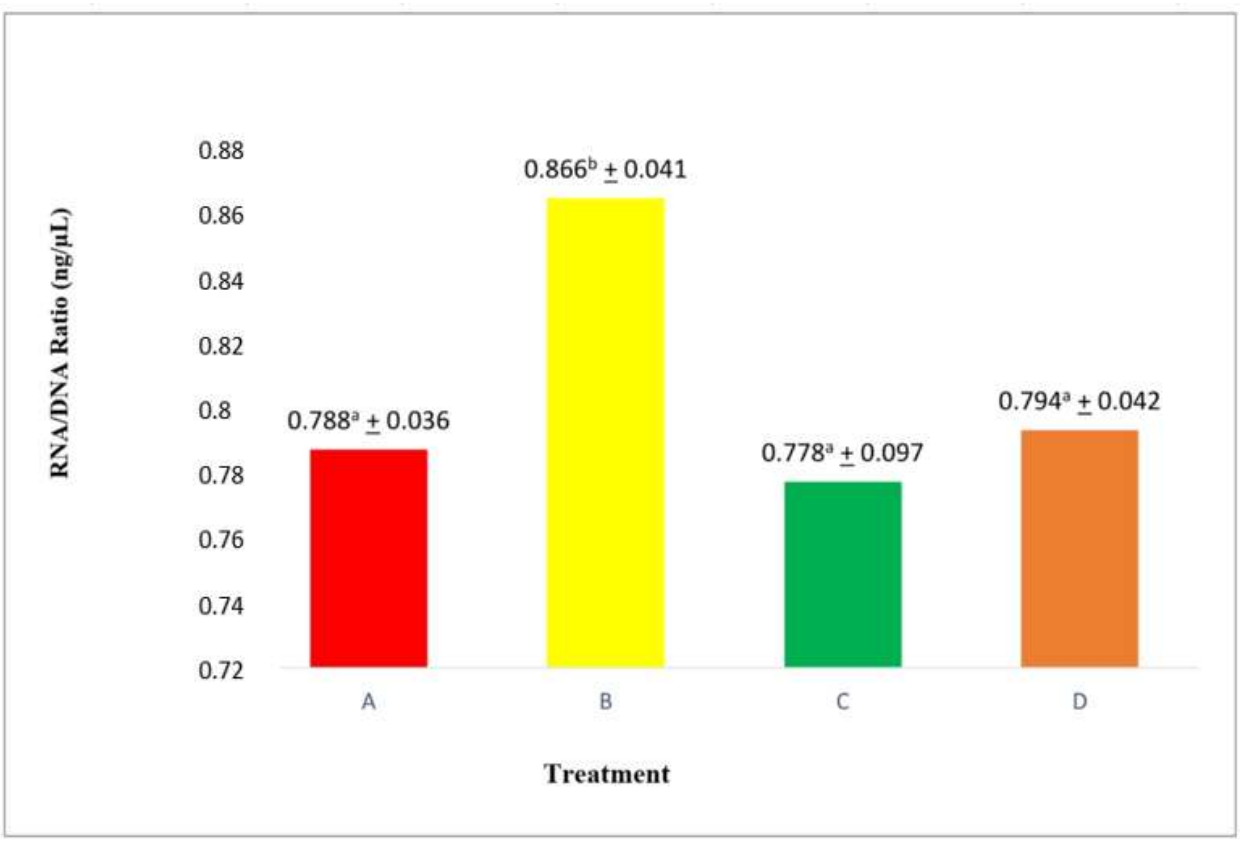
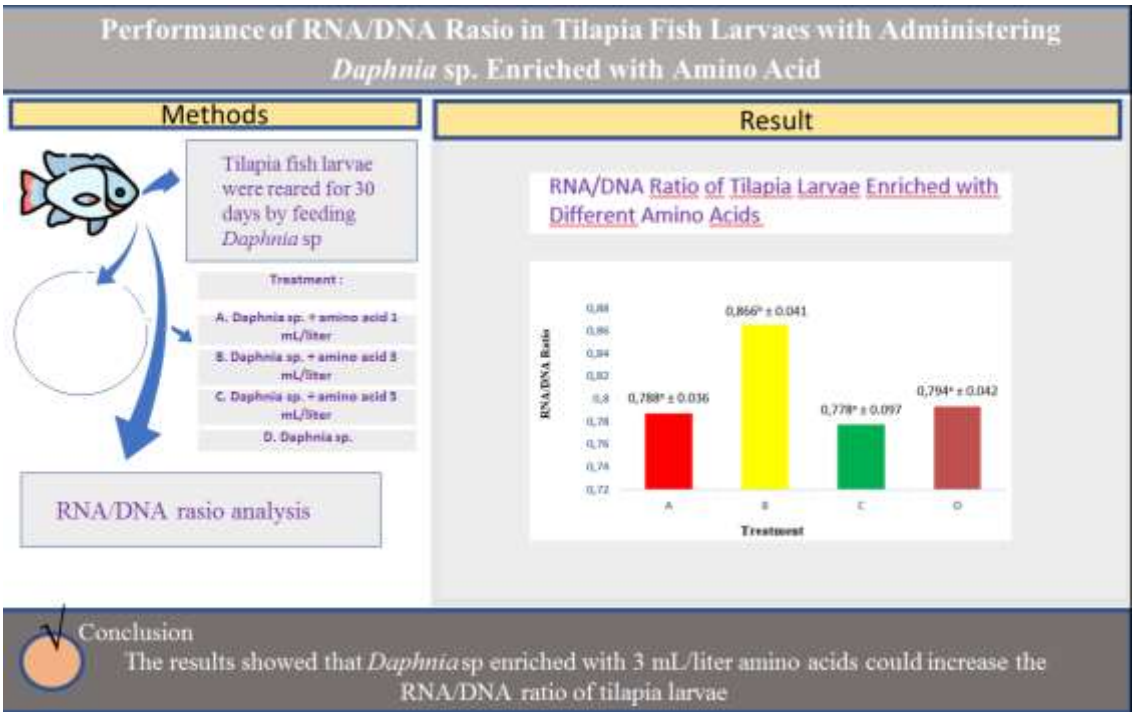


Figure 1. The RNA/DNA Ratio of Tilapia Larvae Enriched with Different Amino Acids

I. GRAPHICAL ABSTRACT



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