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Reproductive responses of female saline-adapted Nile tilapia *Oreochromis* sp. to dietary *Spirulina* sp. supplementation

Respons reproduksi induk betina ikan nila adaptasi salinitas *Oreochromis* sp. terhadap suplementasi *Spirulina* sp. dalam pakan

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ABSTRACT

Saline-adapted Nile tilapia (*Oreochromis* sp.) is a freshwater tilapia fish that was selectively adapted to brackish water aquaculture. However, its sustainable production is often constrained by the suboptimal reproductive performance of female broodstock, creating a bottleneck in seed supply. This study aimed to evaluate the effects of dietary *Spirulina* sp., a microalga rich in protein and bioactive compounds, on key reproductive indices. The experiment was arranged in a completely randomized design (CRD), in which female broodstock were randomly allocated to four dietary treatments containing graded levels of Spirulina supplementation: 0% (control), 1%, 2%, and 3%, with three independent replicates per treatment. The primary productive parameters assessed were fecundity, egg diameter, egg hatching rate, and survival rate. The results demonstrated a significant, dose-dependent improvement in all reproductive parameters ($P<0.05$). The 3% *Spirulina* sp. diet yielded the most substantial outcomes, leading to an increase in fecundity and achieving near-optimal hatching (98.75%) and 7-day larval survival rates (96.27%) compared to the control. Water quality remained stable and consistent across all treatments, confirming that the observed effects were directly attributable to dietary intervention. In conclusion, dietary supplementation with 3% *Spirulina* sp. is a highly effective strategy for enhancing the reproductive performance of female saline-adapted Nile tilapia. This microalga serves as a potent functional feed additive, offering a sustainable approach to improve seed quality and quantity in brackish-water aquaculture systems.

Keywords: brackish water aquaculture, fecundity, Nile tilapia, reproductive performance, spirulina

ABSTRAK

Nila salin (*Oreochromis* sp.) merupakan ikan nila tawar yang memiliki strain khusus yang diadaptasikan secara selektif dan krusial untuk budidaya air payau. Namun, produksi berkelanjutannya sering kali terkendala oleh performa reproduksi induk betina yang suboptimal, sehingga menghambat penyediaan benih. Penelitian ini bertujuan untuk mengevaluasi efek pemberian pakan *Spirulina* sp., mikroalga yang kaya akan protein dan senyawa bioaktif, terhadap indikator reproduksi utama. Percobaan disusun dalam desain acak lengkap (RAL), di mana induk betina secara acak dibagi ke dalam empat perlakuan pakan yang mengandung tingkat suplementasi Spirulina yang bervariasi: 0% (kontrol), 1%, 2%, dan 3%, dengan tiga ulangan independen per perlakuan. Parameter reproduksi utama yang dinilai adalah fekunditas, diameter telur, daya tetas, dan kelangsungan hidup larva. Hasil penelitian menunjukkan peningkatan yang signifikan dan bergantung pada dosis pada semua parameter reproduksi ($P<0,05$). Pakan dengan 3% *Spirulina* sp. memberikan hasil yang paling substansial, menghasilkan peningkatan fekunditas dan mencapai tingkat daya tetas (98,75%) serta kelangsungan hidup larva 7 hari (96,27%) yang mendekati optimal dibandingkan dengan kontrol. Kualitas air tetap stabil dan konsisten di semua perlakuan, yang mengonfirmasi bahwa efek yang diamati disebabkan langsung oleh perlakuan pakan. Kesimpulannya, suplementasi pakan dengan 3% *Spirulina* sp. adalah strategi yang sangat efektif untuk meningkatkan performa reproduksi induk betina nila adaptasi salin. Mikroalga ini berfungsi sebagai aditif pakan fungsional yang kuat, menawarkan pendekatan berkelanjutan untuk meningkatkan kualitas dan kuantitas benih dalam sistem budidaya air payau.

Kata kunci: budidaya air payau, fekunditas, ikan nila, performa reproduksi, spirulina

INTRODUCTION

In fish, dietary composition not only supports somatic development but also regulates endocrine function, gametogenesis, immune response, and stress tolerance (Ahmadifar *et al.,* 2021). Optimization for nutrition uptake is a fundamental determinant of growth, health, and reproductive performance in aquaculture species (Saputra *et al.,* 2018; Azis *et al.,* 2024). Nutritional deficiencies may result in reduced fecundity, poor egg quality, and low larval viability. These challenges become more pronounced under saline culture conditions. Saline-adapted Nile tilapia (*Oreochromis* sp.) is a hybrid species developed through the crossbreeding of superior strains, such as Sultana and GIFT, with locally adapted strains that exhibit tolerance to saline aquatic environments, with optimal salinity levels ranging from 5 to 27 ppt (Vitukawalu *et al.,* 2025).

Saline tilapia in the global market has the potential to reach US$14.4 billion by 2024, prompting significant governmental initiatives, such as the conversion of unproductive shrimp ponds into saline tilapia farms in Indonesia (Setkab, 2024). Despite this potential, challenges such as low egg production per spawning event and a lack of spawning synchrony in broodstock are recognized as major bottlenecks in mass seed production (Little *et al.,* 2025), issues that are often exacerbated by the specific physiological demands of saline environments. This improvement can be achieved through feed manipulation, particularly by incorporating components that contain functional nutrients to support gonadal maturation and overall reproductive processes (Singh *et al.,* 2021). Recent research has underscored the critical role of precise nutritional requirements in these conditions, including experiments on the adaptability and nutritional balances of Nile tilapia in saline waters (Abdelrhman *et al.,* 2020). Consequently, one of the foremost challenges in advancing saline-adapted Nile tilapia aquaculture remains the enhancement of reproductive performance in female broodstock. Where are the indispensable primary source for producing the consistently high-quality seed required to meet this immense market potential.

Several studies have demonstrated that Spirulina supplementation in fish feed. *Spirulina* sp. is one of the blue-green microalgae rich in protein, essential fatty acids, vitamins, and other bioactive compounds that play important roles in promoting fish health and reproductive performance. *Spirulina* sp. has a positive impact on growth and reproductive traits. A combination of *Spirulina* sp. and an oocyte developer significantly improved reproductive performance in Nile tilapia broodstock, specifically for female broodstock (Nainggolan *et al.,* 2014). More recent studies in Java barb (*Osteochilus vittatus*) have shown that dietary *Spirulina* sp. powder can increase the viscerosomatic index and absolute weight gain (Rijal *et al.,* 2023). Furthermore, Youssef *et al.* (2023) observed that although Spirulina supplementation exhibited a positive trend in growth, this effect remained evident.

The nutritional and bioactive components of *Spirulina* sp. have considerable potential in supporting fish reproduction. Additionally, the use of *Spirulina* sp. in the diets of shellfish and other fish species like zebrafish has also been reported to enhance fecundity level, egg quality, and hatching success (Carneiro *et al.,* 2025). The effectiveness of such supplements is often linked to the quality of their cultivation; for instance, enrichment of organic media has been shown to significantly increase microalgal biomass and nutrient density, which are critical for high-quality feed (Rosyadi *et al.,* 2025). Furthermore, reproductive success in tilapia is fundamentally supported by optimal dietary protein and lipid levels, which provide the essential energy for growth and maturation (Niagara *et al.,* 2025). However, studies investigating the effects of *Spirulina* sp. supplementation on the reproductive performance of Saline-adapted Nile tilapia female broodstock remain very limited. Therefore, this study was conducted to evaluate the effectiveness of dietary spirulina supplementation in enhancing reproductive performance in female Saline-adapted Nile tilapia broodstock, with the aim of supporting high-quality and sustainable seed production in intensive aquaculture systems.

Recent evidence also suggests that specific bioactive metabolites, similar to those found in probiotics, can accelerate gonad re-maturation and improve the Gonadosomatic Index (GSI) in female broodstock (Enzeline *et al.,* 2025). Previous research showed that moderate supplementation (25%) supported optimal gonad development and gamete maturation timing in zebrafish, suggesting that spirulina bioactive compounds improve reproductive physiology (Flagiello *et al.,* 2025). The primary objective of this study was to quantify the effects of diet supplementation on reproductive performance parameters of animals treated with different dietary doses of spirulina.

Specifically, we evaluated fecundity (total egg production), egg diameter as an indicator of egg quality, egg hatching rate (percentage of successfully hatched eggs), and survival rate as a measure of offspring viability. By systematically comparing these treatments, the study aimed to establish a clear dose–response relationship between spirulina and reproductive outcomes.

MATERIALS AND METHODS

Time and location of the experiment

This study was conducted at the Takalar Brackishwater Aquaculture Development Center from September to December 2021. The experiment was designed to evaluate the impact of dietary *Spirulina* sp. supplementation on the reproductive performance of female saline-adapted Nile tilapia (*Oreochromis* sp.).

Experimental design

This study employed a completely randomized design (CRD) with four treatments involving different doses of spirulina, each with three replications. The experimental design was as follows: control 0% spirulina, 1% spirulina, 2% spirulina, 3% spirulina. Thus, a total of 12 experimental units were used. The data obtained were analyzed using analysis of variance (ANOVA). If significant differences were observed, further analysis using Tukey's test was performed to determine differences between treatments.

Experimental diets

The diet test used in this study was a commercial pellet feed with a protein content of 30%, enriched with spirulina powder. The Spirulina used was commercial spirulina powder of the Mackay Microfine spirulina brand, with a particle size of 64 mesh. A standardized feed preparation protocol was implemented. To ensure product quality and suitability for experimental use, we performed physical validation, including visual inspection (color uniformity and absence of contamination), texture consistency, odor assessment, and verification of expiration date and packaging integrity. Only batches meeting quality standards were used in feed formulation. The commercial spirulina powder was sieved to ensure uniform particle size before being incorporated into the basal diet at inclusion levels of 0% (control), 1%, 2%, and 3%.

A small amount of distilled water was added to facilitate binding prior to pelleting. The mixing process was carried out using a mechanical mixer to ensure homogeneous distribution. The feed was then air-dried at room temperature (±27°C) to preserve heat-sensitive bioactive compounds. All prepared diets were stored in airtight containers at 4°C until use. The chemical composition of the product is presented in Table 1.

The experimental feed consisted of four groups: no spirulina (control) and spirulina at doses of 1%, 2%, and 3% (based on feed weight). The spirulina powder was first dissolved in 100 ml of freshwater and then evenly sprayed onto the surface of the feed. The selection of these doses was based on previous studies showing the positive effects of spirulina on fish reproduction and seed quality (Youssef *et al.,* 2023). This spirulina can be used because recent studies have demonstrated that 32% protein in combination can significantly enhance growth rates (Niagara *et al.,* 2025).

Application in fish

The test fish used in this study were saline-adapted tilapia developed by the Brackish Water Fishery Development Center (BPBAP), Takalar, originating from hybridization between female Nirwana and Sultana and male **GESIT strain**, followed by selection and adaptation to brackish water conditions. The assessment referred to

Table 1. Nutrient composition of Mackay microfine spirulina product.

No.	Chemical content	Value (%)
1	Protein	58
2	Fats/Lipids	4
3	Ash	13
4	Moisture	8
5	Fiber	7

Note: The nutritional and bioactive composition of Spirulina presented in this table is based on the official technical specifications provided by the manufacturer (Mackay Microfine Spirulina powder). The reported values reflect the manufacturer's declared data.

established ovarian development classifications that distinguish three main stages: immature (A), maturation (B), and mature (C) (Neves, 2009). In this study, broodstock selection corresponded to the mature stage (Stage C) based on macroscopic characteristics, as no histological examination was conducted. The determination of gonadal maturation stage was conducted through a morphological approach, observing the behavior of the Nile tilapia when they were gonadally mature, such as the fish's response, physical characteristics, and their tendency to build nests. This approach was also supported by a review of data on potential broodstock from BPBAP Takalar, as the broodstock used had been reared since they were potential candidates, with males and females separated from the very beginning of their rearing.

Female broodstock were placed according to the experimental units and fed the experimental diet for 10 days. The fish were fed three times a day. Feeding was carried out with restricted feeding based on the body biomass. After the rearing period, the female fish were re-examined and prepared for the spawning process. Spawning was carried out in two weeks by combining male and female broodstock in a prepared spawning pond. Inspections were made every three days to identify females that were incubating eggs. In this study, the observation period for inspection lasted up to 10 days after spawning. The eggs that had been incubated were removed from the female's mouth for further analysis. The eggs were then transferred to a special container to be observed for seven days.

Experimental procedure

Treatment application

The rearing units consisted of four concrete tanks, each equipped with an aeration system. The tanks were cleaned and checked for leaks before use. The fish were acclimated for 10 days and fed a commercial diet at 3% of the biomass per day. During this acclimation, the experimental diets were prepared by dissolving spirulina powder in water according to specific dosages: control: 0% spirulina, 1% spirulina, 2% spirulina, 3% spirulina. After mixing, these treatment diets were then given to the test animals at a rate of 3% per day based on their body biomass. Each tank contained three female broodstock. After the acclimation period, the fish were fed the experimental diet according to the treatment for 10 days.

Spawning

Male and female broodstock that had reached gonadal maturity were placed into a round fiber spawning tank at a 1:1 ratio. Inspections were conducted every three days to detect the presence of eggs. The eggs found were immediately removed, weighed, and transferred to an incubator.

Post-Spawning observation

The eggs obtained were maintained in a special container for seven days. The hatching rate was calculated using the sampling method, which involved taking a 1 mL water sample and counting the number of larvae present. The total number of hatched larvae was calculated based on the total water volume in the incubation container. The container used was an aquarium filled with seawater with a salinity maintained at 15 ppt.

Water quality observation

Water quality in the rearing medium was maintained according to the feasibility standards for Saline-adapted Nile tilapia aquaculture. Waste siphoning and partial water exchange were performed every three days by replacing 20% of the total volume with water of the same salinity to maintain consistency. Water quality parameters were measured twice daily, in the morning (06:00) and afternoon (17:00), including salinity, dissolved oxygen (DO), temperature, pH, and ammonia. Salinity was measured using a hand refractometer, DO using a DO meter, temperature with a thermometer, and pH using a pH meter. Ammonia levels were measured three times during the study: at the beginning, middle, and end of the experimental period. The water quality test results during the experiment are shown in Table 2.

Table 2. Water quality test results during the experimental period.

Water Quality Parameter	Treatment Dose (%)				(BSN, 2009)
	0	1	2	3	
Water Temperature (°C)	27.5-30.5	27.1-30.4	27.5-30.4	27.5-30.1	25–30
Dissolved Oxygen (mg/L)	5.2-6.3	5.3-6.5	5.1-6.5	5.1-6.7	>4
pH	6.8-7.8	6.6-7.4	6.5-7.5	6.5-7.7	6.5-8.5

Water quality parameters remained relatively stable and did not show significant differences among the treatment groups. The values for Dissolved Oxygen (ranging from 5.1–6.7 mg/L) and pH (ranging from 6.5–7.8) fell completely within the optimal ranges recommended by BSN (2009). Although the water temperature across all groups (ranging from 27.1–30.5°C) was observed to occasionally exceed the maximum standard threshold of 30°C, this condition was uniform across all treatments. The stability of these water parameters indicates that the environmental conditions were properly controlled, ensuring that the observed differences in the results were purely an effect of the applied treatments.

Parameters

Experimental variables

This study examined four variables to determine the optimal treatment dosage, namely egg fecundity, egg diameter, egg hatching rate, and survival rate. The data collection techniques for each parameter are described as follows:

Fecundity

Fecundity is a parameter that reflects the reproductive capacity of female broodstock in producing eggs. In this study, fecundity was estimated indirectly by calculating the difference in body weight of the female before and after spawning. This weight difference is assumed to represent the weight of the gonads (ovaries) containing the eggs. Fecundity was calculated using the following formula (Bagenal, 1971);

Note:

Gonad weight = Weight of the female

$$\text{Fecundity (number of eggs)} = \frac{\text{Gonad weight (g)}}{\text{Average weight of a single egg (g)}}$$

broodstock before spawning – Weight of the female broodstock after spawning (grams)

Weight per egg = Average weight of a single egg, calculated by weighing 30–50 randomly selected eggs and dividing the total weight by the number of eggs (grams)

Egg diameter

Measurement of egg diameter was conducted to assess the quality of eggs produced by the female broodstock. Eggs with larger diameters generally contain greater nutritional reserves, which influence hatching success and larval development (Sarmento *et al.,* 2018). Measurements were performed using a binocular microscope equipped with an ocular micrometer with a precision of 0.1 mm. For each experimental unit, a random sample of at least 30 eggs was measured, and the average diameter was calculated.

Egg hatching rate

The hatching rate represents the percentage of eggs that successfully hatched into larvae. This parameter is essential for evaluating the success of spawning and the effectiveness of the treatment on fish reproduction. The calculation was based on the formula by Bagenal (1971):

Note:

Number of hatched eggs = Refers to the

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatched eggs}}{\text{Number of eggs fertilized}} \times 100$$

total number of eggs that successfully developed into larvae after the incubation process. Eggs are considered "hatched" once they have reached the larval stage, typically verified through visual observation or microscopy.

Number of incubated egg = Refers to the total number of fertilized eggs that were prepared and placed in the incubator, regardless of whether they successfully hatched or not.

Survival rate

The survival rate (SR) was used to determine the percentage of larvae that survived after hatching over a specified observation period (7 days in this study). Measurements were conducted in five replications for each experimental unit, and the average value was calculated. The SR value reflects egg quality, incubation success, and the larvae's ability to adapt to the environment from the time of hatching. The calculation followed the formula (Bagenal, 1971):

Note:

Number of live larvae on day 7 = Refers to the

$$\text{Survival rate (\%)} = \frac{\text{Number of live fishes at the end}}{\text{Number of live fishes at initial}} \times 100$$

total number of larvae that remained alive and survived until the 7th day post-hatching. This count was obtained by monitoring larval survival throughout the observation period (e.g., 7 days) and recording the number of surviving larvae at the end.

Initial number of larvae = Refers to the total number of larvae produced from successfully hatched eggs at the beginning of the post-hatching period.

RESULTS AND DISCUSSION

Result

Fecundity

The result of the fecundity measurements under different dietary spirulina treatments is presented in the graph shown in Figure 1. Based on the results, the different doses of spirulina powder diet treatment had a significant effect at the 5% level (P<0.05) on the increase in fecundity. The average fecundity value in the control group was 1353 ± 66.51 eggs, then increased significantly in the 1% treatment to 1767 ± 45.13, increased again in the 2% treatment to 2408 ± 18.08 eggs, and reached the highest value in the 3% treatment, namely 3219.33 ± 37.17. The different letters for each treatment indicate that all treatments were significantly different from each other based on further testing at a significance level of 0.05.

Egg diameter

The results of egg diameter measurements under different dietary spirulina treatments are presented in the graph shown in Figure 2. Based on the results, the different doses of spirulina powder diet treatment had a significant effect at the 5% level (P<0.05) on the egg diameter. The average egg diameter in the control group was 2.61 ± 0.05 mm, then gradually increased in the 1% treatment to 2.71 ± 0.03 mm, 2% to 2.76 ± 0.02 mm, and reached the highest value in the 3% treatment to 2.86 ± 0.07 mm. Where the 3% treatment was significantly different from the control, while the 1% and 2% treatments showed a gradual increase pattern with partial significance.

Egg hatching rate

The results of the egg hatching rate

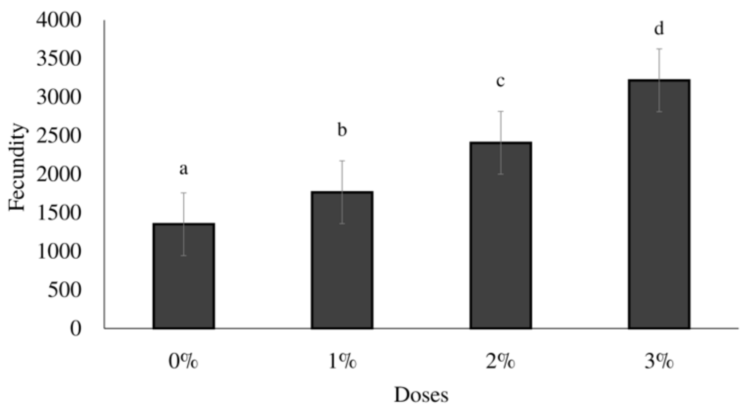

Figure 1. Fecundity in different doses of spirulina powder diet treatment. Boxes represent means ± standard deviation. Different letters above boxes indicate statistically significant differences (Tukey's HSD, P<0.05).

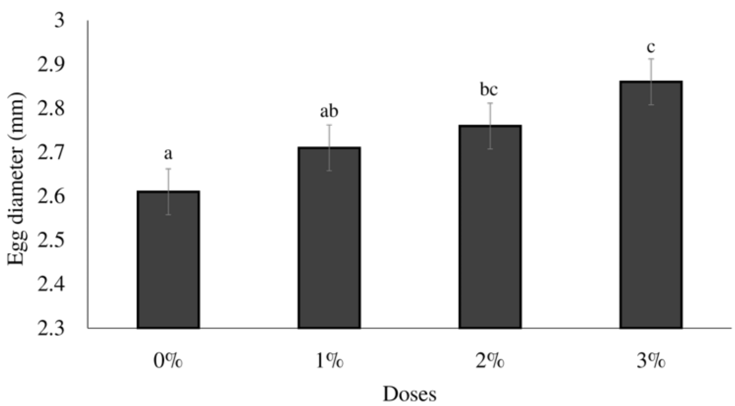

Figure 2. egg diameter in different doses of spirulina powder diet treatment. Boxes represent means ± standard deviation. Different letters above boxes indicate statistically significant differences (Tukey's HSD, P<0.05).

measurements under different dietary spirulina treatments are presented in the graph shown in Figure 3. Based on the results, the different doses of spirulina powder diet treatment had a significant effect at the 5% level (P<0.05) on Egg hatching rate. The average hatchability rate in the control group was 71.63 ± 2.7%, increasing significantly in the 1% treatment to 80.97 ± 1.5%, then in the 2% treatment to 88.77 ± 1.36%, and highest in the 3% treatment at 98.75 ± 0.4%. Further testing showed that the control group differed significantly from the 1%, 2%, and 3% treatments; the 1% treatment differed significantly from the 2% and 3% treatments; and the 2% treatment also differed significantly from the 3% treatment. The different letters confirm that all treatments differed significantly from each other at a significance level of 0.05.

Survival rate

The results of larval survival rate on day 7 post-hatching measurements under different dietary spirulina treatments are presented in the graph shown in Figure 4. Based on the results, the different doses of spirulina powder diet treatment had a significant effect at the 5% level (P<0.05) on survival rate. The average survival rate in the control group was 70.34 ± 1.39%, and in the 1% treatment group it was 74.19 ± 1.86%. The two treatments were not significantly different from each other. However, the 2% treatment showed a significant increase to 85.84 ± 1.78%, which was significantly different from the control and 1% treatments. Meanwhile, the 3% treatment produced the highest value of 96.27 ± 1.17%, which was significantly different from all other treatments (control, 1%, and 2% of spirulina diets).

Regression among fecundity, egg diameter, and hatching rate

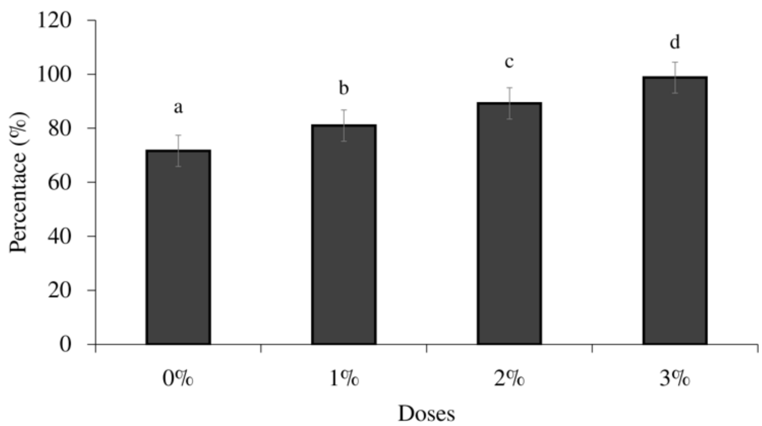

Figure 3. egg hatching rate in different doses of spirulina powder diet treatment. Boxes represent means ± standard deviation. Different letters above boxes indicate statistically significant differences (Tukey's HSD, P<0.05).

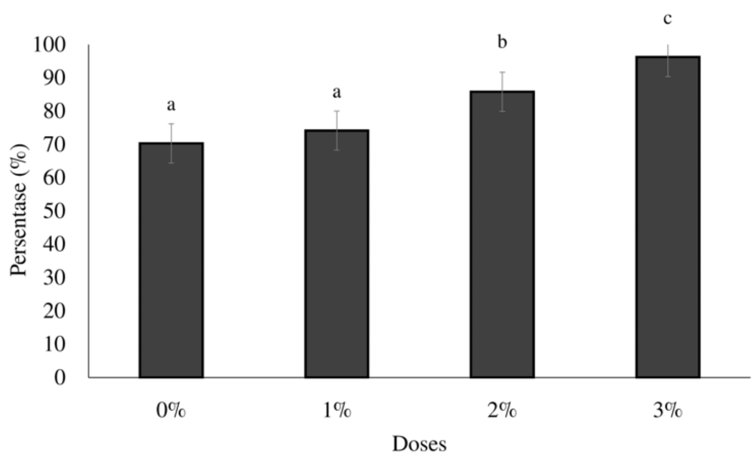

Figure 4. Survival rate in different doses of spirulina powder diet treatment. Boxes represent means ± standard deviation. Different letters above boxes indicate statistically significant differences (Tukey's HSD, P<0.05).

To better understand the relationships among key reproductive performance indicators in broodstock, a regression analysis was conducted to examine the association between fecundity, egg diameter, and hatching rate. This analysis aimed to determine whether variations in egg size and fecundity are related to hatching success, thereby providing a clearer interpretation of broodstock reproductive quality in aquaculture. Linear regression analysis shows a significant positive relationship between fecundity and egg diameter. With a Pearson correlation coefficient (r) of 0.8477 and a coefficient of determination (R^2) of 0.7186. These values indicate that 71.86% of the variation in egg diameter can be explained by the variation in fecundity.

In general, an increase in the number of eggs per parent is followed by an increase in egg size. Furthermore, the relationship between egg diameter and egg hatching rate shows a very strong correlation with an r value of 0.9825 and an R^2 of 0.9653. This means that 96.53% of the variation in egg hatchability can be explained by variations in egg diameter.

Discussion

Fecundity for fish experiment it can be serves as a fundamental metric for broodstock productivity specially for female broodstock. Fecundity directly quantifies the reproductive output essential for the efficiency and profitability of hatchery operations (Pandey, 2024). Enhancement is not only statistically significant ($P<0.05$) but also profoundly meaningful from a practical standpoint. In this study, supplementation with 3% *Spirulina* sp. yielded a remarkable increase in fecundity, reaching 3219 ± 37.17 eggs per female, which represents an approximately increase compared to the control group (1353 ± 66.51 eggs). Compared to the use of spirulina in the diets of shellfish and other fish species has been reported to enhance fecundity, egg quality, and hatching success (Flagiello *et al.,* 2025). These improvements are generally attributed to the nutritional or nutrition

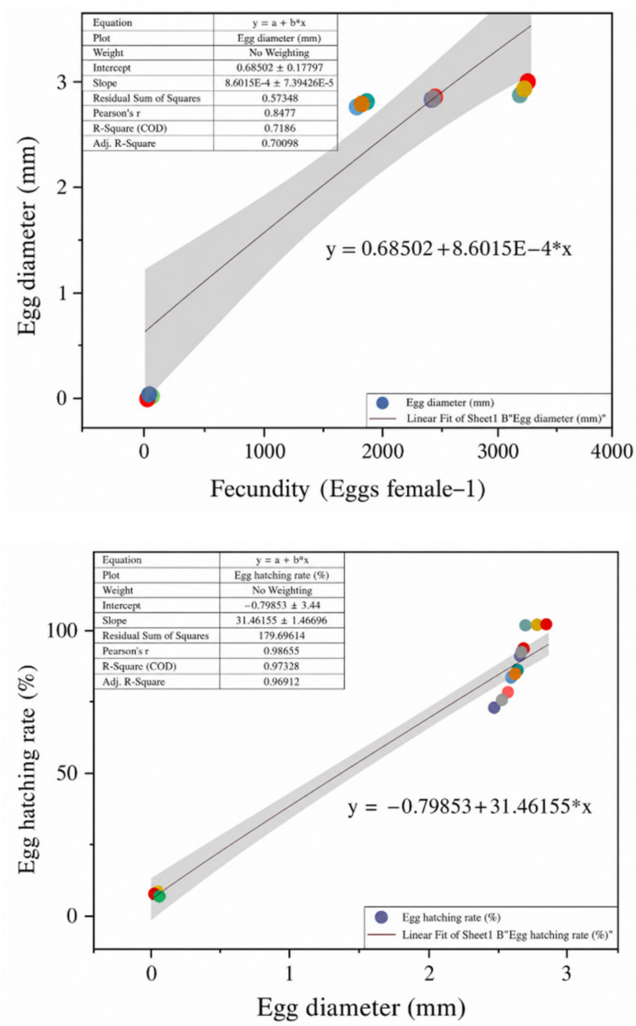

Figure 5. Regression among fecundity, egg diameter, and hatching rate.

content profile and bioactive compounds present in spirulina, including phycocyanin, carotenoids, essential fatty acids, vitamins, and trace minerals, which are known to regulate antioxidant defense systems and reproductive endocrine pathways.

De-Dios *et al.* (2022) for example, spirulina in zebrafish diets improved gonadosomatic index, egg production, fertilization rate, and hatching level compared to controls experiment, indicating enhanced reproductive performance in this model species (Carneiro *et al.,* 2025). Moderate dietary supplementation levels have been shown to support reproductive physiology for aquatic animals. A recent longitudinal study demonstrated that zebrafish fed diets containing moderate spirulina (5–25%) exhibited enhanced survival, improved reproductive behavior, higher spawning success, and optimal gonadal development and timing of gamete maturation, supporting the hypothesis that spirulina bioactive compounds exert functional effects on reproductive physiology. The increase fecundity for fish attributed to a powerful dual-action mechanism of dietary *Spirulina* sp. (Kari, 2025), which help reduce oxidative stress in reproductive tissues and protect developing oocytes from damage. The combined nutritional and protective role suggests that *Spirulina* sp. can act as a functional feed additive to improve broodstock reproductive performance for fish. Dietary supplementation with *Spirulina* sp. may enhance egg production and reproductive efficiency in intensive aquaculture systems or highest density broodstock (Rahman *et al.,* 2023).

Mechanistically, this surge in egg production from female broodstock is driven or delivery by the availability of superior nutritional "building blocks" provided by spirulina (Alagawany *et al.,* 2021). Its high-quality protein (55-70%), with a complete profile of essential amino acids such as arginine, lysine, and methionine, is crucial for the synthesis of vitellogenin (Gajendiran & Narayanan, 2025). Vitellogenin is the yolk protein content precursor synthesized in the liver and transported via the bloodstream to the ovaries for accumulation in developing oocytes in the organism. An abundant supply of these amino acids ensures that the vitellogenesis process operates at maximum capacity, allowing more oocytes to develop concurrently (Lubzens *et al.,* 2010; Tufail & Takeda, 2008). On the lipid front, the gamma-linolenic acid (GLA) content in *Spirulina* sp. a rare omega-6 fatty acid, serves as a direct precursor to prostaglandins. Prostaglandins are vital hormonal signaling molecules that regulate the final maturation of oocytes, ovulation (egg release), and the synchronization of spawning behavior (Nilsson *et al.,* 2020).

Egg diameters for the fish experiment are an important indicator. The gamete quality for female fish broodstock directly correlates with the survival potential of embryos and larvae. In this study, compared to the control group's 2.61 ± 0.05 mm ($p<0.05$), supplementation with 3% *Spirulina* sp. significantly increased egg diameter to 2.86 ± 0.07 mm. However, a more scientifically intriguing observation was the lack of a significant difference at the lower doses of 1% and 2%. The experiment suggests a threshold effect, whereby the nutrients from spirulina at lower doses are likely allocated to the maintenance of the broodstock's somatic functions and basal energy requirements. Only when the supplementation reached a higher concentration (3%) could the surplus nutrients and bioactive compounds be effectively channeled to maximize vitellogenesis to a degree that measurably increased oocyte volume.

This phase involves intense metabolic activity, making oocytes vulnerable to oxidative damage such as lipid peroxidation, where free radicals attack fatty acids in cell membranes. In female tilapia, the increase in oocyte size at the highest spirulina dose may be linked to enhanced cellular protection during gonadal maturation (Służały *et al.,* 2024; Zahran *et al.,* 2024). Such damage can disrupt yolk structure and compromise oocyte integrity. Spirulina contains lipophilic antioxidants, including vitamin E (tocopherol) and β-carotene, which integrate into the lipid-rich membranes of oocytes and neutralize free radicals before damage occurs (Służały *et al.,* 2024; Zahran *et al.,* 2024). This phase involves intense metabolic activity, making oocytes vulnerable to oxidative damage such as lipid peroxidation, where free radicals attack fatty acids in cell membranes.

Protection allows yolk. In female tilapia, the increase in oocyte size at the highest spirulina dose may be linked to enhanced cellular protection during gonadal maturation (Bhattacharya *et al.,* 2024). The protection allows yolk accumulation to proceed more efficiently, resulting in larger and more stable oocytes. Previous studies involve larger eggs represent a greater maternal investment in offspring survival (Burton *et al.,* 2020). Increased yolk reserves provide additional energy for embryo development and early larval stages (McBrid *et al.,* 2015). This energy reserve offers several advantages for larval fish, it can make fish have more time to transition to external feeding, and those from larger eggs are often larger, possess greater energy reserves, and show more advanced organ development (Arevalo *et al.,* 2023; Du & Shine, 2022). Consequently, the fish tend to swim more efficiently and capture prey more effectively. Therefore, dietary spirulina supplementation that enhances egg diameter can support the production of higher-quality larval fish and improve hatchery performance, consistent with previous findings on the positive effects of spirulina on gamete accumulation to proceed more efficiently, resulting in larger and more stable oocytes.

The hatching rate serves as a definitive metric of reproductive performance, reflecting the cumulative success of fertilization, egg quality, and embryonic development. In this experiment, a crucial component of the reproductive performance of female saline-adapted Nile tilapia was dramatically enhanced by dietary *Spirulina* sp.. Experiment data showed hatching rate surged to a near optimal 98.75% ± 0.40% in the 3% supplementation group, a stark and statistically significant contrast to the 71.63% ± 2.70% observed in the control ($p<0.05$). A success rate approaching 100% signifies the near-complete mitigation of embryonic mortality, transforming a major potential bottleneck in hatchery production into a point of exceptional efficiency.

The result from this experiment can demonstrate that the dietary intervention effectively translated into robust, viable embryos. The nutritional content payload delivered by *Spirulina* sp., which is directly fortifies the developing embryo's life support systems in female broodstock. The underlying cause for this enhanced viability can be traced to the superior structural and biochemical quality of the eggs. Specifically, essential fatty acids and phospholipids are incorporated into the chorion and vitelline membranes, creating a more resilient physical barrier against microbial invasion and mechanical stress (Govarthanan *et al.,* 2017; Zahran *et al.,* 2024). This ensures that critical developmental processes, from cell division to organogenesis, proceed with high fidelity, minimizing the risk of fatal developmental errors. Beyond structure, the comprehensive suite of vitamins and minerals provided by spirulina acts as essential cofactors for embryonic metabolic enzymes.

The remarkable hatching rate is not the result of a single factor but a powerful synergy between providing superior nutrition, fortifying structural integrity, and pre-loading the embryo with a robust immune toolkit (Hamed *et al.,* 2024; Zahran *et al.,* 2024). Recent experiment, it is showed the particularly relevant for the aquaculture of saline-adapted Nile tilapia, as indicated in the study's title. Brackish water environments can present additional osmotic stress and a different spectrum of pathogenic challenges to developing fish embryos, specially for tilapia species. The enhanced membrane integrity and passive immunity conferred by dietary spirulina likely make the eggs more resilient to these specific environmental stressors. Therefore, this study confirms that dietary supplementation with *Spirulina* sp. is a highly effective strategy for enhancing the reproductive performance of female tilapia, particularly by ensuring high embryonic viability under the demanding conditions of saline aquaculture systems.

Reproductive performance as value indictaor in aquaculture is not determined by the number of eggs produced or hatched, but more importantly by the proportion of larvae fish such successfully survive in the critical early life stages or phase. Supplementation with *spirulina* sp. give a clear dose dependent response was observed, with the 7-day post-hatch survival rate increasing from 70.34 ± 1.39% in the control group to 85.84 ± 1.77% at the 2% supplementation level, and reaching 96.27 ± 1.17% at the 3% dose ($P<0.05$). Dietary supplementation of *Spirulina* sp. in previous study showed diets significantly can improved larval fish survival. This experiment in substantial level showed the improvement in survival indicates that *Spirulina* sp. supplementation can effectively reduce early larval mortality, which is often a major production constraint in hatchery systems, thereby enhancing overall production efficiency. The improved survival is closely associated with the superior nutritional reserves supplied to fish larval through the yolk sac. The transition from endogenous nutrition to exogenous feeding represents one of the most critical phases in fish larval development.

Larvae produced from *Spirulina* sp. supplemented broodstock likely possess larger and more nutrient rich or content yolk reserves. These reserves, particularly rich in essential fatty acids such as DHA. The DHA content can support the development of important sensory and locomotor structures, including visual organs and swimming musculature, resulting in more active larvae with improved feeding ability (Pilecky *et al.,* 2021).

In addition, greater yolk reserves extend the time available for larvae to successfully initiate exogenous feeding before endogenous energy reserves are depleted (Tu *et al.,* 2022).

Furthermore, *Spirulina* sp. supplementation or dietary supplementation using *Spirulina* sp. may enhance larval fish resilience through the maternal transfer of bioactive compounds to the eggs in female broodstock. Bioactive pigments content such as phycocyanin and carotenoids deposited in the oocytes remain present in larvae after hatching and provide early physiological protection. This maternally derived passive defense plays an important role during the initial post-hatch period when the larval immune system is not yet fully developed (Shastak & Pelletier, 2023). Consequently, larvae may exhibit greater resistance to opportunistic pathogens commonly present in hatchery environments, thereby reducing early-stage mortality. These findings are consistent with previous reports highlighting the strong relationship between broodstock nutrition and larval quality in aquaculture species.

The positive relationship between fecundity and egg diameter on this experiment ($R^2 = 0.7186$) suggests that female fish broodstock producing a higher number of eggs also tend to produce larger eggs. Egg size and fecundity are both positively associated with maternal reproductive success, yet maternal resource limitations result in a trade-off between these two traits (Debes *et al.,* 2025). Theoretically, an increase in egg number results in smaller egg size due to limited energy allocation (Hagmayer *et al.,* 2018). However, the pattern observed in this study indicates otherwise. This result suggests that maternal body size may play a more important role than energy limitation alone.

Larger broodstock likely have greater energy reserves, allowing them to produce more eggs without reducing egg size (McBride *et al.,* 2015; Pandey, 2024). Therefore, broodstock fish size should be considered as an important covariate in further analyses to better understand the relationship between reproductive output and egg characteristics. Therefore, broodstock size should be considered as an important covariate in further analyses to better understand the relationship between reproductive output and egg characteristics (Benkwitt *et al.,* 2021), specifically because female body size variation may directly dictate early life history traits such as feeding ability, growth rates, and morphological development (Takatsu *et al.,* 2022). The relationship between egg diameter and hatchability in female broodstock supplementation shows a very strong correlation ($R^2 = 0.9653$), indicating that egg diameter is the main predictor of hatching success, whereas heavier initial egg weights are directly linked to the production of larger, more improve hatchlings (Dahloum *et al.,* 2024; Peşmen, 2025).

The coefficient of 31.46 shows that small changes in egg diameter have a substantial impact on hatchability. Biologically, eggs with larger diameters have greater yolk volume, thus providing more adequate nutritional reserves for embryo development (Hasan & Khaliduzzaman, 2022). The experiment corelation is find strengths the concept that egg morphometric quality plays an important role in determining early embryo development performance. Conceptually, these research results show that egg diameter acts as a connecting variable between the reproductive capacity of the broodstock (fertility) and the initial success of fish embryo development (hatchability).

CONCLUSION

The supplementation increased fecundity produced larger and higher-quality eggs, and resulted in high reproductive success, as indicated by a hatching rate of 98.75% and a 7-day larval survival rate of 96.27%. These improvements are likely associated with the dual role of spirulina as a nutrient-rich feed ingredient and a source of bioactive compounds that support reproductive physiology. Therefore, the inclusion of 3% *Spirulina* sp. in broodstock diets can be recommended as a practical nutritional strategy to enhance reproductive output, improve larval quality, and increase the productivity of saline-adapted Nile tilapia hatchery systems. Dietary supplementation with 3% *Spirulina* sp. significantly improved the reproductive performance of female saline-adapted Nile tilapia (*Oreochromis* sp.).

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