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BROODSTOCK FECUNDITY AND FRY SURVIVABILITY IN NILE TILAPIA *(Oreochromis niloticus)*: EFFECTS OF DIETARY NUCLEOTIDE SUPPLEMENTATION IN AN ON-FARM FEEDING TRIAL

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Abstract: The rapid expansion of global aquaculture has highlighted the importance of optimizing tilapia broodstock management to address challenges such as low fecundity and fry survival. This study explores the potential of dietary nucleotide supplementation, specifically Nucleoforce Aqua™, in enhancing broodstock fecundity and fry survival within tilapia aquaculture systems. A farm feeding trial, adapted to the principles of practical farm practice, involved administering dietary nucleotides at a 0.05% concentration to tilapia broodstock over a span of thirty (30) days, with the experiment encompassing two distinct treatment groups: T1, supplemented with dietary nucleotides, and T2, a control group without supplementation. Preliminary findings unveil promising trends. Broodstock receiving Dietary Nucleotide exhibited significantly higher fry production and survival rates compared to the control. Analysis of *O. niloticus* fecundity underscores a marked disparity between treatments. T1 yielded an average of 186,000 fry, outperforming T2's 124,000 fry produced from Commercial Diet alone. The Independent Samples T-Test confirmed the statistical significance of this difference, with p-values of 0.004 (one-tailed) and 0.008 (two-tailed), both below the standard significance threshold of 0.05. This result indicates a statistically significant effect of dietary nucleotide supplementation on fecundity. Although fry survival rates between T1 (65%) and T2 (62%) showed minimal differences, the marked 50% increase in fecundity underscores the potential of nucleotide supplementation to enhance reproductive outcomes. These findings suggest that dietary nucleotides can enhance broodstock productivity, addressing challenges in tilapia aquaculture and potentially improving farm sustainability and profitability. This study contributes to the understanding of nutritional strategies in improving broodstock performance and overall aquaculture productivity. Further research is recommended to explore the effects of nucleotide supplementation under varied environmental conditions and to assess cost-effectiveness, enabling the optimization of this technology for broader application in the industry.

Keywords: sustainability, aquaculture, nucleotide supplementation, broodstock management, fecundity, Tilapia fry survival

Introduction

Aquaculture's rapid global expansion, with an annual production of approximately 90.86 million Metric Tons (MT), surpassing other agricultural activities (Bureau of Fisheries and Aquatic Resources [BFAR], 2022) It is projected to supply nearly two-thirds of global fish consumption by 2030, according to the United Nations Food and Agriculture Organization (Food and Agriculture Organization [FAO],

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2018; 2020). In 2022, aquaculture made up 54.15% or 2.35 million MT of the total fisheries output in the Philippines and represented the highest share in the overall fisheries production value with 41.82% or PhP 124.00 billion. On the global stage, the Philippines holds the 11th position in aquaculture production, accounting for 928,820.99 MT or 1.02% of the total output of 90.86 million MT, with the comprehensive value of its aquaculture production, which includes fish, crustaceans, and mollusks was PhP 2.05 billion (FAO, 2022; BFAR, 2022).

The selection of species for aquaculture is primarily influenced by factors such as its availability, legal status, growth rate, and tolerance to different conditions (Rakocy et al., 2005). Within this spectrum of species, Tilapias, particularly Nile Tilapia (Oreochromis niloticus), has been recognized as a priority species in the country, earning the moniker "aquatic chicken" due to its good attributes that make it suitable for aquaculture (Tacio, 2010; BFAR, 2020). The significant dominance of Nile tilapia, constituting approximately 70-80% of global tilapia production (Romana-Eguia et al., 2020), can be attributed to its remarkable qualities, including rapid growth, prolific breeding capabilities, adaptability to captive conditions, resilience to environmental stress, strong market demand, and, notably, a key factor contributing to its success in aquaculture is its ability to readily adopt formulated diets after yolk-sac absorption (Ng and Romano, 2013; Ronald et al., 2014; Munguti et al., 2022).

In the Philippines, tilapia production has risen to 490.96 thousand MT in 2022 (PSA, 2023). Traditionally reliant on capture fisheries, the country has become a global leader in tilapia farming, ranking 6th among major producers and contributing 4.8% to the world's total production in 2021 (Tacio, 2014; FAO, 2020; BFAR, 2023).

Despite tilapia's potential, a 10.62% production decline in 2022 highlights the scarcity of quality fry as a significant obstacle to commercial expansion (Abu-elala et al., 2021; BFAR, 2023). This challenge arises from low fecundity combined with the asynchronous spawning and high mortality rates during first larval feeding stage (El-Sayed, 2006; Tsadik Getinet, 2008; Lupatsh et al., 2010; Roth et al., 2013). Although grow-out phase mortalities have the greatest economic impact due to production costs, first larval feeding mortalities can cause devastating crop losses within days which can account to 90% mortality in some species (Roth et al., 2013; Mengistu et al., 2020)

In response to these challenges, innovative and sustainable methods for aquaculture production have been explored. The need for sustainable intensification of aquaculture production aligns with the global shift towards more efficient practices that overcome the limitations associated with traditional intensive cultivation methods (Navarro-Guille´n et al., 2021). Several strategies have recently been implemented in aquaculture to support sustainable tilapia production, including optimizing broodstock nutrition (Izquierdo et al., 2001) and developing functional feeds (Abu-elala et al., 2021).

Optimized broodstock management and nutrition are of prime importance in aquaculture, as these contribute to enhancing fecundity, breeding success, and larval survival. Proper broodstock nutrition not only supports early larval development during critical stages, such as yolk nourishment, but also maximizes the genetic potential of tilapia for growth, leading to sustainable and efficient juvenile production (Izquierdo et al., 2001; Alemayehu et al., 2018; Bobe, 2015). In Aquaculture, the use of feed additives for optimized nutrition varies significantly, as different species have a unique nutritional requirement. Formulation of fish diets needs to be done very carefully to provide all the essential

nutrients and energy required to meet the physiological demands of growing animals (Hixson, 2014). In this context, the inclusion of nucleotide (NT) as functional feed additive in aquafeeds has gained interest for its potential benefits (Hossain et al., 2016).

Nucleotides, the building blocks of DNA and RNA, are crucial for numerous physiological and biochemical processes, including but not limited to genetic encoding, energy metabolism, and cell signaling (Carver and Walker, 1995; Cheng et al., 2011). While the body can produce nucleotides, their demand increases during periods of rapid growth, stress, disease, and reproduction, classifying them as semi-essential nutrients (Whitehead et al., 2006; Kruger et al., 2018). Supplementing nucleotides through exogenous diet fulfills these heightened needs, reducing stress, promoting growth, and enhancing overall fish health (Cheng et al., 2011; Hossain et al., 2019).

The use of nucleotides is a relatively novel application as a modern feed additive. Nevertheless, multiple research studies have established its positive impact on numerous vital functions in different cultured aquatic animals, which is summarized below in table 1.

Table 1. Summary of recent studies on the effects of dietary nucleotide supplementation on different aquaculture species (2015-2023)

Species	Nucleotide inclusion level	Duration	Observation/s	Reference
Nile Tilapia	0.0, 2.5, or 5.0 g nucleotides (NT)	105 days	1. Growth, health, and resistance↑ (2.5 g NT/kg in both broodstock and offspring diets). 2. The 105-day-old tilapia - biomass, antioxidative status, survival↑; liver lipoperoxidation↓ during bacterial challenge. 3. Offspring - survival ↑; hepatic alanine aminotransferase activity↓ during transport stress and bacterial challenges	do Nascimento et al., 2023
Nile Tilapia	0.4% dietary fermented yeast extract	6 months	1. Dietary inclusion in broodstock diets - seed production, survival, hematological parameters, antioxidant power, intestinal microstructure, and immune- and growth-related genes↑. 2. Fry and Fingerlings - Growth indices↑.	Abu-Elala et al., 2021
European sea bass	0, 500, 1000, and 1500 mg/kg	56 days	1. Inclusion of dietary nucleotides at 500 mg/kg - growth performance, feed utilization, liver enzymatic profile, and histological development of the intestine, liver, and spleen↑. 2. Survival rates across all treatments=.	Magouz et al., 2021
Nile Tilapia	0.0, 2.5, 5.0, 7.5, and 10.0 g NT/kg diet	150 days	1. Dietary inclusions of 5.0–7.5 g NT/kg - Reproductive performance, growth, and overall health↑. 2. At 7.5 g NT/kg - Egg production per gram of spawning female, glycogen content in ovaries, hemoglobin levels and antioxidant enzyme activities (SOD and GST)↑. 3. Redox balance, liver energy metabolism, and crude protein in ovaries↑. 4. Spawning rate females↓; Growth rates↑ (7.5–10.0 g NT/kg).	De Lima et al., 2020
Stiped Catfish	250 g/ton and 500 g/ton diets	8 weeks	1. Growth performance, haematological and biochemical indices, and immune responses↑ (500 g/ton). 2. Resistance↑ to *Pseudomonas aeruginosa*; cumulative mortality rates↓.	Yaseen et al., 2020
Rainbow trout	0.15% in the form of 5′-monophosphate (IMP, AMP, GMP, UMP, CMP)	15 weeks	1. Growth performance and feed utilization =. 2. Polyunsaturated fatty acid composition in the liver and muscle tissues of rainbow trout↑.	Ridwanudin et al., 2019

Amberjack	0.6% in gradually replaced FM with soybean-based diets	60 days	1. Digestibility, immune responses, stress resistance, and intestinal health condition↑. 2. Alternative protein utilization↑.	Hossain et al., 2018
Pacific white shrimp	0, 2, 4, and 6 g/kg diets	30 days	1. Reproductive performance, including increased fecundity, egg diameter, and latency period↑; acid profile, particularly total n-3 polyunsaturated fatty acids in the ovaries↑. 2. Health parameters - total hemocyte count, plasma glucose, total protein, cholesterol, and triglycerides↑. 3. Growth performance =.	Arshadi et al., 2018
Nile tilapia	0, 1, 2, 4 and 8 g kg^{-1} diet	60 days	1. Growth performance, feed utilization, haematological and innate immunity↑; 2. Disease resistance against S*treptococcus agalactiae*↑.	Kader et al., 2018
Nile Tilapia	0, 0.05, 0.15, and 0.25%NT in diets	30 days	1. Haematology, antioxidant activity, innate immunity, expression of intestinal cytokines, and disease resistance against *Aeromonassobria*↑	Reda et al., 2018
Rui	0, 5, 10 and 15 g kg^{-1} diet	60 days	1. Nucleotide supplementation at 10 g kg^{-1} - Growth performance and immune response↑; survival offish against *A. hydrophila* disease↑.	Baidya et al., 2015
Hybrid Tilapia	0, 120, 240, 360, 480 and600 mg NT/kg	10 weeks	1. Weight gain and feed efficiency=. 2. Inclusion level of 120–240 mg NT/kg - Immune responses and survival against *S. iniae* infection↑.	Shiau et al., 2015
Nile tilapia	0; 0.5; 1.0; 2.0 and 4.0 g kg	60 days	1. Growth performance, feed utilization, haematological profile and immune response=. 2. Resistance to A. hydrophila↑.	Barros et al. (2015)

The symbol (↑) represents an increase or enhancement in the specified response or a positive effect due to nucleotide supplementation; (=) indicates no change or no significant difference, while (↓) signifies a decrease.

Despite these promising findings, data regarding the dietary inclusion of NT in fish broodstock and its impact on the offspring's quality remain scarce (do Nascimento et al., 2023). Existing studies lack emphasis on the impact of nucleotide supplementation on seed production and reproductive performance (Abo-State et al., 2017). Additionally, there is insufficient data on potential age/size-related responses and certain physiological reactions of fish when exposed to external stressors (Hossain et al., 2019).

A recent field trial conducted by do Nascimento et al., (2023) demonstrated that purified NT supplementation specifically in tilapia broodstock improved early development, antioxidative status, and liver integrity in offspring at 45 and 105 days old. However, this trial was conducted in a greenhouse with controlled water temperature maintained at 28°C using a heater, the optimal temperature for tilapia farming (Romana-Eguia et al., 2020). This controlled environment aided in eliminating the impact of external stressors on the experimental conditions. This opens a chance to examine the knowledge gap regarding the influence of commercially available nucleotide products during the broodstock conditioning period for the tilapia breeders in a farm environment, where external factors like weather and temperature play a role.

In this regard, this study aimed to evaluate the impact of dietary nucleotide supplementation, specifically using Nucleoforce Aqua™, on the broodstock fecundity and fry survivability of tilapia *(Oreochromis niloticus)* under farm conditions. Specifically, this study sought to: (1) assess the effects of dietary nucleotide supplementation on the fecundity of broodstock; (2) evaluate its influence on fry

survivability; and (3) identify practical recommendations for improving tilapia aquaculture practices to achieve higher yields and promote long-term sustainability.

Materials and Methods

Experimental Facilities

The farm trial was conducted at Sta. Maria Tilapia Fish Farm located in Bangyas Calauan, Laguna, where groundwater served as the primary water source. Throughout the experiment, the only water parameter monitored was the water temperature. The entire experiment followed the farm management practices established by the farm owner. The methodologies involved were daily water exchange rate of 20% per pond, and the fish were exposed to the ambient light conditions as part of the farm's standard procedures. The trial was performed in 2021 during the period from 2nd of April to 15th of June under typical farm conditions.

Trial Pond Preparation

Four earthen ponds, each covering an area of 200 m², were utilized for tilapia *(Oreochromis niloticus)* breeding. The water depth in these ponds was maintained at one meter. To prepare for the breeding process, a complete drainage of all four ponds was conducted to capture all existing fish and eliminate potential predators and undesired species. After draining, the pond bottoms were leveled, creating a slope towards the drainage structure to ensure effective water removal. Prior to refilling the ponds, Tea Seeds were employed over a two-day period to eradicate any remaining predators present in the pond ecosystem.

In each earthen pond designated for the feeding trial, six 10 m² hapa net were installed. Additionally, a separate pond was dedicated to the setup of four fine-meshed hapa nets, each measuring 12 m². These smaller hapa nets served as a temporary nursery for the collected fry before they were transferred to the designated nursery tanks.

Experimental Treatment and Feeding Regime

Nucleoforce Aqua™ is a commercially available nucleotide supplement which was developed by Bioiberica in Spain. It contains 34% free nucleotides sourced from inactivated yeast extract, with an 80% pyrimidine and 20% purine content (Bioiberica 2019; El-Nokrashy et al., 2020; Yaseen et al., 2020). The dietary levels of Nucleoforce Aqua™ supplement were applied in accordance with the commercial producer's prescribed rate in the current study.

The dietary nucleotide supplement was manually prepared every feeding. During the preparation, 0.05% (12.5g/25kg) of Nucleoforce Aqua™ per ton of commercial feeds was dissolved in a 500 ml water. The diluted Nucleoforce Aqua™ was then added to the commercial feeds (Tateh feeds), with 36 % crude protein (CP), 5 % crude lipids, 8% fiber, 12% crude ash, and 12% moisture, as shown in table 2. The experimental feeds were sun dried for one hour before feeding.

During the feeding trial, two feeding treatments were employed: Treatment 1 (T1), which consisted of commercial feed supplemented with 0.05% dietary nucleotide, and Treatment 2 (T2), which involved

using only the commercial feed without supplementation, this serves as the control. Each treatment was conducted with a replicate to ensure the reliability and validity of the results. Brood fish were fed manually at a rate of 2% of their body weight twice daily. Broadcast feeding technique was employed during the feeding trial. Continuous feeding was done for 30 days.

Table 2: Composition of Nucleoforce Aqua™ and Tateh feeds

Content	Nucleoforce Aqua™	TATEH
Kjeldahl Nitrogen	11.5%	
Crude Protein (NKj x 6.25)	73%	36%
Moisture	4%	12%
Ash	13.4%	12%
Crude Lipids		5%
Crude Fiber		8%

Experimental Fish and Condition

Tilapia *(Oreochromis niloticus)* broodstock was procured from Sta. Maria Tilapia Fish farm at Bangyas Calauan Laguna. A total of 1,344 apparently healthy tilapia breeders, 224 male and 1,120 female, per pond were used for this experiment. Weight ranges from 150-250 grams each.

The fish were conditioned for seven (7) days prior to the feeding trial. Manual sexing was employed to carefully sort the male and female breeders, which were weighed on their initial stocking. Following the sorting process, the female breeders were placed into four different treatment ponds. Throughout the 7-day conditioning period, the male breeders were exclusively stocked in hapa nets due to their lower quantity compared to the female breeders. During this period, the broodfish were fed twice daily, allowing them to eat until satiation, using the same commercial feed that the farm utilizes. Once the conditioning period concluded, the male breeders were transferred from the hapa nets to the treatment ponds. The breeders were stocked with a sex ratio of 1:5, male and female respectively. During the feeding trial, each treatment pond housed 336 individuals or 56 sets of breeders.

Rearing of larvae

Subsequent to the initial 15-day period, rigorous monitoring was implemented to observe fish spawning within the treatment ponds, focusing on identifying the presence of fry near the perimeters of these ponds. Each day at 7 am during the fry collection routine, scoop nets were employed to gather the fry, promptly transferring them to the designated hapa nets. Following the collection process, the fry were then relocated to the designated nursery ponds.

To facilitate efficient management, two separate concrete tanks, namely FT1 and FT2, were designated for the fry collected from T1 and T2, respectively, and these tanks were intended to function as nurseries for the gathered fry. A manual counting procedure was implemented during each stocking event to ensure accurate quantification. The collected fry from both T1 and T2 were provided with a diet exclusively composed of fry mash for a period of 15 days.

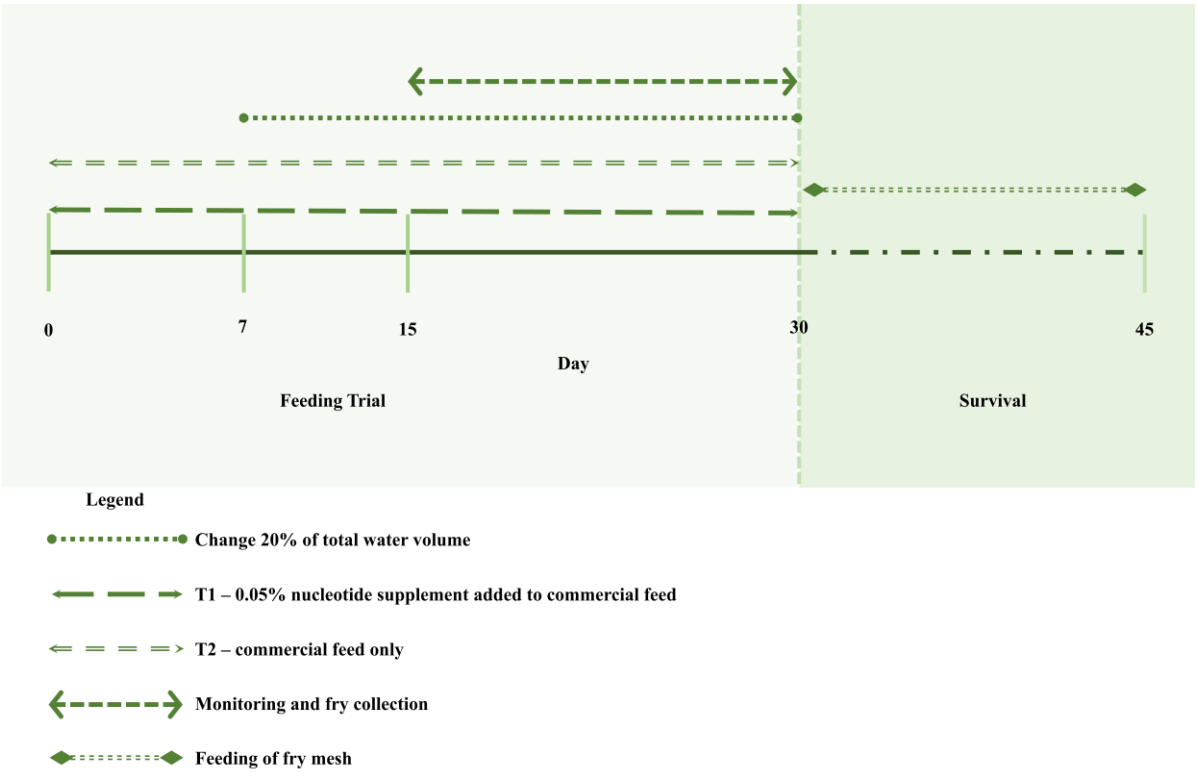

Figure1: Water and feeding management of the pond culture

Data Collection

The data on fecundity in terms of number of fry was obtained by manually counting the fry every stocking. After 15 days of fry mash feeding, the final sampling was done by counting the remaining fry to determine the survival rate for each treatment. It was calculated using the formula below.

$$\textbf{Survival rate (\%)} = \frac{\text{Number of fry after 15 days of rearing}}{\text{Initial Number of fry during stocking}} \times 100$$

Statistical Analysis

Data was analyzed statistically to determine whether the supplementation of 0.05% (12.5g/25kg) dietary nucleotides (Nucleoforce Aqua™) to tilapia *(Oreochromis niloticus)* broodstock fecundity was significant. To assess the significant difference in fecundity across treatments, the gathered data was examined for significant differences ($P<0.05$) using SPSS Independent Samples t-test. The t-test was selected as it is ideal for assessing significant differences between two independent groups, making it the most efficient method for this analysis.

Results and Discussion

Fecundity

The assessment of fish fecundity is significant in aquaculture, as it provides essential data for sustainable practices by determining the reproductive capacity of individual broodfish, including the number of eggs and fry produced (Hossain et al., 2019). This data serves as a foundation for optimizing breeding protocols and ensuring sustainable production levels in aquaculture systems.

In this study, the comparison of tilapia broodstock fecundity, in terms of fry produced was evaluated. The inclusion of dietary NT at 0.05% in the commercial feed (T1) demonstrated a substantial advantage over the control group (T2), which received only commercial diet. As presented in Table 3, T1 exhibited a significantly higher average fry production of 186,000 fry compared to T2's average of 124,000 fry. This notable difference emphasizes the positive impact of nucleotide supplementation on enhancing broodstock fecundity.

Table 3: Fecundity of Tilapia in terms of fry produced

TREATMENT	FRY PRODUCED		TOTAL	TREATMENT MEAN
	R1	R2		
T1 (with nucleotide supplementation)	190,000	182,000	372,000	186,000
T2 (commercial feeds only)	128,000	120,000	248,000	124,000

*R1 and R2 refer to replicate 1 and replicate 2, respectively, as each treatment in the study included two replicates to ensure reliability and accuracy of the results.

From a statistical standpoint, the Independent Samples T-Test results validate the observed differences. The p-values for one-tailed (0.004) and two-tailed (0.008) tests were both below the standard significance threshold of 0.05, confirming a statistically significant effect of dietary nucleotide supplementation on fecundity. These p-values indicate a high degree of confidence that the enhanced fecundity in T1 was directly attributable to nucleotide supplementation, rather than random variation.

Table 4: Result of Independent Samples T-test on Fecundity of Tilapia

Independent Samples Test
t-test for Equality of Means

		t	df	Significance		Mean Difference	Std. Error Difference
				One-Sided p	Two-Sided p		
FECUNDITY	Equal variances assumed	10.960	2.0	.004	.008	62000.000	5656.854
	Equal variances not assumed	10.960	2.0	.004	.008	62000.000	5656.854

These findings align with existing literature on the application of dietary nucleotide supplementation in aquaculture. Studies on various fish species, such as halibut, haddock (Gonzalez Vecino, 2005),

Japanese flounder (Cheng et al., 2011), and Nile tilapia (Hossain et al., 2019) have shown improved fecundity, egg quality as well as larval survival with nucleotide-enhanced diets. Gonzalez-Vecino (2005) further identified improved lipid mobilization during oogenesis as a key mechanism behind this enhanced reproductive performance, reinforcing the broader applicability of these results across diverse aquaculture systems.

Fry Survival

Survival rates in aquaculture are critical indicators of both efficiency and sustainability. In a typical freshwater grow-out farm a low survival rate averaging 55% can be observed (Serd, 2024). In contrast, modern farm that incorporate technological advancements in farm management, employ specialized equipment, and implement stringent biosecurity protocol, reported a survival rate averaging between 70% and 90%, (BFAR, 2022).

Studies by Abu-Elala et al. (2021) and Ringo et al. (2012) have shown that nucleotide-enriched diets can positively impact broodstock productivity and fry health, potentially leading to better survival rates. However, the current farm trial findings as shown in Table 4 were comparable, FT1 have 65% and FT2 was 62% which imply that both treatments gave the same effect on the survival of fry.

Table 5: Survival Rate of Tilapia Fry after 15 days of Fry Mash diet only

TREATMENT	FRY COLLECTED	FRY SURVIVAL	SURVIVAL RATE (%)
FT1 (Fry from T1)	372,000	241,800	65%
FT2 (Fry from T2)	248,000	153,760	62%

Despite the lack of a significant difference on fry survival under high heat index conditions, the farm trial results suggest that dietary nucleotide supplementation could still be a beneficial practice for tilapia farmers in rearing tilapia under favorable environmental conditions.

Moreover, it is vital to highlight that this experiment was conducted during the dry season, adding an important environmental context to our findings. The high heat index in Laguna, Philippines, during the experiment in June 2021, which averaged 37.4°C (99.3°F) according to the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA), could have influenced the survival rate recorded. This is significantly higher than the average heat index for the month of June, which is 33.8°C (92.8°F). Tilapia growth performance and fry survival are known to decline at temperatures exceeding the ideal range of 27-31°C (Romana-Eguia, 2020), potentially affecting the outcomes of the study.

The alignment of our results with existing literature emphasizes the significant role of nucleotide supplementation in strengthening the stress resistance and overall resilience of tilapia, particularly when exposed to challenging conditions, such as sudden temperature changes. It can be observed that a higher immune response or stress response to challenging environments in those supplemented with dietary nucleotide, increasing its survival during the period of exposure to high temperature and other external factors affecting the survival of tilapia fry.

Conclusion

The 30-day farm feeding trial demonstrated that nucleotide supplementation at a 0.05% inclusion level positively influenced broodstock fecundity, suggesting its potential to enhance aquaculture productivity. While survival rates for both treatments were comparable, the increased fecundity in the nucleotide-supplemented group emphasizes the potential benefits of this dietary intervention. The results highlight the adaptability and practical application of nucleotide supplementation in aquaculture systems, particularly in improving broodstock productivity and supporting the immune response tilapia fry.

However, it is important to acknowledge the limitations of this study, particularly the small sample size, the circumstance that it was conducted under a single, extreme farm condition. Further research is needed to assess the effectiveness of nucleotide supplementation under varying environmental factors such as temperature, salinity, and oxygen levels. Expanding studies with larger sample sizes, longer trial durations and across multiple farm sites would provide more reliable data and enhance the applicability of results. Exploration on varying nucleotide inclusion levels would also be essential to optimize supplementation under different conditions. Additionally, conducting a cost-benefit analysis is crucial to ensure that this supplementation approach remains economically viable for aquaculture farms in the long term.

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Declaration of Interest Statement

The authors declare that they have no conflict of interests.

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Appendix

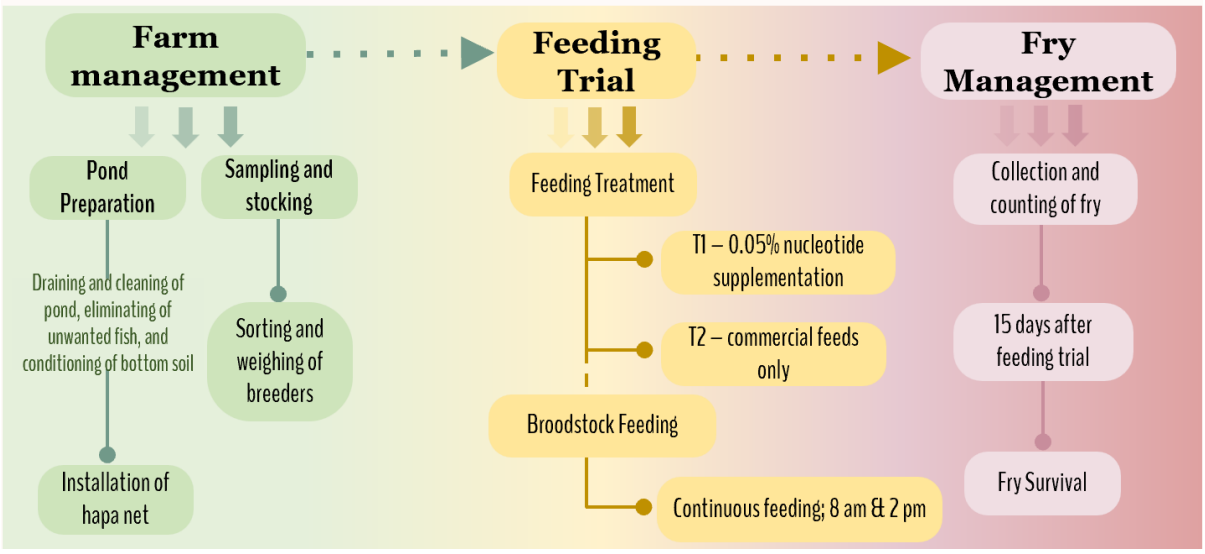

Figure 2: Schematic diagram of the method used during the study.