Comparative evaluation of animal-based oils, fish oil, poultry oil, and pork oil as a lipid source in Larval performance of Anabas koi (*Anabas testudineus, Bloch 1792*)

P. Gokulnath[1*], S. Aanand[2], S. Athithan[3], P. Velmurugan[4]

[*1] PG Scholar, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi, India- 628 008.

[2] Assistant Professor and Head, Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Bhavanisagar, Erode, India - 638451.

[3] Professor and the Head of Department, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Department of Aquaculture, Fisheries College and Research Institute, Thoothukudi, India- 628 008.

[4] Assistant Professor and Head i/c, Theni Centre for Sustainable Aquaculture, Theni CeSA, Theni Dt.

Corresponding author information:

Corresponding Author Name: P. Gokulnath

Affiliation of Corresponding Author:

* PG Research Scholar, Fisheries College & Research Institute, Thoothukudi.

ABSTRACT

The present investigation focused on evaluating the influence of animal-based lipid sources incorporated into Black Soldier Fly (BSF) meal-based diets on the nursery performance of *Anabas testudineus*. Eight experimental diets were designed using fish oil, poultry oil, and pork oil at 5% and 7% inclusion levels (T1–T6), together with two rapeseed oil controls: one without vitamin E (C1) and another with vitamin E (C2). Nursery performance parameters, including larval length, body weight, specific growth rate (SGR), and survival, were examined. Significant differences ($p < 0.05$) were recorded across treatments. In this trial, T2 showed the greatest increase in final length (25.82 ± 0.18 mm), weight (201.85 ± 0.74 mg), and SGR (13.57 ± 0.02%/day), followed by T4, while the lowest values occurred in C2. Survival was highest in T4 (2,297 ± 81 fry) and lowest in C1. The better performance of C2 compared to C1 confirmed the antioxidant contribution of vitamin E during the nursery stage. Overall, 7% fish oil was the most effective lipid source, while 7% poultry oil also emerged as a promising substitute in BSF-based diets to improve larval performance of *A. testudineus*.

Keywords: Anabas, BSF-meal, Animal Lipids, Nutrition, Larval rearing

1.INTRODUCTION

Aquaculture has become a critical industry for global food security, particularly as demand for seafood continues to rise. Among the various species cultivated, *Anabas testudineus*, commonly known as the climbing perch, is of increasing importance due to its adaptability to diverse environmental conditions and its nutritional value. One of the most crucial factors influencing the success of aquaculture operations is the nursery phase, where early-life nutrition can significantly impact the growth, survival, and long-term productivity of farmed species. Recent advancements in aquaculture nutrition have emphasized the role of dietary lipids, particularly animal-based oils, in supporting larval development and enhancing survival rates. (Ansari et al., 2021). Lipids not only serve as energy sources but also provide essential fatty acids that are critical for the development of cellular membranes and overall health. (Priyadarshana et al., 2021).

This study investigates the comparative effects of various animal-based oils, including fish oil, poultry oil, and pork oil, incorporated into Black Soldier Fly (BSF) meal-based diets on the larval performance of *Anabas testudineus* during the nursery phase. The experiment evaluates several key parameters, such as growth rate, survival, and specific growth rate (SGR), to determine the most effective lipid source for promoting optimal performance during early life stages (Tran et al., 2022). While fish oil has been traditionally regarded as a high-quality lipid source for aquaculture feeds due to its rich content of long-chain polyunsaturated fatty acids (LC-PUFAs), alternative sources like poultry and pork oils are gaining attention for their potential cost-effectiveness and sustainability. These alternatives may offer viable solutions in reducing reliance on marine-based resources, which are often subject to supply uncertainties and environmental concerns (Rana et al., 2015).

Furthermore, the inclusion of antioxidants such as vitamin E has been shown to mitigate oxidative stress, which can otherwise limit the efficacy of lipid sources in fish diets (Infante-Villamil et al., 2021). The role of antioxidants in enhancing the nutritional quality of feeds and improving the robustness of larvae is well-documented (ICAR-CIFA, 2016). This study aims to fill the gap in knowledge regarding the comparative efficacy of these animal-derived lipid sources and their interactions with antioxidant supplementation, specifically in the context of *Anabas testudineus* larval rearing.

The findings of this research hold the potential to inform future dietary formulations for *Anabas testudineus* aquaculture, providing a foundation for more sustainable and cost-efficient practices in nursery rearing. By focusing on lipid quality and its impact on early growth and survival, this study contributes to the broader field of aquaculture nutrition, where optimizing early-life nutrition is key to enhancing productivity and sustainability

2. MATERIALS AND METHODS

This study examined the influence of various animal-derived lipid sources incorporated into BSF-based diets to determine the most effective option for nursery performance and larval rearing of Anabas koi.

2.1 Nursery rearing of Anabas koi under respective dietary treatments

2.1.1 Experimental Design

The experiment was carried out in biofloc-based circular tanks, each measuring 3 m in diameter and 1 m in height. The design included six dietary treatments along with two controls: one without vitamin E (C1) and another supplemented with vitamin E (C2). Each treatment was replicated, resulting in a total of 16 tanks. Before use, the tanks were thoroughly cleaned with detergent, sun-dried, rinsed, and then filled with fresh water (Fig. 1).

2.1.2 Experimental Animals

Freshly hatched Anabas koi larvae obtained from induced breeding tanks were initially acclimated in small containers provided with mild aeration. Once the fry reached the free-swimming stage (Fig. 2), they were transferred into nursery tanks at a uniform stocking density of 100 individuals per tank (Fig. 3). The rearing phase continued for 21 days, during which the fry were raised until the fingerling stage. Weekly sampling was performed to monitor growth. At each interval, five fish were randomly collected from every tank and measured for total length (cm) and body weight (g) (Figs. 11, 12). These data were subsequently used to evaluate larval growth performance across the dietary treatments.

2.1.3 Preparation of Experimental Diets

Feed ingredients were initially ground, sieved, and sun-dried to reduce their moisture content (Fig. 4). The respective treatment lipids were incorporated (Figs. 9–12), and the mixtures were homogenized using a high-speed blender. The powdered diets were standardized to maintain 10% moisture content, ensuring their stability and shelf life. Standard procedures were followed to analyze the proximate composition of the diets, including crude protein, crude lipid, fiber, ash, gross energy, and moisture. The feed ingredients and their proximate composition are summarized in Tables 3 and 4.

2.1.4 Feeding Regime

During the initial stage, larvae were fed daily at 10% of their body weight, divided into three equal meals over 21 days. As the larvae increased in size, the feeding rate was gradually adjusted relative to biomass to minimize overfeeding and to maintain water quality. Feeding was carried out manually, with close monitoring to avoid wastage (ICAR-CIFA, 2016).

2.1.5 Length–Weight Measurement in Nursery Rearing

Throughout the nursery phase, fry were sampled once every week (Figs. 13, 14). From each tank, five individuals were randomly collected with a hand net and lightly anesthetized using tricaine methane sulfonate (MS-222) at 50 mg/L (Carter et al., 2011) before measurements were taken. Total length (cm) was determined using a digital caliper, while body weight (g) was recorded with an electronic balance (Figs. 11, 12). After sampling, the fry were subjected to a mild potassium permanganate ($KMnO_4$) dip to minimize stress before being returned to their respective tanks. At the conclusion of the 21-day trial, survival (%) was estimated based on the number of fish remaining. Growth performance was evaluated using the Specific Growth Rate (SGR) following Froese (2006), as presented in Table 1.

$$SGR\ (\%/day) = [(ln\ Wf - ln\ Wi)/days] \times 100$$

where ln Wf is the natural logarithm of final weight and

ln Wi is the natural logarithm of initial weight.

The recorded data were used to compare growth performance under different dietary treatments (ICAR-CIFA, 2016).

2.2 Water Quality Assessment

Throughout the experimental period, water quality in the rearing tanks was monitored at two-week intervals. Parameters such as water temperature, dissolved oxygen (DO), pH, ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), inorganic phosphate (PO_4), free carbon dioxide (CO_2), total alkalinity, total hardness, total dissolved solids (TDS), total suspended solids (TSS), and electrical conductivity (EC) were measured following the standard protocols outlined by APHA (2005). The mean values of these water quality parameters obtained during the trial are presented in Table 2.

2.3 Statistical Analysis

The experimental data collected were subjected to statistical analysis using one-way ANOVA in the Statistical Package for the Social Sciences (SPSS, version 20.0) to determine significant differences at the 5% probability level. Post-hoc comparisons among treatment means were carried out using Duncan's Multiple Range Test (DMRT) (IBM SPSS Statistics for Windows, version 20.0; Armonk, NY: IBM Corp.).

3. RESULTS

Nursery rearing performance of Anabas koi

The results of the nursery rearing trial are presented in Table 1. All measured growth and survival parameters exhibited statistically significant differences ($p < 0.05$) among the treatments, indicating that dietary lipid source had a pronounced effect on larval development and viability. The comparison of nursery rearing parameters across different dietary treatments is graphically represented in Figures 15, 16. Survival rate, a primary indicator of hatchery performance and larval robustness, differed significantly ($p < 0.05$) among dietary treatments. The highest survival was observed in T4 (Poultry oil 7%) 55.3 ± 1.2%, followed by T6 (Pork oil 7%) 52.7 ± 1.0% and T2 (Fish oil 7%) 42.8 ± 1.3%, all of which outperformed the control groups. In contrast, T3 (Poultry oil 5%), 30.4 ± 1.6% and T1 (Fish oil 5%), 41.8 ± 1.4% showed lower survival rates. The lowest survival was recorded in C1 (Control without vitamin E), 33.7 ± 1.1%, indicating the importance of both lipid and antioxidant supplementation in promoting larval robustness.

In terms of final body weight, larvae from T2 (Fish oil 7%) again showed the highest ($p < 0.05$) mean value (201.85 ± 0.74 mg), significantly outperforming all other treatments. The next highest weights were seen in T4 (Poultry oil 7%), 165.45 ± 0.63 mg, and T6 (Pork oil 7%), 154.60 ± 0.51 mg. T3 (Poultry oil 5%) 98.75 ± 0.48 mg, T5 (Pork oil 5%) 115.90 ± 0.58 mg, and T1 (Fish oil 5%) 131.20 ± 0.47 mg showed moderate gains, while the lowest final weight was in C1 (Control without vitamin E) 92.35 ± 0.48 mg, indicating the limited nutritional adequacy of the basal feed.

The highest SGR was recorded in T2 (Fish oil 7%) 13.57 ± 0.02%/day, followed by T4 (Poultry oil 7%) 12.60 ± 0.03%/day, T6 (Pork oil 7%) 12.23 ± 0.04%/day, and T1 (Fish oil 5%) 11.65 ± 0.03%/day. The lowest growth rates were found in T3 (Poultry oil 5%), 10.70 ± 0.05%/day, and C1 (Control without vitamin E),

10.66 ± 0.14%/day, emphasizing the metabolic importance of both lipid quality and vitamin E in early fish development. The number of surviving fry, which integrates survival rate and stocking density, also differed significantly ($p < 0.05$) between treatments. T4 (Poultry oil 7%) 2,297 ± 38 and T2 (Fish oil 7%) 2,294 ± 31 recorded the highest surviving fry counts, followed by T6 (Pork oil 7%) 1,035 ± 28. The lowest surviving fry count was observed in C1 (Control without vitamin E), 131 ± 13, supporting the conclusion that both lipid supplementation and antioxidant inclusion are essential during larval rearing.

Among the tested lipids, T2 (Fish oil 7%) proved most effective, likely due to its superior fatty acid profile and bioactive lipid fractions essential for larval growth and survival. T4 (Poultry oil 7%) and T6 (Pork oil 7%) also supported good performance, suggesting their potential for partial replacement strategies. In contrast, the control diet (C1), devoid of BSF and lipid enrichment, was markedly inferior across all parameters, highlighting the importance of both high-quality protein and lipid inclusion during early larval stages.

4. DISCUSSION

The nursery period has a significant impact on long-term survival and productivity in *Anabas testudineus* aquaculture. In the treatments, T2 (Fish oil 7%) had a considerably higher final weight (201.85 mg) and specific growth rate (13.57%/day) due to the presence of long-chain polyunsaturated fatty acids (LC-PUFAs), specifically DHA and EPA. These essential fatty acids have been shown to improve brain development, cellular membrane fluidity, and energy usage in early life stages of fish (Hamre et al. 2013; Rainuzzo et al. 1997). Earlier studies have demonstrated similar enhancements in larval performance across both freshwater and marine taxa in response to adequate n-3 HUFA supplements. (Tocher 2010; ICAR-CIFA 2016). In T4 (poultry oil 7%) treatment had the best survival rate (55.3%), indicating its potential as an effective and sustainable alternative lipid source during the larval stage. Although poultry fats lack a HUFA profile, the enhanced survival may be attributed to the high caloric content and digestibility of poultry lipids (Gonçalves and Cyrino 2014). The synergistic inclusion of poultry and microalgal oils in the diet had compensated for HUFA deficiencies in marine finfish larvae, showing successive results in nursery feed formulations (Carvalho et al. (2020).

Studies on *A. testudineus* larviculture highlight the importance of nutritional balance, appropriate feeding regimes, and water quality in determining post-hatch survival and deformity rates (Amornsakun et al. 2005). Due to the limited availability of essential fatty acids in saturated animal lipids, pork oil–based diets (T6) supported moderate growth, and lower survival. The use of black soldier fly (BSF) meal as a protein source, together with functionally balanced lipid sources with antioxidants, has shown promising results in enhancing early larval development. Hence, fish oil at 7% (T2) remains the most efficient for encouraging development, while poultry oil 7% in T4 offers an alternative source for enhancing survival and lowering feed costs in *A. testudineus* nursery diets. (Belghit et al. 2019; Makkar et al. 2014; Ahmed et al. 2015).

5. CONCLUSION

This research highlights the critical importance of dietary lipid sources in improving larval performance of *Anabas testudineus.* Diets containing 7% fish oil (T2) yielded the greatest gains in growth and survival, largely due to their rich composition of long-chain polyunsaturated fatty acids (LC-PUFAs). Poultry oil at 7% (T4) proved to be a promising and sustainable substitute, showing moderate success in nursery growth and fry survival. In

contrast, the use of pork oil resulted in comparatively poor outcomes, underscoring its limited nutritional value. Supplementation with vitamin E played a key role in reducing lipid oxidation and further improving nursery performance. Overall, these results emphasize the potential for formulating nutritionally balanced, economical, and sustainable aquafeeds that minimize dependence on marine-based resources while supporting effective hatchery practices for freshwater fishes.

6. CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

7. STATEMENT AND DECLARATIONS

The experiment was conducted at the Erode Bhavanisagar Centre for Sustainable Aquaculture (EBCeSA), Bhavanisagar, Erode District – 638451, Tamil Nadu, India. All procedures involving experimental animals were carried out in compliance with the guidelines for animal care and ethics established by Tamil Nadu Dr. J. Jayalalithaa Fisheries University (TNJFU). To minimize stress prior to dissection, fish were anesthetized using tricaine methane sulfonate (MS-222) at a concentration of 40 mg/L (Topic Popovic et al., 2012).

The authors confirm that there are no financial conflicts of interest or personal associations that could have influenced the findings presented in this study.

8. AUTHOR CONTRIBUTIONS

Conceptualization: P Gokulnath, S Aanand; Methodology: P Gokulnath, S Aanand; Conduction of Research: P Gokulnath, S Aanand; Writing – original draft preparation: P Gokulnath; Writing – review and editing: P Gokulnath, S Aanand, S Athithan, P Velmurugan; Supervision: P Gokulnath, S Aanand, S Athithan, P Velmurugan.

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10. DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are not publicly available due to institutional restrictions, but are available from the corresponding author on reasonable request due to confidential information.

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Fig. 1

Fig. 2

Fig. 3

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Fig. 9

Fig. 10

Fig. 11

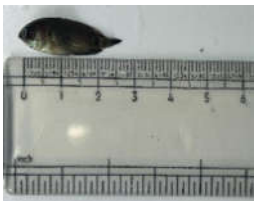

Fig. 13

Fig. 14

Fig.1 - Cleaning and setting up of tanks / Fig.2 - 5 day old fry of Anabas koi / Fig.3 - Fry stocked into nursery pond / Fig.4 - Seiving and weighing of feed ingredients / Fig.5, 6, 7 - Supplementation of Animal lipids in fish feed / Fig.8 - Supplementation of Vit-E in fish feed / Fig. 9,10 - Feed preparation / Fig. 11,12 – Length, weight measurement of fry / Fig. 13,14 – Sampling of Nursery rearing fry.

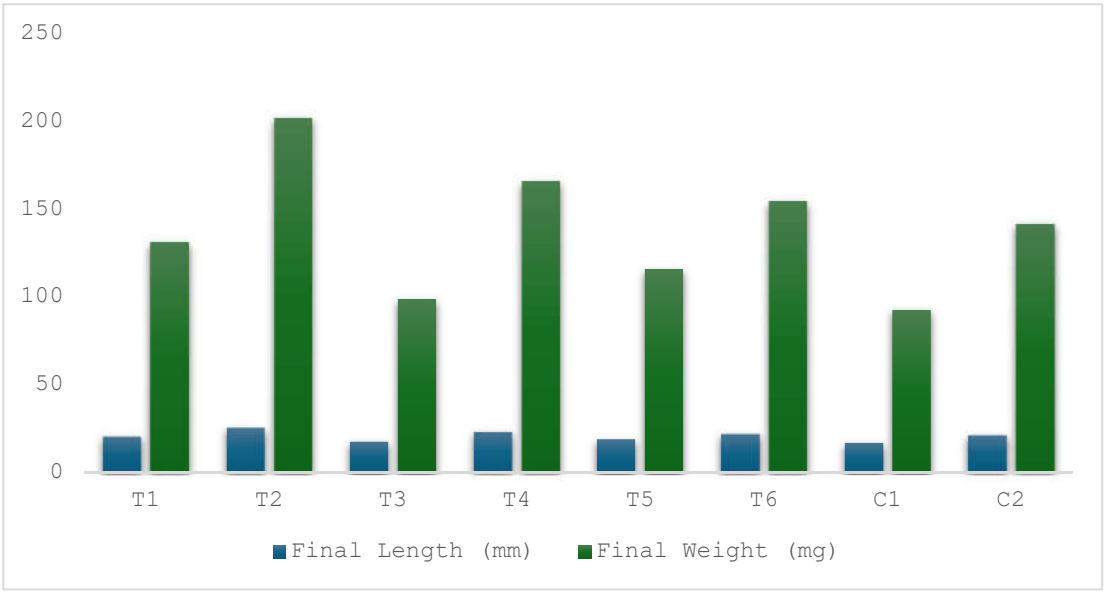

Fig. 15 Nursery rearing performance of Anabas koi

a,b,c,d values on error bar differ significantly (p <0.05)

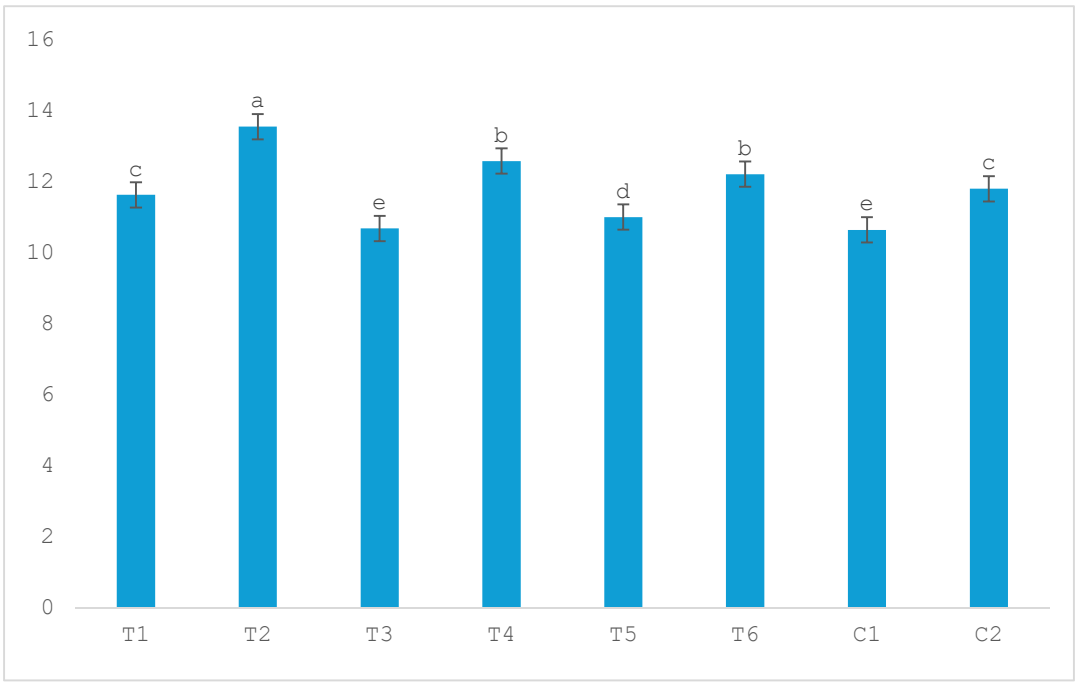

Fig. 16 SGR (%/day) in Nursery rearing of Anabas koi

a,b,c,d values on error bar differ significantly (p <0.05)

Different superscript letters within a row indicate significant differences among treatments based on one-way ANOVA followed by Duncan's multiple range test (p < 0.05).

Table 1. Nursery rearing parameters of experimental fishes

Treatment	Initial Fry	Final Length (mm)	Final Weight (mg)	SGR (%/day)	Survival (%)	Surviving Fry
T1	2,529 ± 114	20.85 ± 0.13^{c}	131.20 ± 0.47^{c}	11.65 ± 0.03^{c}	41.8 ± 1.4^{c}	1,057 ± 67^{c}
T2	5,360 ± 214	25.82 ± 0.18^{a}	201.85 ± 0.74^{a}	13.57 ± 0.02^{a}	42.8 ± 1.3^{c}	2,294 ± 96^{a}
T3	1,803 ± 97	17.92 ± 0.28^{e}	98.75 ± 0.48^{e}	10.70 ± 0.01^{e}	30.4 ± 1.6^{d}	548 ± 41^{e}
T4	4,156 ± 162	23.40 ± 0.15^{b}	165.45 ± 0.63^{b}	12.60 ± 0.03^{b}	55.3 ± 1.2^{a}	2,297 ± 81^{a}
T5	1,212 ± 82	19.25 ± 0.25^{d}	115.90 ± 0.73^{d}	11.02 ± 0.02^{d}	38.2 ± 1.5^{c}	463 ± 39^{d}
T6	1,964 ± 88	22.35 ± 0.27^{b}	154.60 ± 0.82^{b}	12.23 ± 0.04^{b}	52.7 ± 1.1^{b}	1,035 ± 56^{b}
C1	388 ± 30	17.10 ± 0.26^{e}	92.35 ± 0.52^{e}	10.66 ± 0.01^{e}	33.7 ± 1.2^{d}	131 ± 12^{f}
C2	1,122 ± 76	21.65 ± 0.16^{c}	141.70 ± 0.39^{c}	11.82 ± 0.03^{c}	51.5 ± 1.2^{b}	578 ± 38^{c}

*Means with different superscripts in the same column differ significantly ($p < 0.05$) by Duncan's Multiple Range Test (DMRT). Values are expressed in terms of Mean ±SD.

Where T1 - Fish oil 5%, T2 - Fish oil 7%, T3 - Poultry oil 5%, T4 - Poultry oil7%, T5 - Pork oil 5%, T6 - Pork oil 7%, C1 – Control without Vit E, C2 – Control with Vit E.

Table 2. Water quality parameters.

The results of water quality analysis at every fortnight intervals are given below in (mean ± SD).

Si.No	Parameter	Nursery rearing (mean ± SD)
1.	pH	8.25 ± 0.05
2.	Temperature (°C)	25.84 ± 1.07
3.	Dissolved Oxygen (ppm)	7.81 ± 0.58
4.	Total Alkalinity (ppm)	102.36 ± 18.92
5.	Total Dissolved Solids (ppm)	0.46 ± 0.11
6.	Total Suspended Solids (ppm)	0.72 ± 0.48
7.	Total Hardness (ppm)	229.14 ± 15.26
8.	Free CO_2 (ppm)	3.58 ± 1.43
9.	Ammonia (ppm)	0.49 ± 0.15
10.	Nitrite (ppm)	0.21 ± 0.02
11.	Nitrate (ppm)	4.12 ± 0.64
12.	Phosphate (ppm)	2.18 ± 0.42

13.	Electrical Conductivity (µS/cm)	0.51 ± 0.07

Where ppm - parts per million.; µS/cm - microsiemens per centimeter, ◦C - degree Celsius.

Table 3. Feed ingredients and the composition of Anabas koi.

The required feed ingredients for experimental diets for 1kg are given below.

Ingredients	C1	C2	T1	T2	T3	T4	T5	T6
Fish meal (g)	270	270	270	270	270	270	270	270
BSF larval meal (g)	180	180	180	180	180	180	180	180
Soyabean meal (g)	120	120	120	120	120	120	120	120
Rice bran (g)	250	250	250	250	250	250	250	250
Cassava starch (g)	150	150	150	150	150	150	150	150
Vit and Min mix (g)	10	10	10	10	10	10	10	10
Vit – E (mg/kg)	0	100	100	100	100	100	100	100
Rapeseed oil (ml)	20	20	20	20	20	20	20	20
Fish oil (ml)	0	0	50	70	0	0	0	0
Poultry oil (ml)	0	0	0	0	50	70	0	0
Pork oil (ml)	0	0	0	0	0	0	50	70

Table 4. Proximate composition analysis

The results of proximate analysis of different experimental diets are given below.

Treatment	Protein (%)	Lipid (%)	Fibre (%)	Moisture (%)	Ash (%)
T1	33.49	7.65	6.46	10.73	10.32
T2	33.65	9.33	6.92	10.52	10.43
T3	32.47	6.93	6.13	10.27	10.62
T4	32.65	8.72	6.67	9.84	10.72
T5	31.49	6.7	6.26	9.46	11.05
T6	33.07	8.23	6.58	10.35	10.61
C1	31.07	3.40	5.38	11.20	9.36
C2	33.47	3.54	5.56	11.32	9.12