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The Effect of Dietary Protein Level and Amino Acid Supplementation on the Nile Tilapia (*Oreochromis niloticus*) Nursering Performance Under Biofloc System Conditions at a Cold Suboptimal Water Temperature

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ABSTRACT

This study was conducted to evaluate the effect of dietary protein level and amino acid supplementation on tilapia nursering performance under biofloc system conditions at a cold suboptimum temperature. Four experimental diets were organized; 30% CP diet as positive control (30P), 22% CP diet as negative control (22P), 22% CP diet supplemented with 1% lysine (22PL) and 22% CP diet supplemented with 0.5% threonine (22PT). Three replicates of each treatment were tested for each formulation of diet under the conditions of the biofloc system. 12 experimental tanks (1000L) were used, each of which was filled with 400 L and stocked with 50 fries (6.20±0.01 gm/fish). The experiment extended for 97 days. The weight gain (WG), feed intake (FI), feed conversion ratio (FCR), and specific growth rate (SGR) of fish fed the 22PT diet had the highest significant values (11.10, 16.95, 1.55, and 1.40, respectively). Furthermore, the 22PT diet groups showed the highest wet whole-body and dried microbial floc protein contents. While, the fish groups fed the 22PT diet had the lowest total ammonia nitrite (TAN) values. Therefore, when tilapia fish are subjected to nursering in cold, suboptimal water, a low-protein diet enhanced with threonine can boost the biofloc system's performance.

INTRODUCTION

The global aquaculture should witness an increase in its annual growth rate to mitigate the shortage in protein food supply, particularly in the developing countries **(Subasinghe, 2005; Gutierrez-Wing & Malone, 2006; Matos *et al.*, 2006)**. To determine the aquaculture potential of a species, studies should be conducted to manage the culture conditions for optimizing growth under different production systems, including fish farming and integrated agri-aquaculture systems **(Deacon, 1997; Suloma & Ogata, 2006; Kimera *et al.*, 2021a, b)**. Water quality can be improved with biofloc technology (BFT), which involves adding a carbon source to the production units to encourage the growth of heterotrophic bacteria that uptake nitrogen. Hence, the ammonium concentration would decrease more rapidly than nitrifying bacteria

(Widanarni *et al.*, 2012; Hargreaves, 2013). By keeping the water quality in the culture unit at a suitable level, BFT reduces the amount of water used in aquaculture units. As a result, it generates inexpensive, highly protein-rich bioflocs that aquatic organisms can eat **(Crab *et al.*, 2007, 2009, 2010; El-Shafiey *et al.*, 2018; Mabroke *et al.*, 2021; Suloma *et al.*, 2021)**. Moreover, According to **Avnimelech *et al.* (1999)**, single cell proteins derived from inexpensive carbon and nitrogen sources can partially replace the pricey commercial protein sources found in aquaculture feeds. Compared to the non-BFT ponds fed with 30% crude protein, **Avnimelech (2011)** assessed that the highest rates of growth performance and protein utilization were recorded for the tilapia reared under BFT ponds and fed on 20% crude protein. **Azim and Little (2008)** noted that, fish fed 24% CP under BFT condition showed higher growth rate compared to fish fed 35% and reared in clear-water conditions. In addition, **Khalil *et al.* (2016)** detected no significant differences in the growth performance rates of Keeled mullet (*Liza carinata*) fish fed on 25% CP, 30% CP and 35% CP diets under biofloc system. Similarly, when *L. vannamei* was raised in unfiltered pond water and fed on 25% and 35% CP, **Tacon *et al.* (2002)** found no significant differences in the growth performance. Additionally, no significant differences were detected between the specific growth rates of *P. monodon* fed on 25% CP and that fed 40% CP in an extensive shrimp culture system or biofloc system **(Hari *et al.*, 2004)**.

The perfect temperature range for the Nile tilapia is from 26 – 30°C. Given that the ideal temperature for raising *O. niloticus* is 28°C, raising this species at a cold, suboptimal temperature of 22°C resulted in a decrease in feed utilization, which was followed by the detection of a critical decline in fish development **(Azaza *et al.*, 2008)**. In general, the temperature, feeding, and voluntary development, as well as the lethal temperature, are all significantly affected by hereditary qualities and nutrition **(Abdel-Ghany *et al*., 2019)**. On the other hand, **Crab *et al.* (2009)** investigated the effectiveness of BFT on maintaining good water quality in over-wintering ponds for the tilapia as a biological approach to overcome its problems rapidly, with respect to the nursery phase. **Boyd (1998)** and **Wilen *et al.* (2000)** reported that, temperature affects the dissolved oxygen in water and added that both microbial community and the cultured species affect fish development.

Therefore, this study was conducted to address the impact of the interaction between reducing the dietary protein level and the supplementation of lysine and threonine on water quality and growth performance of the Nile tilapia (*Oreochromis niloticus*) under the biofloc condition system at cold suboptimum temperature during the nursery phase.

MATERIALS AND METHODS

This study was carried out at Cairo University's Faculty of Agriculture's Fish Nutrition Lab in the Animal Production Department.

Experimental design and diets

The Nile tilapia (*Oreochromis niloticus*) specimens were obtained from a private hatchery, Kafr El-Sheikh Governorate, Egypt, and were transported alive in tanks to the Fish Nutrition Lab. The fries were acclimatized for two weeks in two 3m^3 tanks. Four experimental diets were addressed as follows: 30% CP diet forming the positive control (30P), 22% CP diet performing the negative control (22P), 22% CP diet supplemented with 1% lysine (22PL), and 22% CP diet supplemented with 0.5% threonine (22PT). Three replicates of each treatment were tested for each formulation of diet under the conditions of the biofloc system. No artificial light was used in the greenhouse. Every 15 days, fish in each replicate tank were weighed, and the amounts of daily diet and carbon source (starch) were readjusted according to the fish weight throughout the experiment that extended for 97 days.

The experimental diets were formulated using soybean, fish meal, gluten, yellow corn, and sunflower oil ingredients. They were supplemented with amino acids (Lysine and Threonine), vitamins and minerals. The compositions of the diets (% dry matter bases) including their ingredients and nutrients are presented in Table (1). The dry ingredients were combined into a uniform blend for each of the diets that were tested. After that, sunflower oil was added, and to get uniformly sized pellets, a small mincer with a narrow breadth was used. The result was then kept in storage at 8ºC until it was put into tanks.

Experimental conditions

Twelve experimental tanks of 1000L were used, each of which was filled with 400L of water. Each tank was filled with a stock of 50 fries (6.20±0.01gm/ fish). The tanks were aerated by air stones and concentrated with 0.5HP ring blower to maintain oxygen rate at 5-6 mg/l. Tanks were stored under an enclosed- structure greenhouse and covered by polycarbonate sheets without using heaters.

The heterotrophic bacteria were activated by the daily addition of starch, which was utilized as a carbon source to keep the C/N ratio at 1:10 **(Avnimelech, 1999)**. Starch was completely mixed with water cultured tank in a beaker before spreading to tank surfaces at day time. Carbohydrate under the characteristic light and aeration conditions are the most reasonable factors that sustain biofloc development and improvement **(Azim & Little, 2008)**. Fish were fed twice per day at 9am and 5pm, and biofloc volume was measured twice a week (Monday and Thursday).

Water quality

Values of water temperature and pH were determined using Lovibond® Tintometer® water testing device and Milwaukee ph600 pocket pen. Total suspended solids, TAN (NH_3-N), nitrite (NO_2-N) and nitrate (NO_3-N) values were recorded weekly using Lovibond® Multidirect device. Moreover, the Biofloc volume was measured weekly after 15-20 minutes of sedimentation using Imhoff cone **(Avnimelech & Kochba, 2009)**. Alkalinity was measured by titration with sulphuric acid (0.02 N) against the sample solution (50ml) until the pH value reaches 4.5 **(Boyed & Tucker, 1992)**.

Growth parameters

SGR (%) = [ln(FW) – ln(IW)/ N] ×100, fish (n = 50) of each replicate was weighed every fifteen days to estimate the growth parameters, including weight gain (%), feed conversion ratio (FCR) and specific growth rate (%) (SGR). Weight gain (%) = (FW–IW), feed given (DW)/body weight gain (WW), where FW is final weight; IW is initial weight; DW is dry weight; WW is wet weight; ln is natural log, and N is the number of culture days; all were the methods used to evaluate the growth of fish under study.

Table 1. Formulation and proximate composition (% dry weight basis) of the experimental diets

Ingredient (%)	30P	22P	22PL	22PT
Soyabean meal[a]	36	22.8	22.8	22.8
Fish meal[b]	12	7.6	7.6	7.6
Gluten[c]	6	3.8	3.8	3.8
Corn[d]	36.9	56.7	55.7	56.2
Vegetable oil[e]	6	6	6	6
Minerals & Vitamins[f]	2	2	2	2
Salt[g]	0.5	0.5	0.5	0.5
Vitamin C[i]	0.05	0.05	0.05	0.05
PHT[j]	0.05	0.05	0.05	0.05
CMC[k]	0.5	0.5	0.5	0.5
Lysine[l]	----	----	1	----
Threonine[m]	----	----	----	0.5%
Total %	100	100	100	100
Diet composition				
Moisture (%)	11.60	8.60	7.98	7.03
Protein (%)	31.5	23.2	23.0	23.1
Lipids (%)	9.59	5.22	5.44	5.82
Ash (%)	6.95	2.31	2.42	3.80
Total carbohydrates[n]	40.36	59.17	55.86	61.25
Gross energy (kcal/kg)[o]	4360.58	4359.02	4442.28	4357.18

a; Soyabean meal, Food Technology Research Institute, Ministry of Agriculture, Giza, Egypt; **b;** Fish meal **c;** Gluten (60-63% P) Al-Ahram for food industries **d;** imported yellow corn from Argentina **e;** Vegetable oil commercial food-grade **f;** Minerals + vitamins, multimix, all essential vitamins + minerals for layer fatting feed **g;** Salt commercial food-grade **I;** Vitamin C L(+) ascorbic acid $C_6H_5O_6$; M=176.13 gm/mol POCHSA- POLAND **j;** PHT Butylated Hydroxy Toluene 99% **k;** CMC carboxy methyl cellulose sodium sail (high viscosity)laboratory reagent Oxford Lab Chem **l;** Lysine **m;** Threonine, **n**; total carbohydrate content was determined by the difference: total carbohydrate=100−(% crude protein+% crude fat+% total ash+%moisture); **o;** dietary gross energy was calculated using the conversion factors of 5.6, 9.4, and 4.2 kcal/kg for protein, lipids and carbohydrates, respectively (Hepher et al., 1983).

Proximate composition

The proximate composition of fish, diets and floc meal samples was defined after completing the experiment, following the method of **AOAC (1995)**. The moisture content was recorded by drying the samples at 105°C (Binder oven, E series 28, Germany) to a constant weight, and the difference in the sample weight constitutes its moisture content. Ash content was determined by incinerating the samples in a muffle furnace at 600°C for 3h. Total carbohydrate content was assessed following the successive equation: total carbohydrate = (100 − % crude protein + % crude fat + % total ash + % moisture). Crude lipid was determined by soxhlet extraction using ether (boiling point, 40–60°C) as a solvent. Furthermore, crude protein was analyzed using the method of Kjeldahl **(AOAC 2016)**.

Table 2. Essential amino acids content of the experimental diets

Amino acid	30P	22P	22PL	22PT	Tilapia requirement*
Arginine	1.85	1.17	1,17	1.17	1.18
Histidine	0.76	0.51	0.51	0.51	0.48
Isoleucine	1.30	1.19	1.19	1.19	0.87
Leucine	2.80	2.1	2.1	2.1	0.95
Lysine	1.76	1.14	2.14	1.14	1.45
Methionine & Cystine	1.13	0.65	0.65	0.65	0.90
Phenylalanine	1.50	1.04	1.04	1.04	1.05
Threonine	1.10	0.87	0.87	1.37	1.05
Valine	1.70	1.20	1.20	1.20	0.78

* **Santiago and Lovell (1988)**

Live food production (zooplankton)

Successful larvae culture requires sufficient live food resources such as zooplankton, as a starter food in hatcheries. The BF system promotes the production of these zooplankton communities, and the densities of these organisms depend on the level of protein and volume of the biofloc. For the estimation of the densities of zooplankton in different treatments, five liters of water were filtered from the subsurface layer of each site through a standard plankton net with 55µm mesh size. The collected samples were preserved immediately in plastic jars using 5% formalin solution. A sub-sample of one milliliter was brought into the lab and examined using a binocular compound Olympus microscope in a counting cell (Rafter Sedwick Cell). For each sample, this process was carried out thrice, and the average was calculated using the following equation **(APHA, 2005)**

$$\text{No./m}^3 = \frac{C \times V'}{V'' \times V'''}$$

Where;
C = average number of organisms counted
V ′ = volume of the concentrated sample, mL
V ″ = volume counted, mL
V ‴ = volume of the grab sample, m^3

The density of each group was expressed as individual/ L. Organisms were identified to species level using the descriptions of **Ruttner-Kolisko (1974)**, **Koste (1978)**, **Shiel and Koste (1992)**, **Einsle (1996)** and **Smironov (1996)**.

Statistical analysis

IPM SPSS Statistics 20.0 was the software used for all statistical analyses. One-way ANOVA was utilized to analyze the data. In order to preserve data integrality, the odd replicate value was excluded from the statistical analysis. To find differences between experimental groups at a significant difference of $P < 0.05$, Duncan's multiple range test was employed **(Duncan, 1955)**.

RESULTS

Water quality

Table (3) displays the parameters related to water quality values. The average value of water temperature during the experimental period ranged from 20- 30^{o}C (Fig. 1). While, the average pH value in all the tanks was 8.2, showing no significant difference among treatments during the period of the experiment (Fig. 2). Moreover, the recorded values of total nitrogen ammonia (TAN) ranged from 0.1 to 0.2 mg/L. The fish fed the 22PT diet had the lowest value, indicating that there were no changes in the stream during the study period (Fig. 3). Throughout the experiment, the biofloc volume witnessed an increase, showing significant differences among the treatments after the tenth week (Fig. 4). Fish fed the 22PT diet had the highest biofloc volume (16.8 ml/l) at the end of the culture period, while fish fed the 22PL diet had the lowest (11.81 ml/l).

Notably, the values of the total suspended solids TSS ranged from189.9 to 219.9 mg /l (Fig.4). Additionally, the alkalinity values varied from 259.4 to 287.7 mg/l; the 22PT treatment produced the highest value of 287.7 mg/l (Fig. 5).

Table 3. Water quality parameters of different experimental treatments

Variable	30P	22P	22PL	22PT
Temperature (°C)	24.4±2.8 (20.0-29.6)	24.4±2.6 (20.0-29.1)	24.3±2.6 (20.0-28.7)	24.5±2.9 (20.0-30.9)
pH	8.2±0.4 (8.1-8.3)	8.2±0.2 (7.9-8.6)	8.2±0.2 (7.9-8.7)	8.3±0.2 (7.9-8.7)
TAN (mg/L)	0.2±0.1^{a} (0.0-0.6)	0.2±0.2^{a} (0.0-0.8)	0.2±0.1^{a} (0.0-0.7)	0.1±0.1^{b} (0.0-0.3)
Nitrite (mg/l)	0.1±0.2 (0.0-0.3)	0.1±0.1 (0.0-0.3)	0.1±0.1 (0.0-0.3)	0.1±0.1 (0.0-0.2)
Floc volume (ml/l)	15.2±12.9 (1.5-65.0)	14.4±15.1 (0.5100.0)	11.8±8.7 (0.2-50.0)	16.8±18.1 (0.2-80.0)
TSS (mg/l)	219.9±52.9 (135.0-355.0)	194.8±53.7 (115.0-355.0)	189.9±46.9 (96.0-276.0)	205.0±81.9 (75.0-392.0)
ALK (mg/l)	259.4±98.5 (118.8-486.2)	266.9±100.5(1 18.8-486.2)	275.0±100.5 (123.2-506.0)	287.7±96.1 (145.2-508.2)

Values are mean 1 ±SD range

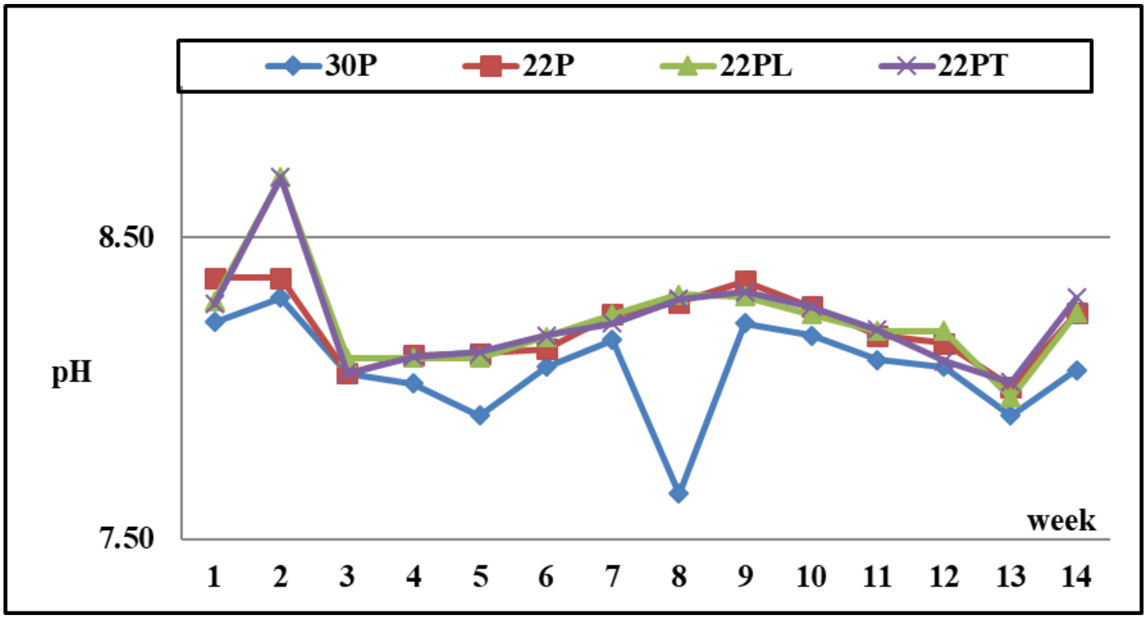

Fig. 1. A histogram showing pH values in all treatments during the study period

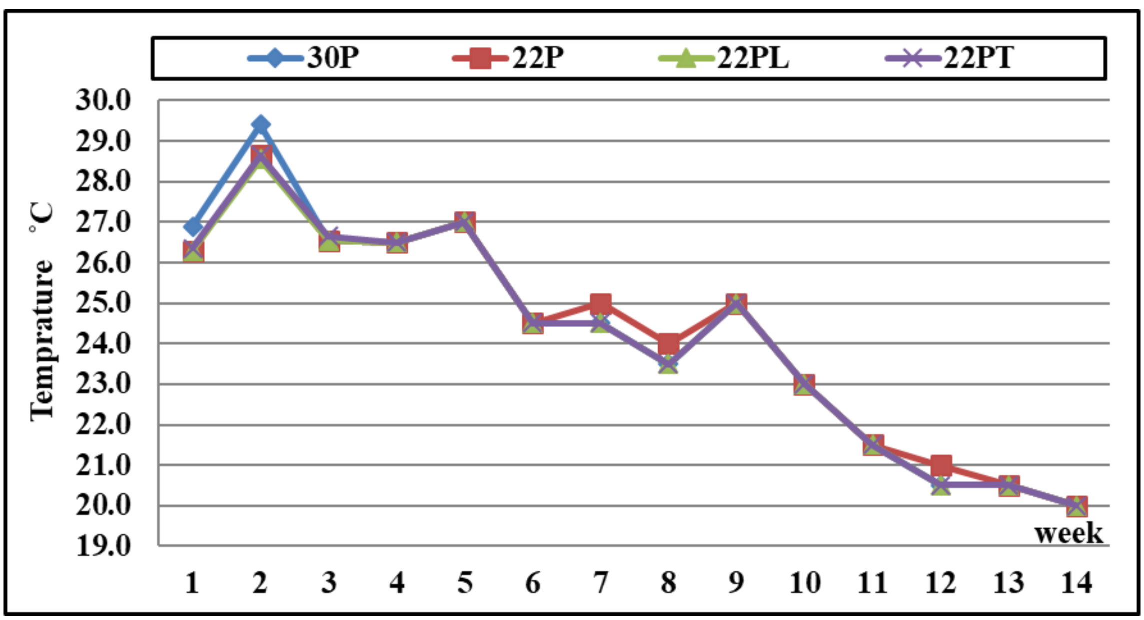

Fig. 2. A histogram showing temperature values ˚C in all treatments during the study period

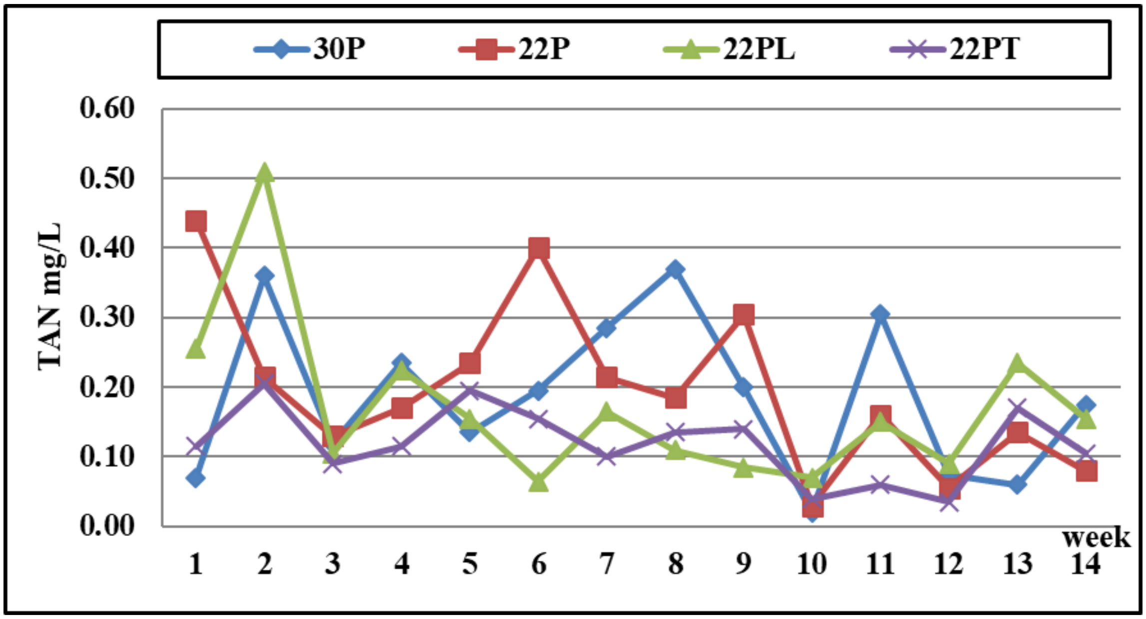

Fig. 3. A histogram showing TAN values in all treatments during the study period

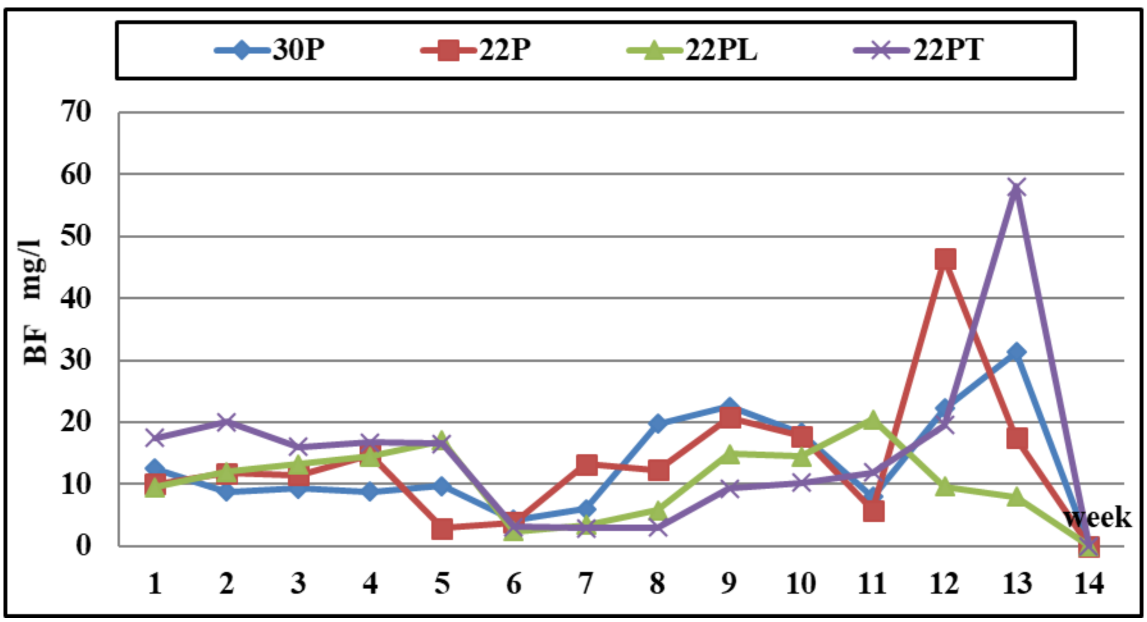

Fig. 4. A histogram showing BF volume in all treatments during the study period

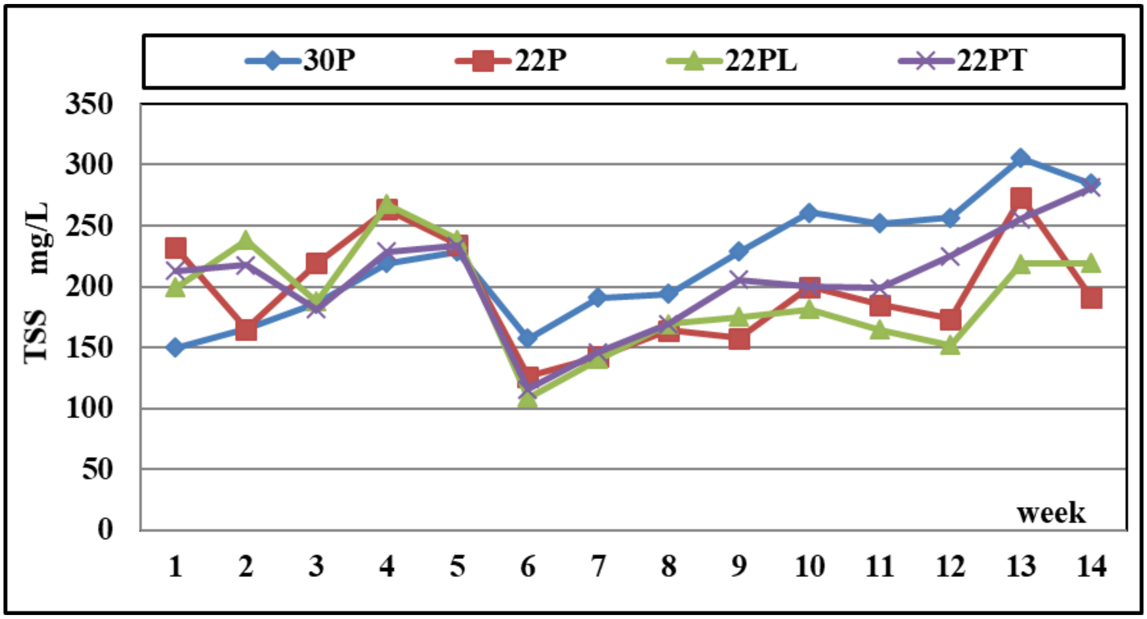

Fig. 5. A histogram showing TSS values in all treatments during the study period

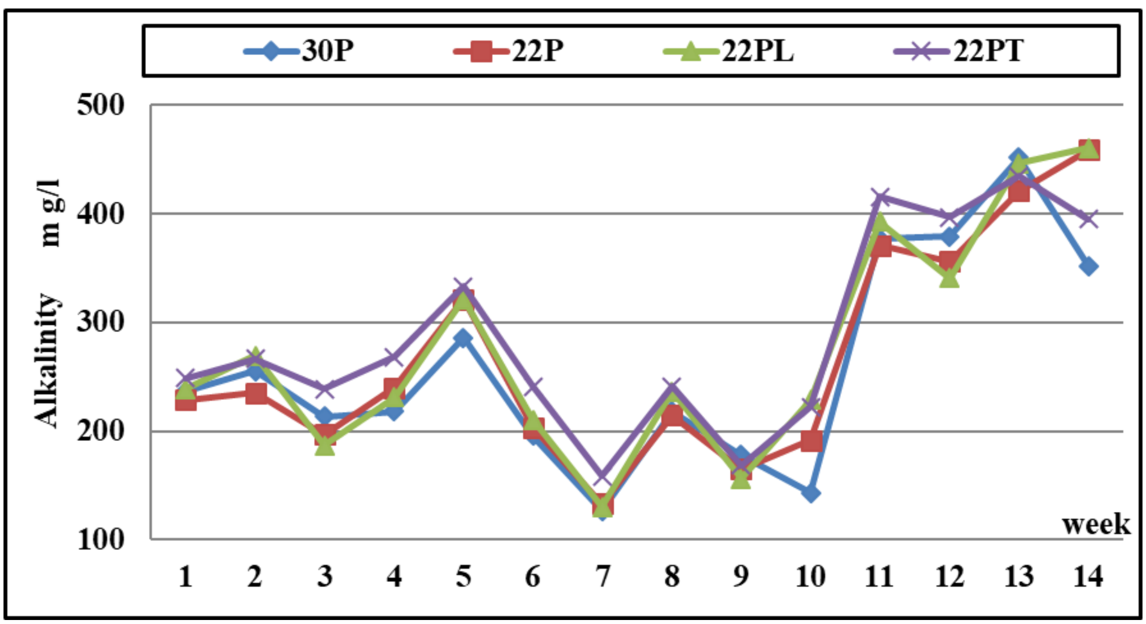

Fig. 6. A histogram showing alkalinity values in all treatments during the study period

Growth performance and feed utilization

Table (4) shows the growth performance parameters of fish under study. The 30P and 22PT treatments recorded higher values of WG (11.20 and 11.10, respectively) and SGR (1.4 and 1.4, respectively); whereas, the lowest values of FI and FCR were recorded for the 22PT treatment.

Table 4. Growth feed efficiency of the Nile tilapia fed the experimental diets for 94 days

Variable	30P	22P	22PL	22PT
Mean initial weight (g/fish)	6.20±0.00	6.25±0.70	6.25±0.50	6.25±0.50
Mean final weight (g/fish)	17.40±1.00 [a]	14.90±0.60 [ab]	13.30±0.20 [b]	17.35±1.45 [a]
Weight gain (g/fish)[1]	11.20±1.00 [a]	8.65±0.55 [ab]	7.05±0.07 [b]	11.10±1.40 [a]
Feed intake (g)	20.85±0.35 [a]	18.40±0.50 [b]	17.50±0.20 [b]	16.95±0.63 [b]
FCR (feed: gain)[2]	1.90±0.28 [ab]	2.15±0.05 [ab]	2.50±0.00 [a]	1.55±0.25 [b]
SGR[3]	1.40±0.10 [a]	1.15±0.05 [ab]	1.00±1.00 [b]	1.40±0.10[a]

Means in the same row with different superscripts are significantly different (P<0.05) by Duncan's test.

[1]Weight gain (WG) = final body weight (g) − initial body weight (g)
[2]Feed conversion ratio (FCR) = feed intake (g)/ body weight gain (g)
[3]Specific growth rate (SGR) = (in final body wt. − in initial body wt.)/ feeding days × 100

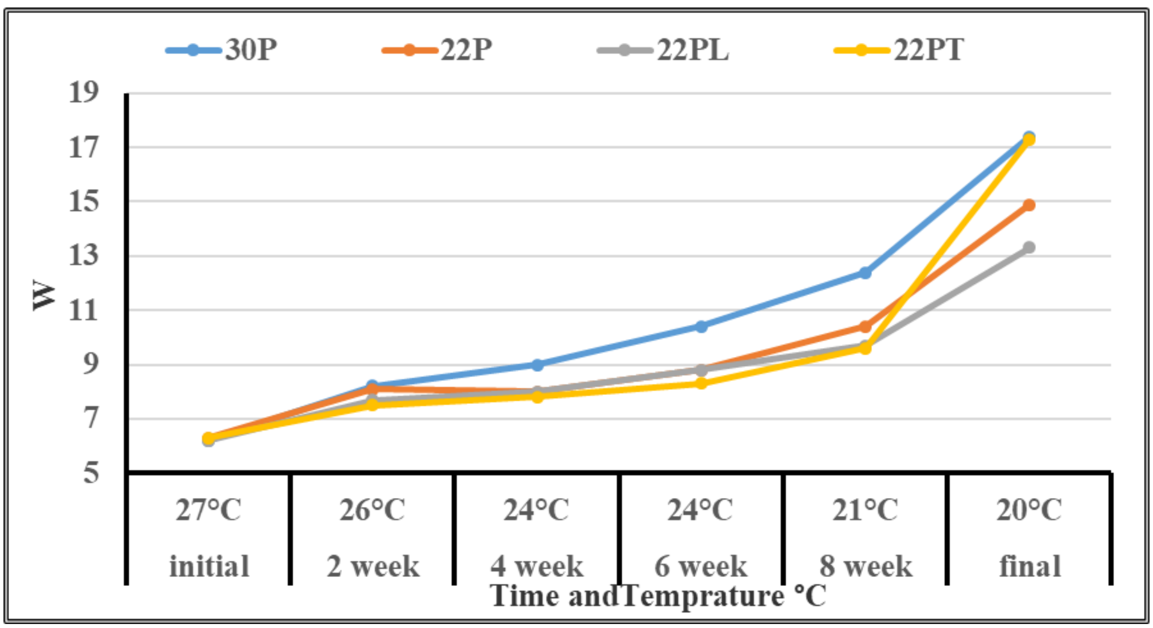

Fig. 8. A histogram showing the relation between weight and time in all treatments during the study period

Whole fish proximate composition

The whole fish chemical composition is illustrated in Table (5). Fish fed the 30P diet had the highest moisture value, while fish fed the 22PT diet had the lowest value, with no discernible difference between the treatments. The highest protein value (17.5%) was recorded in fish fed 22PT treatment, while the lowest (11.7%) was registered for those fed 22PL, showing no significant difference among the treatments. Remarkably, the highest lipid percent was recorded in fish fed 30P, 22P and 22PT; whereas, the lowest significant difference of the whole fish protein was in fish fed 22PL treatment. In addition, the highest ash percentage (5.2) was noted in fish fed 22PT diet, while the lowest (3.3%) was for the 22PL treatment, recording no significant difference among the treatments.

Table 5. Whole fish chemical composition affected by experimental diets for 97days

Variable	30P	22P	22PL	22PT
Moisture (%)	71.6±1.3	71.8±0.8	77.7±3.7	66.5±4.9
Protein (%)	15.2±0.7	15.3±0.3	11.7±3.0	17.5±3.4
Lipid (%)	8.7±0.7^{a}	8.9±0.4^{a}	6.6±0.8^{b}	8.5±0.7^{ab}
Ash (%)	4.4±0.1	4.0±0.2	3.3±1.1	5.2±0.9

Means in lipid row with different superscripts are significantly different ($P< 0.05$) by Duncan's test.

Biofloc proximate composition

Table (6) shows the biofloc chemical composition. The highest moisture value was recorded in the 30P treatment, while the lowest value was assessed in fish fed 22PL diet, showing no significant difference between the treatments. The highest protein values of 30.5% and 29.3% were recorded in fish fed 22PT and 22PL treatments, respectively, without recording any significant difference between the treatments. Nonetheless, the lowest protein value (27.5%) was reported in the 22P treatment without significant difference among the treatments. It is noteworthy mentioning that, the highest lipid percentage was recorded in fish fed 30P diet, while the lowest was observed in fish fed 22P diet without significant difference among all the treatments. Additionally, fish fed on 30P diet registered the lowest ash percentage, showing no significant difference among the treatments.

Table 6. Chemical composition of BF affected by experimental diets for 94 days

Variable	30P	22P	22PL	22PT
Moisture (%)	11.81±0.66^{a}	9.77±0.01^{ab}	8.51±0.63 ^{b}	9.00±1.13 ^{ab}
Protein (%)	28.4±0.3	27.5±1	29.3±1	30.5±1.7
Lipid (%)	1.5±0.1	1.2±0.2	1.4±0.1	1.3±0.3
Ash (%)	15.84±0.53^{b}	24.80±1.26 ^{a}	23.94±2.04^{a}	26.30±2.04^{a}

Means in the lipid row with different superscripts are significantly different ($P< 0.05$) by Duncan's test.

Live food production (zooplankton)

Table (7) displays the findings of the zooplankton density and composition. Only two phyla—Rotifera and Ciliophora—were found in all of the biofloc experimental tanks.

Three taxa were identified under phylum Rotifera, including *Anuropsis fissa, Colurella* sp., and *Monostyla* species, while only one species of phylum Ciliophora was detected, namely: *Tintinnidium pusillum.* Markedly, the lowest density of Rotifera was recorded in 30P and 22PT treatments, whereas the highest density was noted in 22P and 22PL treatments. On the other hand, the lowest total count for phylum Ciliophora was found in 30P treatment, while the highest was in 22PL treatment. In general, the 30P treatment showed the lowest total count of Ciliophora and Rotifera. Additionally, the total count increased with the decrease in the dietary protein levels in 22P, 22PL and 22PT treatments.

Table 7. Zooplankton composition and density in the biofloc treatment tanks (No./L)

Zooplankton taxa	30P	22P	22PL	22PT
Rotifera				
Anuropsis fissa	106±34	108±93	180±0	25±5
***Colurella* sp.**	9±9	99±72	54±18	60±60
***Monostyla* spp.**	487±26^{b}	1033±123^{b}	4239±125^{a}	610±42^{b}
Total density	602	1,240	4,473	695
Ciliophora				
Tintinnidium pusillum	279±261	762±282	2511±2439	715±695
Total density	881	2002	6984	1410

Means in the same row with different superscripts are significantly different ($P< 0.05$) by Duncan's test.

DISCUSSION

Water quality

The current results indicate that all water quality parameters under the biofloc system conditions are suitable for the production of the Nile tilapia according to recommendation figured in the study of **Emerenciano *et al.* (2017)**. The experimental diets did not have significant effect on water quality, except for TAN. In contrast to the findings of **García-Ríos *et al.* (2019)**, who found a significant effect of protein levels on water quality, the dietary protein levels had no effect on the TAN level. The addition of threonine to the low protein diets decreased the TAN level significantly.

Moreover, **Zidan *et al.* (2017)** reported that the optimal water temperature for fish culture ranged from 25.1– 30.6ºC. In the present study, a descending trend was recorded in water temperature along the study period, recording a water temperature average of 24.4^{o}C in all tanks, with values ranging from 20.0 - 30.9^{o}C. **De Almeida *et al.* (2021)** postulated that the tilapia suboptimum temperature is 20.8ºC. In the present study, water temperature experienced a gradual decrease within the normal range till the 8th week. With the start of week eight, water temperature began to decrease reaching the suboptimal temperature. The pH values showed no significance, or specifically, a stream change occurred during the period of experiment (8.2 for all treatments). The pH readings fell between the typical ranges needed to raise tilapia in a biofloc system. This result is consistent with the findings of **El-Sayed (2006)** and **El-Sherif and El-Feky (2009)**, who found that the optimal pH range for tilapia is between 7 and 8. Conversely, the alkalinity values varied between 259.4 and 287.7 mg/l, indicating no statistically significant variations across all treatments. This indicated the biofloc system's ability to function as a buffer within the recommended range for aquaculture systems **(Huet, 1986; Boyd & Tucker, 1998; Wurts, 2003)**. Alkalinity was remarkably high in all treatments, especially in 22PT treatment, which recorded the highest value indicating that the amount required of $NaHCO_3$ would witness a decline. The findings of **Azim and Little (2008)**, who noted fluctuations in the alkalinity values (80–250 mg/l), which point to a reduction in buffering capacity, are not consistent with this finding. Thus, a frequent input of sodium bicarbonate (NaHCO3) is required to avoid pH oscillation in BFT. In the present study, the levels of TAN were distinctive and low, especially in tanks with 22PTdiet. The decline in TAN may be attributed to the threonine supplementation. This explanation is in consonance with that of **Michelato *et al*. (2016)** who reported that, threonine addition increases the retention of both protein and amino acids, causing rapid growth of the Nile tilapia. In this context, **Walton (1985)** and **Abidi and Khan (2008)** recorded that threonine increases the metabolic rate and decreases ammonia excretion.

The values of TSS in the present study (75- 392) are within the recommended limits for BFT standard conditions recommended in the study of **Avnimelech (2011**). However, **Azim and Little (2008)** and **Silva *et al.* (2018)** noticed TSS reaching up to 1,000 mg/L in different studies with the tilapia fed different CP concentrations.

Growth performance and feed utilization

Under the present study conditions, the tilapia fed 22PT diets showed a compensatory effect when subjected to suboptimal temperature. At the beginning of the treatment, a slight weight gain was observed, which dramatically increased by time, and that may be related to threonine addition. In this essence, **Wohlfarth and Hulata (1983)** noted that, the temperature below the suboptimum level restricts the adequate growth of fish. They also mentioned that normal feed intake stops below 20.8ºC and that reproduction stops when temperature reaches 22.8ºC. In addition, **De Almeida *et al.* (2021)** reported that, the tilapia feeding, swimming, and vital physiological functions decrease at 20ºC, while **Michelato *et al*. (2016)** indicated that threonine is essential for growth, protein, and amino acid retention of the large Nile tilapia. Additionally, **Lem-me (2003)** noticed that, threonine is a critical essential amino acid for fish development serving as an antecedent of non-essential amino acids, such as serine and glycine. He concluded that the effects of dietary threonine were more expressive on protein and amino acids retention. **Veldkamp *et al.* (2000)** notified that, commercial male turkeys fed diets supplemented with amino acid threonine at low temperature witnessed a significant reduction in FCR, while the high temperature showed no response. This outcome is consistent with the observation made by **Ferguson *et al.* (2003)** that growing pigs' reaction to threonine is influenced by the temperature of their surroundings. **Avnimelech (2011)** reported that feed rations in biofloc tilapia systems can be lowered to at least 20% compared to conventional non BFT. In their study on shrimp, **Xu and Pan (2014)** deduced that, the BFT proved its effectiveness in decreasing the protein levels (from 30 to 20 percent). **Ogello *et al.* (2014**) proclaimed that, in bio-flocs technology (BFT), lakes are a potential food source for fish. In fact, the BFT can be considered as a self-sustaining biotechnology machine since it produces food concurrently; however, it is an ignored resource in the aquaculture industry. **Megahed (2010)** and **Kim *et al.* (2016)** mentioned the possibility of reducing CP levels under the biofloc system. The aforementioned authors confirmed that the decline in the level of this protein did not cause any loss of performance regarding shrimp in BFT. To illustrate, **Day *et al.* (2016)** recorded the role of biofloc as a supplementary feed component of high nutritional quality, especially in terms of protein, with respect to several aquaculture species. It is worthy to mention that, the best FCR value was recorded in tanks fed 22PT.

Chemical composition of fish

In the current study, the 22PT treatment recorded the highest value in protein and ash contents, and the lowest in lipid and moisture contents, reflected in the dry matter level. The results indicate that supplementing threonine amino acid in fish diets may improve fish nutritive value. **Helland *et al*. (2013)** found a linear increase in the crude protein and quadratic impact on whole-body humidity, unrefined lipid, and ash of Atlantic salmon bolstered expanding levels of threonine. **Zhao *et al.* (2020)** stated that, dietary threonine improved the growth of hybrid catfish and enhanced muscle protein content as well. Notably, increased ash values on wet basses may be attributed to the addition of threonine to the experimental diet. Furthermore, the increase of dry matter on wet basses may be due to the addition of threonine to the experimental diet. **Becerril *et al.* (2017)** recorded that, some fish species frequently observed in biofloc can provide good protein, lipids and carbohydrate contents.

Biofloc proximate composition

The 22PT treatment recorded the highest nutritional value compared to the other treatments, due to the high percentage of protein, ash and dry matter. **Tacon *et al.* (2002)** and **Ballester *et al.* (2010)** noted that, biofloc quality in terms of fatty acid profile and protein content seems to be affected by the system input and microbial floc as a good source of vitamins and minerals that can supply the needs of those nutrients. The average ash content of floc meal in the present study varied from 15.8 to 26.3%, which is within the ranges found in other studies **(Azim *et al.*, 2008; Emerenciano *et al.*, 2013b)**. Hence, the increased protein values on wet basses may be related to the supplementation of amino acids to the experimental diets. This perspective aligns with that of **Mabroke *et al.* (2019)** who observed that, even when floc meal accounted for 50% of the total ingredients, the amino acid content of the various experimental diets satisfied the tilapia's needs.

Live food production (zooplankton)

Successful larvae culture (fish seed production) requires sufficient live food resources such as rotifers, copepods, cladocerans and other zooplankton, as a starter food in hatcheries **(Ogello *et al.*, 2019)**. According to **Ogello *et al.* (2020)**, the small size (to facilitate ingestion by fish larvae), high digestibility, palatability, and nutritional completeness of live food are the reasons why fry and fingerling cultures prefer it. The use of high density microalgal pastes, whose culture protocol is costly, delicate, and stressful, is the conventional method of producing live food **(Ogello *et al.*, 2018)**. Therefore, new protocols have been developed **(Ogello *et al.*, 2017)** for mass production of live food resources using BFT. The bioflocs form the basis of the food chain in aquatic ecosystems. Therefore, bioflocs are responsible for the initial nutrient cycling process in the aquatic ecosystems **(Avnimelech, 2007**; **Avnimelech & Kochba, 2009)**. High densities (> 1200 individuals' ml $^{-1}$) of single strains of rotifers *Proales similis* **(Kagali *et al.*, 2018)** and *Brachionus rotundi- formis* **(Ogello *et al.*, 2018)** were produced using fish wastes diet (FWD) in BFT units. In a different study, BFT was used in an outdoor setting to obtain high densities of mixed zooplankton communities, including rotifers, copepods, and cladocerans **(Ogello *et al.*, 2019)**. Thus, BFT seems to be a significant step toward enabling year-round pre-planning of fish seedling production in aquaculture facilities. Because of their higher nutritional content, bioflocs can be used as nutritional supplements in addition to encouraging a faster population density of live food resources. Ever since research has shown that biofloc paste contains essential polyunsaturated fatty acids (PUFAs) **(Ogello *et al.*, 2018)**. The paste can be used as an enrichment emulsion to live food resources (zooplankton) and larval fish, thus reduce or eliminate the use of expensive commercial enrichment emulsions. Currently, live food resources are first supplemented with expensive commercial emulsions in Asian and European hatcheries. The biofloc emulsion could be better than other homemade emulsions (of fish oil and yolk sac), which have short shelf life that limits their application in aquaculture. The biofloc PUFAs are more protected against oxidation, and provide a variety of other natural nutrients that meet the species-specific nutritional requirements of the cultured fishes **(Harel *et al.*, 2002)**.

Generally, in the present study, the lowest densities of Rotifera and Ciliophora were recorded in the high protein treatment (30P), and increased with decreasing the dietary protein levels 22P, 22PL and 22PT.

CONCLUSION

The present study concluded that addition of threonine amino acid to the low protein diets improved the growth performance, feed utilization, water quality and the tolerance to thermal stress during the nursery phase of *O. niloticus*. Therefore, it is recommended to use a low protein diet (low price feed) supplemented with threonine as a tool to improve biofloc system performance during tilapia nursering at a cold suboptimal water temperature.

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