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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

Khaled H. Salama [1*], Shimaa Henish [2], Khadra A. Mohamed [4], Marian G. Nassif [2], Marwa M. El-Naggar[3], Magdy T. Khalil [3], Ashraf Suloma[5]

1. Basic Sciences Dept., Institute of Environmental Studies and Research, Ain Shams University, Egypt.
2. National Inst. of Oceanography and Fisheries (NIOF), Egypt.
3. Zoology Dept, Fac. Sci, Ain Shams University, Egypt
4. Aquatic Environment, National Center of water Research.
5. Fish Nutrition Lab (FNL), Animal Production Dept, Faculty of Agriculture, Cairo University, Egypt.

* Corresponding author: salamera61@hotmail.com

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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 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). Formulated diets were tested under biofloc system conditions in three replicates for each treatment. 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. Results revealed that fish fed on 22PT diet recorded the highest significant values of weight gain (WG), feed intake (FI), feed conversion ratio (FCR) and specific growth rate (SGR) (11.10, 16.95, 1.55 and 1.40, respectively). Additionally, the 22PT diet groups recorded the highest protein content of dried microbial floc and wet whole-body. While the lowest total ammonia nitrite (TAN) values were recorded for the fish groups fed on 22PT diet. Thus, under biofloc system conditions, a low protein diet supplemented with threonine can improve biofloc system performance during the tilapia nursering in cold suboptimal water temperature.

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)**. Biofloc technology (BFT) is a technique of enhancing water quality by supplementing the production units with a carbon source to promote nitrogen uptake through heterotrophic bacterial growth. Hence, the ammonium concentration would decrease more rapidly than nitrifying bacteria **(Widanarni *et al.*, 2012; Hargreaves, 2013)**. BFT minimizes water usage in aquaculture units through maintaining adequate water quality within the culture unit. Thus, it produces low cost bioflocs rich in protein serving as a feed for aquatic organisms **(Crab *et al.*, 2007, 2009, 2010; El-Shafiey *et al.*, 2018; Mabroke *et al.*, 2021; Suloma *et al.*, 2021)**. Moreover, **Avnimelech *et al.* (1999)** postulated that, the expensive commercial protein sources in aquaculture feeds can be partially substituted with single cell proteins originated from cheap carbon and nitrogen sources. Compared to 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, **Tacon *et al.* (2002)** recorded no significant differences in the growth performance of *L. vannamei*, reared in unfiltered pond water and fed on 25% CP and 35% CP. Additionally, no significant differences were detected between the specific growth rates of *P. monodon* fed on 25% CP and that fed 40% CP in 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, a decrease in feed utilization was observed when this species was reared at a cold suboptimal temperature of 22°C, and subsequently a critical decrease in fish development was detected **(Azaza *et al.*, 2008)**. In general, the temperature feeding and voluntary development ceases, as well as the lethal temperature; all are 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 conducted in Fish Nutrition Lab., Animal Production Department, Faculty of Agriculture, Cairo University.

Experimental design and diets

The Nile tilapia (*Oreochromis niloticus*) specimens were obtained from a private hatchery, at 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). Formulated diets were tested under biofloc system conditions in three replicates for each treatment. No artificial light was used in the greenhouse. Every 15 days, fish in each replicate tank were weighed and the amount 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). All tested diets were handled by mixing the dry fixings into a homogenous blend. Then, sun flower oil was added, and a small mincer with small breadth was used to get uniform-sized pellets. Then, the outcome was stored at 8ºC until stocked in tanks.

Experimental conditions

Twelve experimental tanks of 1000 L were used, each of which was filled with 400 L of water. Each tank was filled with a stock of 50 fries (6.20±0.01 gm. /fish). The tanks were aerated by air stones 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.

Starch was used as a carbon source, which was added daily to maintain the C/N proportion at 1:10, activating the heterotrophic bacteria **(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). This was achieved as follows: weight gain (%) = (FW–IW), FCR= feed given (DW)/body weight gain (WW) Where; FW= final weight, IW= initial weight, DW= dry weight, WW= wet weight, ln= natural log, and N = number of culture days.

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 estimation of the densities of zooplankton in different treatments, five liters of water were filtered from 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. In the laboratory, a sub-sample of 1 ml was transferred to a counting cell (Rafter Sedwick Cell) and examined under a binocular compound Olympus microscope. 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

All statistical analyses were performed using IPM SPSS Statistics 20.0 software. Data were analyzed using one-way ANOVA. Odd replicate value was omitted during statistical analysis to save data integrality. Duncan's multiple range test was used to identify differences among experimental groups at a significant difference of ($P <0.05$) **(Duncan, 1955)**.

RESULTS

Water quality

The values of water quality parameters are presented in Table (3). The average value of water temperature during the experimental period ranged from 20 and 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. It was noticed that, the lowest value was recorded in fish fed 22PT diet, showing no stream changes 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). At the end of the culture period, the highest biofloc volume (16.8 ml/l) was recorded in fish fed on 22PT diet, whereas the lowest (11.81 ml/l) was recorded in fish fed on 22PL diet.

Notably, the values of the total suspended solids TSS ranged from189.9 to 219.9 mg /l (Fig.4). Furthermore, the alkalinity values ranged from 259.4 to 287.7 mg/l, and the highest value (287.7 mg/l) was recorded in 22PT treatment (Fig. 5).

Table 3. Water quality parameters of different experimental treatments

Variable	30P	22P	22PL	22PT
Temperature (ºC)	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

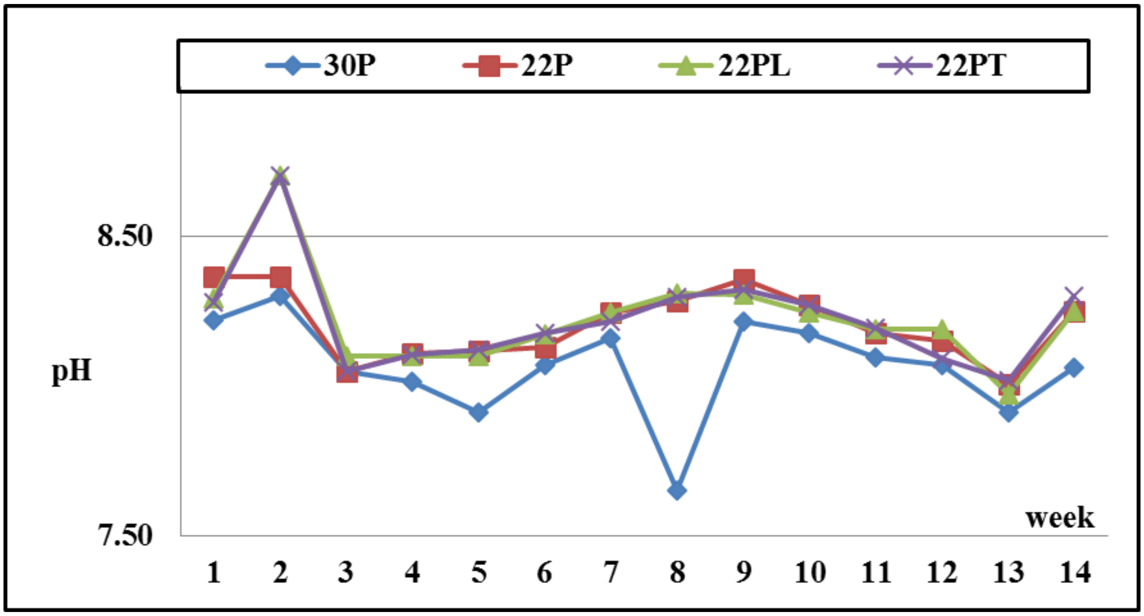

Fig. 1. A histogram showing pH values in all treatments during the study period

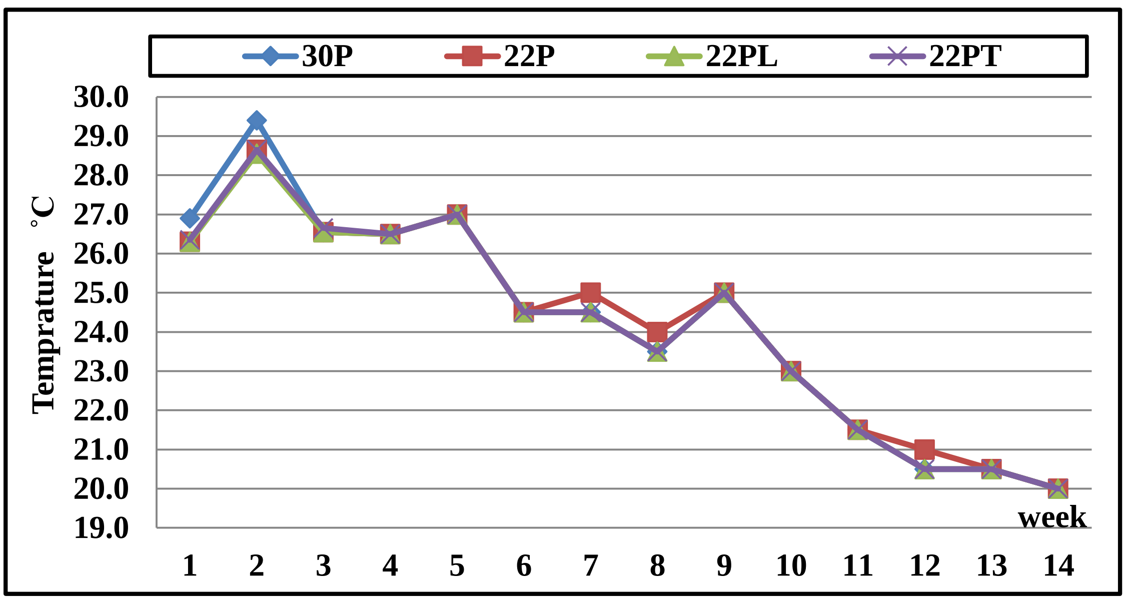

Fig. 2. A histogram showing temperature values ˚C in all treatments during the study period

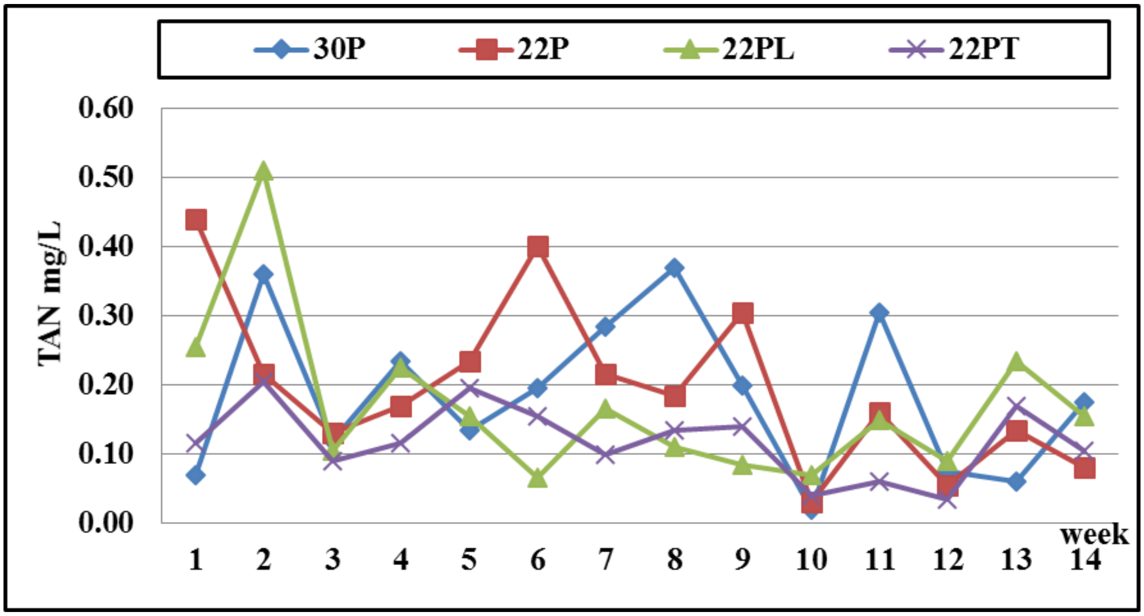

Fig. 3. A histogram showing TAN values in all treatments during the study period

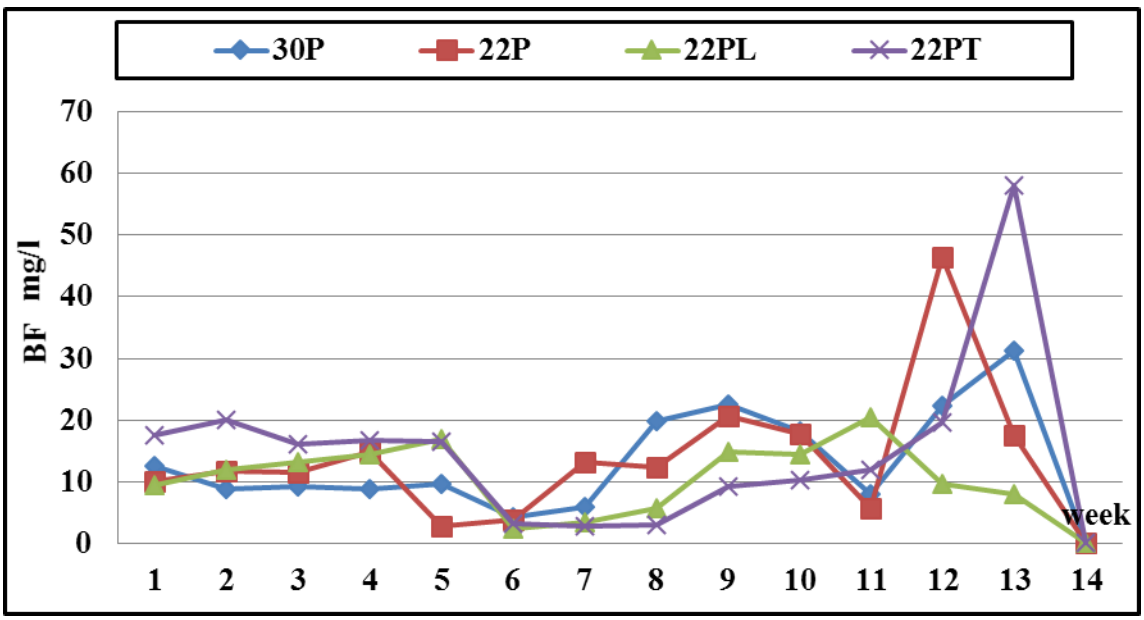

Fig. 4. A histogram showing BF volume in all treatments during the study period

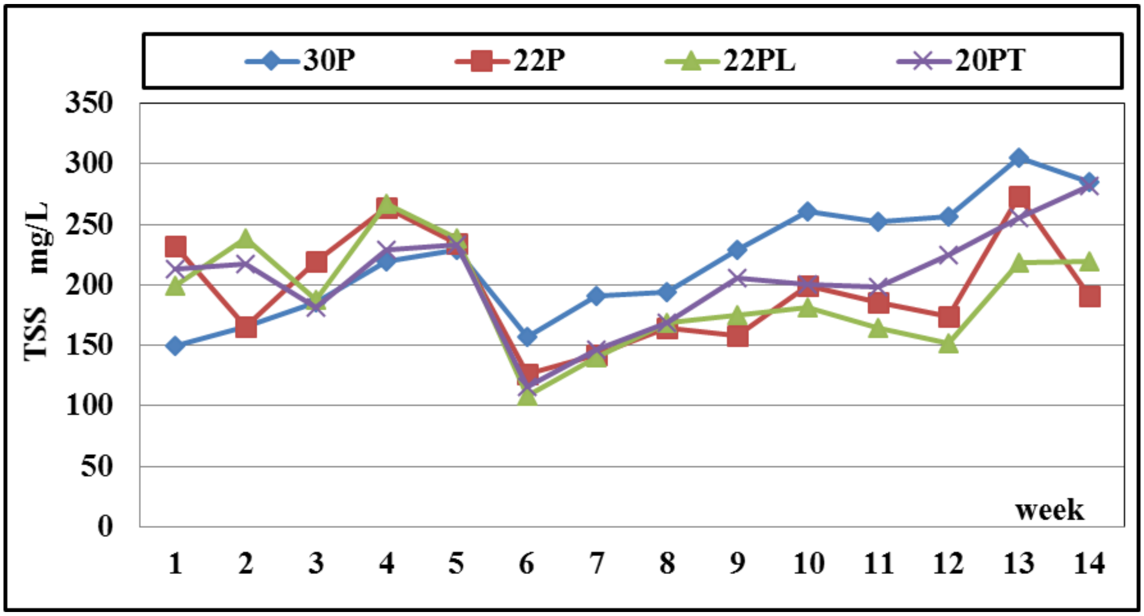

Fig. 5. A histogram showing TSS values in all treatments during the study period

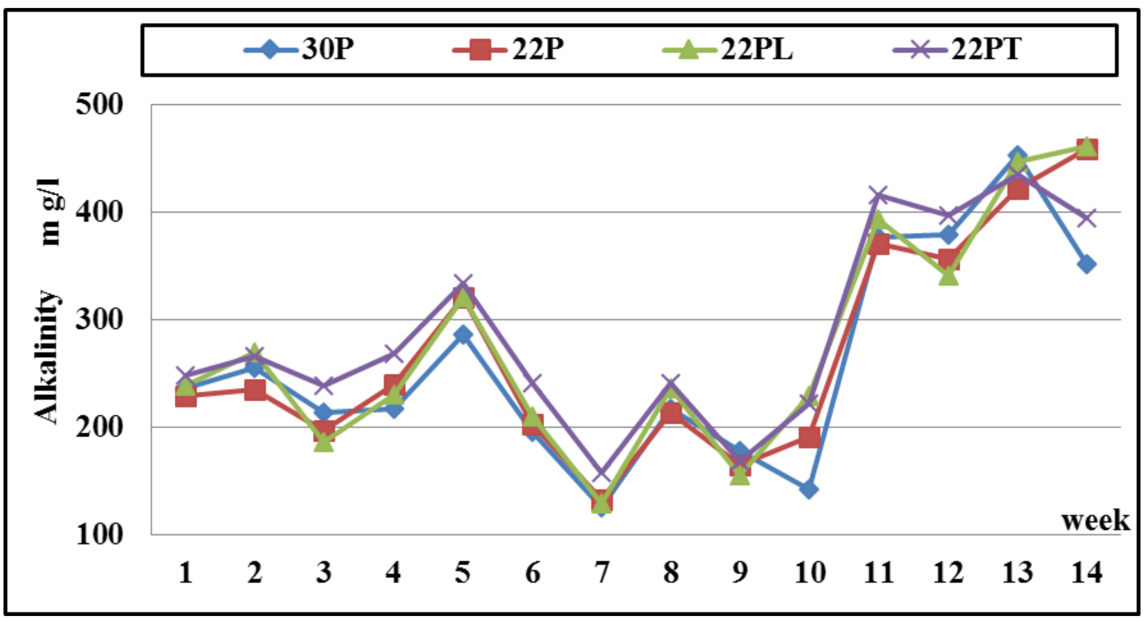

Fig. 6. A histogram showing alkalinity values in all treatments during the study period

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

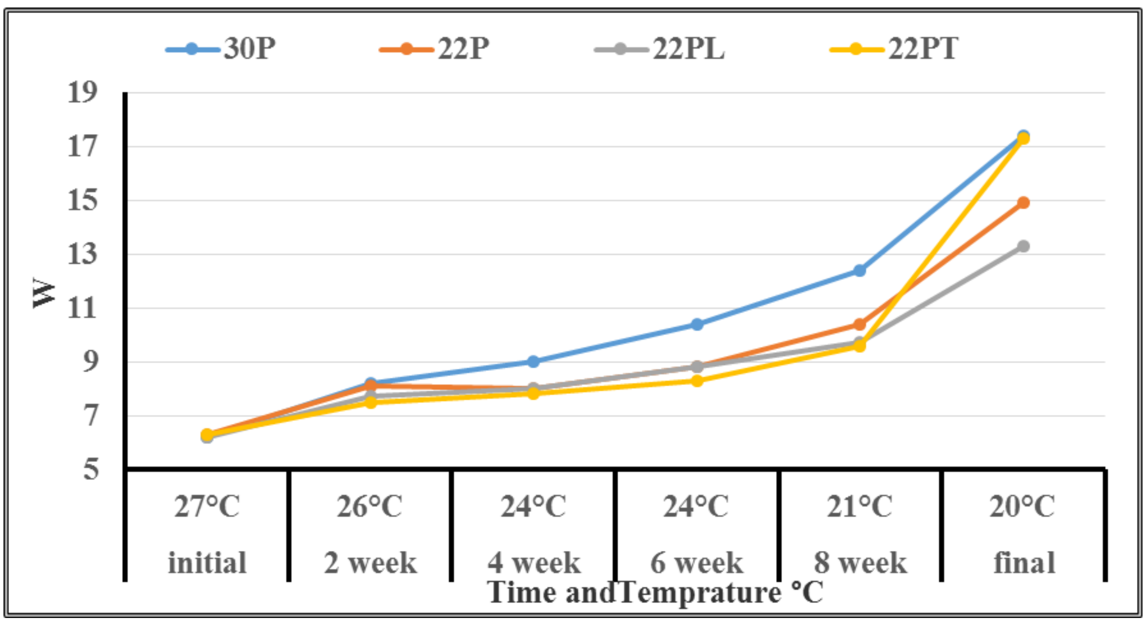

Fig. 8. A histogram showing the relation between weight and time in all treatments during the study period

Whole fish proximate composition

The whole fish chemical composition is illustrated in Table (5). The highest moisture value was recorded for fish fed 30P diet, while the lowest value was for those fed 22PT diet without showing any significant difference among 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)

The results of the zooplankton density and composition are presented in Table (7). Among all the biofloc experimental tanks, only two phyla were identified; namely, Rotifera and Ciliophora. 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. Generally, the lowest total count of Rotifera and Ciliophora was recorded for 30P treatment. 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

Zooplankton taxa	30P	22P	22PL	22PT
Rotifera				
***Anuropsis fissa*/L**	106±34	108±93	180±0	25±5
***Colurella* sp./L**	9±9	99±72	54±18	60±60
***Monostyla* spp./L**	487±26^{b}	1033±123^{b}	4239±125^{a}	610±42^{b}
Total density/L	602	1,240	4,473	695
Ciliophora				
***Tintinnidium pusillum*/L**	279±261	762±282	2511±2439	715±695
Total desity /L	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 figures of **Emerenciano *et al.* (2017)**. The experimental diets did not have significant effect on water quality except for TAN. The dietary protein levels showed no effect on the TAN level, which disagrees with the result of **García-Ríos *et al.* (2019)** who detected a significant effect of protein levels on water quality. The addition of threonine to the low protein diets decreased 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 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)** reported 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 values were within the normal ranges for rearing the tilapia under the biofloc system. This finding concurs with those of **El-Sayed (2006)** and **El-Sherif and El-Feky (2009)** who determined the ideal pH value for the tilapia within ranges from 7 to 8. On the other hand, the alkalinity values ranged from 259.4 to 287.7 mg/l, showing no significant differences among all the treatments which demonstrated the buffering capacity of the biofloc system within the recommended range of 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. This result disagrees with that of **Azim and Little (2008)** who observed oscillations in the alkalinity values (80–250 mg/l), which indicates a decrease in buffering capacity. Thus, 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 & Khan (2008)** recorded that threonine increases the metabolic rate and decreases ammonia excretion.

The values of TSS in the present study (75- 392) were 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 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 added that reproduction ceases when temperature reaches 22.8ºC, and that normal feed intake ceases below 20.8ºC. 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 significant reduction in FCR, while high temperature showed no response. This result agrees with the finding of **Ferguson *et al.***

(2003) who recorded that, the environmental temperature has an effect on the response of growing pigs to threonine. **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 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 aquaculture industry. **Megahed (2010)** and **Kim *et al.* (2016)** mentioned the possibility of reducing CP levels under 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. In the present study, the average ash content of floc meal ranged between 15.8% and 26.3%, which is within the ranges reported in previous 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 percepective is in line with that of **Mabroke *et al.* (2019)** who noted that, the amino acid content of different experimental diets covered the requirements of the tilapia even when the floc meal reached 50% of the total ingredients.

Live food production (zooplankton)

Successful larvae culture (fish seed production) require sufficient live food resources such as rotifers, copepods, cladocerans and other zooplankton, as a starter food in hatcheries **(Ogello *et al.*, 2019)**. The preference of live food for fry and fingerling culture is attributed to the small size (for ease of ingestion by fish larvae), high digestibility, palatability and nutritional completeness **(Ogello *et al.*, 2020).** The conventional method of live food production involves the use of high density of microalgal pastes, whose culture protocol is expensive, fragile and stressful **(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 aquatic ecosystems **(Avnimelech, 2007**; **Avnimelech & Kochba, 2009)**. High densities (> 1200 individuals' ml $^{-1}$) of single strains of rotifers i.e. *Brachionus rotundi- formis* **(Ogello *et al.*, 2018)** and *Proales similis* **(Kagali *et al.*, 2018)** were produced using fish wastes diet (FWD) in BFT units. In another study, high densities of mixed zooplankton communities i.e. rotifers, copepods and cladocerans were obtained using BFT under outdoor conditions **(Ogello *et al.*, 2019)**. Therefore, BFT appears to be a major leap toward making pre-planning of fish seedling production in aquaculture facilities feasible throughout the year. Besides promoting faster population density of live food resources, the bioflocs can be used as nutritional supplements due to the higher nutritional factors. Since studies have established the presence of essential PUFAs in biofloc paste **(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 cold suboptimal water temperature.

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