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Reducing Nitrogenous Waste in RAS culture of GIFT Fingerlings Through Dietary Manipulation of Protein and Lipid Levels

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Abstract

Recirculating aquaculture systems (RAS) offers several advantages, such as increased production and water conservation, but they can lead to elevated nitrogenous waste levels due to their intensive nature. This 60-day feeding trial investigated the effects of six hetero-nitrogenous and hetero-caloric diets on genetically improved farmed tilapia (GIFT) fingerlings reared in RAS. Six diets, with decreasing crude protein from 40% to 30% and increasing lipid levels from 7% to 12% were formulated and designated as: T1 (40% CP, 7% lipid), T2 (38% CP, 8% lipid), T3 (36% CP, 9% lipid), T4 (34% CP, 10% lipid), T5 (32% CP, 11% lipid), and T6: 30% CP, 12% lipid). GIFT fingerlings (initial weight 5.05 ± 0.03 g) were stocked evenly into eighteen RAS tanks, maintaining stocking density of 6000 g/m^3. Results showed a significant ($p < 0.05$) reduction in total ammonia-nitrogen, nitrite-nitrogen (NO_2-N), and nitrate-nitrogen (NO_3-N) levels in the culture water from T1 to T6. However, phosphorus (PO_4^{3-}) levels did not show a significant difference ($p > 0.05$) among the different treatment groups. The gill superoxide dismutase (SOD) and catalase (CAT) activities, indicators of oxidative stress, were elevated ($p < 0.05$) in T5 and T6, while liver CAT activity, crucial for hydrogen peroxide detoxification, also increased significantly ($p < 0.05$). The T4 group (34% CP, 10% lipid) exhibited significantly ($p < 0.05$) higher specific growth rate and lower NH_3-N, NO_2-N, and NO_3-N levels compared to other groups. Therefore, a diet of 34% CP and 10% lipid is recommended for GIFT fingerlings in RAS to promote growth and to minimise nitrogenous waste.

Keywords: Dietary intervention, low protein, high lipid, nitrogenous waste, antioxidant enzymes

Introduction

Aquaculture has emerged as the most efficient producer of edible protein and remains the fastest-growing primary food production sector in the world. However, over the past few decades, the environmental impact of aquaculture has gained increasing attention. The rapid expansion of aquaculture practices results in the release of high effluents, resulting in issues such as water contamination, spread of diseases, destruction of mangroves, eutrophication, and salinisation of land and water (Boyd, 2003). Furthermore, the limited availability of land for aquaculture presents another challenge. To address these issues, a more intensive type of aquaculture technology named recirculating aquaculture system (RAS), has gained attention. RAS offers higher fish production even in a limited environment and also minimise the discharge of wastewater into the ecosystem (Badiola, Basurko, Piedrahita, Hundley, & Mendiola, 2018; Joseph & Augustine, 2020). Despite being water-efficient, RAS is known for releasing nutrient-rich effluents during partial water exchanges, which include high levels of total ammonia nitrogen (TAN), nitrite-N (NO_2-N) and nitrate-N (NO_3-N), that can threaten biodiversity in the ecosystem (Martins et al., 2010).

Ammonia-N excretion is the primary concern in RAS culture tanks. Accumulation of ammonia in the closed environment can lead to toxic conditions that

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negatively affect fish health, growth and stress responses (Li, Wen, Zhao, Li, & Zhu, 2016). A major cause of higher ammonia-N release is the preferential use of dietary protein by fish as an energy source. Surplus protein or amino acids in the diet are catabolised and used to fuel intermediary metabolism (Teles, Couto, Enes, & Peres, 2020), which either increases TAN levels in culture water or converted into glucose or triglycerides for energy storage (Teles et al., 2020). Feeding fish with a high protein diet in RAS can exacerbate this issue, leading to higher accumulation of dietary origin TAN (Mota, Limbu, Martins, Eding, & Verreth, 2015). Thus, a strategy to reduce nitrogenous waste through the reduction of digestible protein levels in the diet (Mihalca, Tita, Tita, & Mihalca, 2010) must be employed. Background studies reveal that lipids can more effectively conserve protein for growth in fish, compared to carbohydrates, referred to as the protein-sparing action (Kaushik, Doudet, Médale, Aguirre, & Blanc, 1995; Sagada et al., 2017). This reduces the breakdown of dietary protein, which in turn lowers feed costs and decreases ammonia excretion. Moreover, the dietary protein is utilised exclusively for growth with minimal inevitable catabolism, resulting in improved growth and nutrient utilisation (Sagada et al., 2017; Thirunavukkarasar et al., 2022).

For this study, a suitable species compatible with the space-efficient RAS model was selected. Generally, the Genetically Improved Farmed Tilapia (GIFT) strain of Nile tilapia (*Oreochromis niloticus*) is considered as one of the best fish for aquaculture, owing to its numerous key attributes. The GIFT culture has tremendous market potential, faster growth rate, efficient feed conversion, and is well-suited for high stocking density conditions (El-Sayed, 2006). Additionally, tilapias are adaptable to a variety of diets, and contribute to lower nutrient loading in the effluent (Alebachew et al., 2022). It also has high export potential to countries like the USA, Africa and Japan (TNAU Agritech Portal, 2015). These traits make GIFT strain the most suitable choice for culturing in RAS.

RAS significantly demonstrates its ability at supporting high stocking densities compared to other aquaculture systems. Studies have been conducted to optimise the stocking density of tilapia in RAS, which have shown higher net production compared to open aquaculture systems. Gullian-Klanian and Arámburu-Adame (2013) concluded that Nile tilapia (*Oreochromis niloticus*) fingerlings stocked at 400 to 500 fish/m^3 exhibited high performance, reaching a biomass production of 37 kg/m^3. Additionally, studies have been conducted on optimising the stocking density, aimed at lowering TAN, nitrite, and phosphorous output in culture water. The stocking density of GIFT tilapia in RAS for minimal release of nitrogen and phosphorus was optimised by Patel (2021), which was found to be 3833 g/m^3 (250 fingerlings/m^3). Thus, the present study aims to optimize growth performance and minimize TAN excretion in RAS culture tanks with high density GIFT stocking.

However, explicit studies on the impact of such dietary interventions in GIFT, particularly in RAS environments, have not been conducted. Therefore, this study seeks to investigate the effects of decreasing dietary protein levels and increasing dietary lipid levels on total ammonia nitrogen excretion by GIFT tilapia reared in RAS. These findings can help improve diet formulations for rearing GIFT in RAS, enhancing both economic and environmental sustainability of tilapia culture with optimal fish growth and water quality.

Materials and Methods

The experimental system used in this study consisted of 18 glass tanks (50 cm x 40 cm x 30 cm, 60 L capacity), each maintained at a water volume of 50 L. Integral to the system was a 1000 L mechanical gravel bed filter, capable of removing particles smaller than 50 μm, which also facilitated biological filtration through nitrification processes (Fig. 1). A submersible pump (50 W, JANISONS PET PRODUCTS, F_{max} = 2500 L/h; H_{max} = 3 m) was used to circulate water from the reservoir tank to the culture tanks at a flow rate of 0.42 m^3/hr. Regulators controlled the water flow in each culture tank (Fig. 1). Regular water replenishment accounted for evaporation losses. Aeration was maintained at adequate levels, and all operational parameters were consistently monitored. Before initiating the experiment, fish were fed to satiation on a daily basis, utilising a control diet.

GIFT (*Oreochromis niloticus*) juveniles of same age and size were procured from MPEDA (Marine Products Export Development Authority), Cochin and stocked into the previously disinfected tanks filled with fresh water in the Fish Nutrition, Biochemistry and Physiology (FNBP) wet lab of

ICAR- Central Institute of Fisheries Education (CIFE), Mumbai. During an acclimatisation period of 15 days, the fishes were fed with a commercial diet of 0.8 mm diameter (35% CP) thrice daily till satiation. This period also allowed the establishment of nitrogenous bacteria essential for system efficacy.

Six hetero-nitrogenous, hetero-caloric experimental diets with graded level of crude protein (CP) and lipid were formulated (Table 1), prepared using practical ingredients (Table 1). Initially, the major ingredients were pulverized, weighed and mixed with CMC (Carboxy methyl cellulose) to form a dough. The dough was then autoclaved for steam cooking and pelletised into 1.2 mm diameter pellets using an automatic pelletizer. All the treatment diets were prepared using the same procedure ans then dried in a tray drier (45^{o}C) to reduce moisture content to less than 10%. The diets were labelled and stored in airtight containers at 4^{o}C until use.

The treatment diets are designated as: T1 (40% CP, 7% lipid), T2 (38% CP, 8% lipid), T3 (36% CP, 9% lipid) T4 (34% CP,10% lipid), T5 (32% CP, 11% lipid) and T6 (30% CP, 12% lipid). However, the protein requirement of Nile Tilapia, with a size of <20g, is 40% (NRC, 2011). Hence, the protein content in the diet T1 was set at 40%, gradually reduced by 2% in each subsequent diet. This variation in protein-lipid levels helped identify the optimal protein-lipid balance for growth and energy needs, minimizing wastage of protein resources.

Each treatment (T1, T2, T3, T4, T5 and T6) was set in triplicates using a completely randomised design (Table 2). The feeding trial was conducted in pre-washed and disinfected rectangular tanks of the RAS system. Initially, the experimental setup was run with a sample fish and a control diet to allow the nitrogenous bacteria to grow before the start of the experiment. A total of 1080 GIFT fingerlings were evenly stocked into each experimental tank, which had a 60L capacity (containing 50L of water), with a stocking density of 6000 g/m^3 (1200 fish/m^3), randomly. Fish were hand-fed thrice daily (9:00, 13:00, and 17:00 hours) to satiation for 60 days.

Determination of TAN, NO_3-N and NO_2-N of culture water were carried out daily (Spectro quant, Germany). Phosphorous was determined using

Table 1. Formulation of different experimental diets prepared to feed GIFT juveniles in RAS

Ingredients (%)	T1	T2	T3	T4	T5	T6
Soybean	30	27.15	24.45	21.65	18.85	16
GNOC	30	27.15	24.45	21.65	18.85	16
MOC	23	23	23	23	23	23
Wheat flour	4.75	4.75	4.75	4.75	4.75	4.75
DORB	6	10.4	14.6	19	23.4	27.90
Veg oil	1.8	2.45	3.05	3.65	4.25	4.85
Fish oil	1.8	2.45	3.05	3.65	4.25	4.85
Vitamin-mineral mixture[1]	1.5	1.5	1.5	1.5	1.5	1.5
CMC[2]	1.08	1.08	1.08	1.08	1.08	1.08
BHT[3]	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride	0.03	0.03	0.03	0.03	0.03	0.03
Stay C[4]	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin E	0.01	0.01	0.01	0.01	0.01	0.01

T1: 40% CP and 7% lipid in the diet; T2: 38% CP and 8% lipid in the diet; T3: 36% CP and 9% lipid in the diet; T4: 34% CP and 10% lipid in the diet; T5: 32% CP and 11% lipid in the diet; T6: 30% CP and 12% lipid in the diet; [1]Composition of the Vitamin-mineral mixture (quantity/kg): Vitamin A, 55,00,000 IU; Vitamin D_3, 11,00,000 IU; Vitamin B_2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Ascorbic acid, 2500 mg; Vitamin B_6, 1,000 mg; Vitamin B_{12}, 6 mg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 mg; Selenium, 125 mg; [2]CMC, carboxymethyl cellulose; [3]BHT, butylated hydroxytoluene; [4]Stay C, ROVIMIX® STAY-C®35 (DSM in Animal Nutrition & Health)

Table 2. Experimental design (completely randomised design) for evaluating the effect of dietary manipulation

Treatments	Particulars
T1	(40% Protein + 7% Lipid)
T2	(38% Protein + 8% Lipid)
T3	(36% Protein + 9% Lipid)
T4	(34% Protein + 10% Lipid)
T5	(32% Protein + 11% Lipid)
T6	(30% Protein + 12% Lipid)

All treatments are in triplicates.

Phosphate Test Kit, Model PO-23 (Dar et al. 2022). Other water quality parameters, such as dissolved oxygen (DO) was examined by using a DO probe (Aquafin, India). The temperature and pH were measured using a digital thermometer (Thermoscientific, USA) and a pH probe (Aquafin, India), respectively.

The initial and final wet weights of the fish were measured after one-day starvation at both the beginning and end of the experiment. During the final sampling (after 60 days), three fish from each tank was anaesthetised by the standard method (clove oil, 50 μL/L) (Jana et al., 2021) and taken for dissection over a cold plate. The tissue samples were collected in 0.25M sucrose solution to prepare a 5% homogenate using a tissue homogenate (REMI Equipments, Mumbai, India). The homogenate was then centrifuged at 5000 rpm at 4°C for 10 min (Prakash et al., 2023), and the supernatant was stored at -20°C for analysis of oxidative stress enzymes.

Specific growth rate (SGR) was calculated using the standard formula (Talukdar et al., 2021).

$$SGR = \frac{\ln \text{ of final wet weight (g)} - \ln \text{ of initial wet weight}}{\text{culture period (days)}} \times 100$$

Superoxide dismutase (SOD) was measured according to the method described by Misra and Fridovich (1972), based on the enzymatic reaction involving the oxidation of epinephrine, which forms a red-coloured adrenochrome. A 50 μL sample was mixed with 1.5 mL of 0.1M carbonate - bicarbonate buffer (pH 10.2) containing 0.057g EDTA. Then, 0.5 mL epinephrine (3mM) was added to initiate the reaction, and the change in optical density (OD) was

Table 3. Antioxidant enzyme activities of GIFT juveniles reared in RAS fed with different experimental diets

Treatments[1]	SOD[2]		Catalase[3]	
	Gill	Liver	Gill	Liver
T1	$11.29^{a} \pm 0.3$	11.97 ± 0.5	$5.83^{a} \pm 0.02$	$8.07^{a} \pm 0.33$
T2	$11.17^{a} \pm 0.11$	11.76 ± 0.82	$5.86^{a} \pm 0.1$	$8.25^{a} \pm 0.16$
T3	$11.21^{a} \pm 0.07$	10.93 ± 0.14	$5.92^{a} \pm 0.05$	$8.48^{ab} \pm 0.2$
T4	$11.52^{ab} \pm 0.24$	12.15 ± 0.61	$5.98^{ab} \pm 0.05$	$8.87^{bc} \pm 0.17$
T5	$11.88^{ab} \pm 0.07$	12.31 ± 0.6	$6.27^{bc} \pm 0.03$	$9.12^{c} \pm 0.08$
T6	$12.15^{b} \pm 0.36$	12.52 ± 0.28	$6.37^{c} \pm 0.21$	$9.36^{c} \pm 0.09$
SEM	0.12	0.22	0.06	0.13
Contrast analysis, *p-value*				
Overall	0.046	0.424	0.010	0.002
Liner	0.004	0.240	0.000	0.000
Quadratic	0.141	0.246	0.186	0.835

All values are expressed as Mean ± SE (n=3). The mean values in the same column with different superscripts differ significantly ($p < 0.05$).

[1]T1: 40% CP and 7% lipid in the diet; T2: 38% CP and 8% lipid in the diet; T3: 36% CP and 9% lipid in the diet; T4: 34% CP and 10% lipid in the diet; T5: 32% CP and 11% lipid in the diet; T6: 30% CP and 12% lipid in the diet; [2]SOD (superoxide dismutase) activity is expressed as 50% inhibition of epinephrine autooxidation/mg protein/min; [3]Catalase activity expressed as nanomoles H_2O_2 decomposed/mg protein/min.

measured immediately at 480nm for 3 min, at 30-second intervals using a Shimadzu–UV spectrophotometer. SOD activity was expressed as the amount of protein required to inhibit 50% of epinephrine auto-oxidation).

Catalase (CAT) was measured using the method described by Takahara et al. (1960). A 10-50 µL tissue homogenate was added to 2.45 mL of 50 mM phosphate buffer (pH 7.0), and the reaction was initiated by adding 1.0 mL of H_2O_2 solution. The kinetic change in absorbance was measured immediately at 240 nm for 3 min at 15-second intervals. CAT activity was expressed as nanomoles of H_2O_2 decomposed per mg protein per minute.

Data analysis was performed using SPSS 22.0 software for Windows. One-way analysis of variance (ANOVA) was applied, and the resultant values were expressed as mean ± standard error. Duncan's multiple range test was used for the post-hoc analysis ($p < 0.05$).

Results & Discussion

The levels of TAN (Fig. 2), NO_2-N (Fig. 3) and NO_3-N (Fig. 4) are graphically represented to understand the variation between the different treatment groups. These parameters exhibited significant differences ($p < 0.05$) with varying levels of protein and lipid in the diets. TAN concentration decreased significantly ($p < 0.05$) from T1 (0.24 ppm) to T6 (0.08 ppm), as dietary protein decreased and lipid levels increased. Similarly, NO_2-N (0.29 to 0.13 ppm) and NO_3-N levels (1.08 to 0.72 ppm) have also followed a similar trend. The reduction in nitrogenous wastes across treatments indicates a response to lower dietary CP. However, phosphorus (PO_4^{3-}) did not show any significant difference ($p > 0.05$) between the different treatment groups (Fig. 5). Water temperature in the culture tanks varied from 27.5ºC to 28.6ºC. The pH ranged between 7.25 to 7.65, while DO values varied from 5.7 to 6.5 ppm throughout the experiment.

The effect of low protein and high lipid levels on the growth of GIFT fingerlings is outlined in Fig. 6. The bar chart compares the SGR across six different treatments (T1 to T6), with significant variations observed ($p < 0.05$). The SGR showed a clear increasing trend ($p < 0.05$) from the T1 group to the T4 group, reaching its peak in T4 group, which was fed a diet containing 32% protein and 10% lipid. However, the SGR declined in the T5 and T6 groups. The lowest SGR values ($p < 0.05$) were found in the T1 group, followed by the T2 group.

The oxidative stress response of the GIFT juveniles, measured by the activities of SOD and CAT in the gills and liver, is represented in Table 3. The SOD activity in the gills followed a linear trend, showing significant differences ($p < 0.05$) across treatments. The highest activity was observed in the T6 group, indicating higher ROS production. In contrast, the SOD activity in the liver did not differ significantly ($p > 0.05$), indicating a consistent anti-oxidative capacity. Similar levels of SOD activity were maintained in the liver in spite of the dietary variations. The CAT activity in both gill and liver has shown an overall and linear trend, with significantly ($p < 0.05$) higher activity in the T5 and T6 groups. The CAT activity did not differ among the initial treatments, i.e. from T1 to T4, indicating that the fish health was in optimal condition at those dietary levels.

Recirculating Aquaculture System (RAS) is a technology primarily designed to conserve resources like water in fish farming. It recycles water by removing dissolved or suspended solid waste from fish culture. Total Ammonia Nitrogen (TAN) is a major dissolved waste released into the aquaculture system. However, it will be converted to nitrite and then to nitrate by nitrifying bacteria in biological

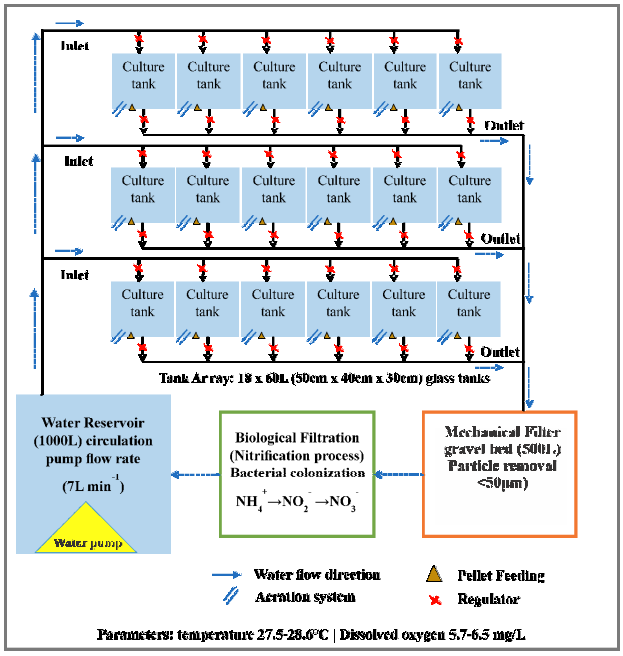

Fig. 1. Layout of experimental RAS Setup

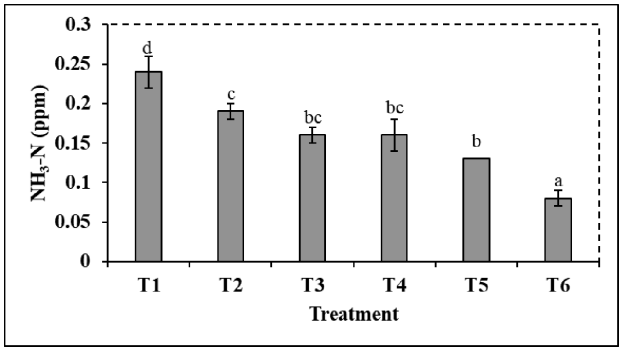

Fig. 2. Ammonia-N release in different treatment groups.

Treatments are represented as follows: T1: 40% CP and 7% lipid in the diet; T2: 38% CP and 8% lipid in the diet; T3: 36% CP and 9% lipid in the diet; T4: 34% CP and 10% lipid in the diet; T5: 32% CP and 11% lipid in the diet; T6: 30% CP and 12% lipid in the diet. Different superscripts above each bar signify statistical differences among treatments (p<0.05).

filters, which are considered to be less toxic. However, excess nitrate in culture tanks can have chronic health and welfare impacts on the fish (Davidson, Good, Welsh, & Summerfelt, 2014). Reduction of nitrate to ammonia through the DNRA (Dissimilatory nitrate reduction to ammonia) pathway (Kamp, Høgslund, Risgaard-Petersen, & Stief, 2015) may also pose a possible risk to the health of cultured fish. DNRA is a biological process by a number of heterotrophic bacteria or some fungi, which involves reduction of nitrate to ammonium under anoxic conditions. Therefore, reducing TAN levels is crucial in enhancing the efficiency of RAS, as it is the most toxic among the dissolved solids generated in RAS.

In this study, the estimated TAN values from different treatment tanks indicate that the fish fed with higher dietary protein levels produced more nitrogenous waste due to increased protein breakdown and excretion. Meanwhile, the group fed with 30% protein and 12% lipid showed the lowest nitrogen excretion, leading to lower TAN levels in the culture water. This suggests that lipids were used as the primary energy source in fish-fed high lipids and low protein. Similarly, NO_2-N and NO_3-N levels decreased from the T1 to T6 group, indicating that the nitrification from ammonia also reduced with reduction in dietary protein and ammonia excretion. However, phosphorus (PO_4^{3-}) levels did not vary ($p > 0.05$) across the different treatment groups, indicating that utilization of the dietary phosphorous by the experimental fish was similar in all the treatment groups.

On the other hand, growth performance was higher in the group fed with 34% protein and 10% lipid. The lower growth rate in the initial treatments may have been due to the higher TAN content in the culture water. Generally, decreasing protein levels in diets leads to decreased growth (Lee, Jeon, & Lee, 2002). However, if the dietary protein is higher than required, it is diverted towards catabolic pathways and used as an energy source, resulting in higher ammonia levels in the culture tanks, which negatively impacts growth. Reduced growth in the

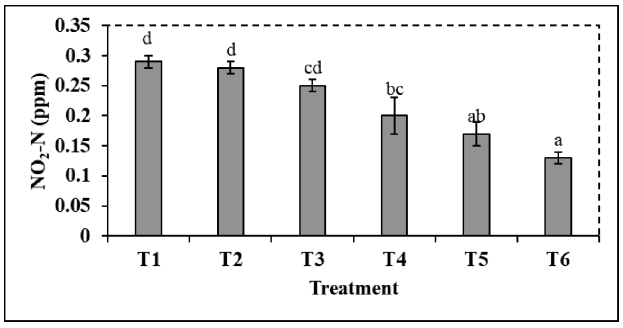

Fig. 3. Nitrite-nitrogen release in different treatment groups.

Treatments are represented as follows: T1: 40% CP and 7% lipid in the diet; T2: 38% CP and 8% lipid in the diet; T3: 36% CP and 9% lipid in the diet; T4: 34% CP and 10% lipid in the diet; T5: 32% CP and 11% lipid in the diet; T6: 30% CP and 12% lipid in the diet. Different superscripts above each bar signify statistical differences among treatments (p<0.05).

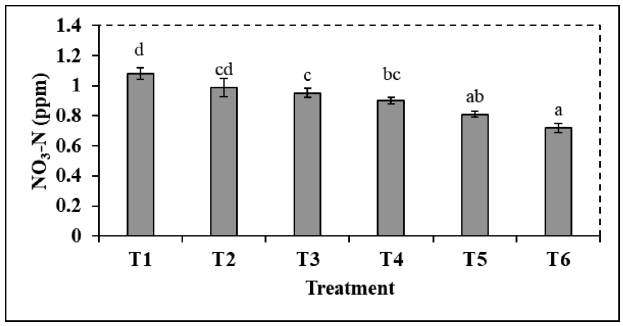

Fig. 4. Nitrate-nitrogen release in different treatment groups.

Treatments are represented as follows: T1: 40% CP and 7% lipid in the diet; T2: 38% CP and 8% lipid in the diet; T3: 36% CP and 9% lipid in the diet; T4: 34% CP and 10% lipid in the diet; T5: 32% CP and 11% lipid in the diet; T6: 30% CP and 12% lipid in the diet. Different superscripts above each bar signify statistical differences among treatments (p<0.05).

groups with lower protein content indicate the failure to meet the nutritional requirements of the cultured fish. These results are consistent with the findings of Li, Liu, Jiang, Zhu, and Ge (2010); Orire and Sadiku (2011); Huang, Wen, Li, Li, & Zhu (2016); Wang et al. (2018); & Godoy-Olmos et al. (2022).

These results indicate the optimisation of the diet along with an effective reduction in nitrogenous waste release into the culture water. The results are consistent with the statements of Huang, Aalto, Dalsgaard, and Pedersen (2024). They found that increasing carbon-to-nitrogen (C:N) ratio in the dietary feed helped in the efficient reduction of TAN release into the Recirculating Aquaculture System (RAS), demonstrating that non-protein energy sources have a protein-sparing effect in rainbow trout (*Oncorhynchus mykiss*). Further, Godoy-Olmos et al. (2022) also explained that the diets optimised to the requirements of the fish help reduce ammonia excretion in the culture tanks. To our knowledge, this study is the pioneering work in optimising dietary protein and lipids for optimal growth of GIFT fingerlings in RAS, which targets the reduction of ammonia excretion by the fingerlings into the culture water.

SOD and CAT are important antioxidant enzymes that indicates the level of stress experienced by fish. SOD serves as the first line of defence against superoxide radicals by converting them into hydrogen peroxide (H_2O_2), which is then further eliminated by CAT (Jiang et al., 2016). In this study, SOD activity in the gills increased only when the fish were fed diets with more than 10% lipids, suggesting an enhanced ability to cope with stress when fed with up to 10% lipids. Notably, SOD activity was higher in the groups fed with low protein and high lipid levels (30-32% protein, 11-12% lipid), indicating an increased oxidative stress burden due to higher dietary lipids or increased oxidative load in peripheral tissues. A similar trend was observed in *Onychostoma macrolepis* (Gou, Chang, Deng, Ji, & Zhou, 2019) and Asian red-tailed catfish (*Hemibagrus wyckioides*) (Deng, Zhang, Sun, Zhang, & Mi, 2021) and in turbot (*Scophthalmus maximus* L.) (Zhang et al., 2022). SOD activity in the liver did not vary across the treatments, which may be due to the fish's ability to scavenge superoxide radicals even in the presence of dietary variations. Similarly, CAT activity in the gills exhibited a pattern analogous to that of SOD activity in the gill, as it converts the H_2O_2 produced by SOD into water and oxygen. Elevated CAT activity in the 11% and 12% lipid-fed groups was an adaptive response to higher oxidative stress (Liu et al., 2023). The high dietary lipids inhibited the antioxidant capacity of GIFT juveniles, resulting in lipid peroxidation and oxidative damage (Sheikhzadeh, Tayefi-Nasrabadi, Oushani, & Enferadi, 2012; Liu et al., 2023). CAT activity in the liver displayed a significant difference ($p < 0.05$), emphasizing the liver's role in detoxifying reactive oxygen species generated by dietary oxidative substrates such as excess lipids. A previous study with similar results inferred that the liver CAT has a greater role in hydrogen peroxide detoxification

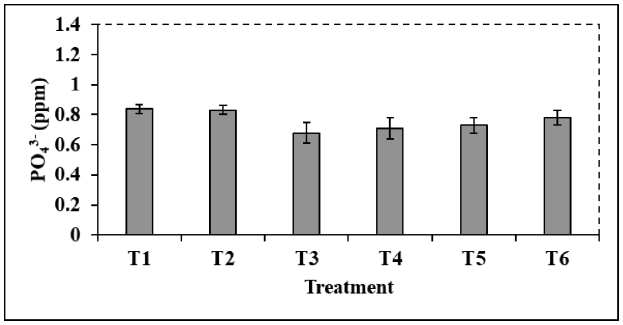

Fig. 5. Phosphorous release in different treatment groups. *Treatments are represented as follows:* T1: 40% CP and 7% lipid in the diet; T2: 38% CP and 8% lipid in the diet; T3: 36% CP and 9% lipid in the diet; T4: 34% CP and 10% lipid in the diet; T5: 32% CP and 11% lipid in the diet; T6: 30% CP and 12% lipid in the diet.

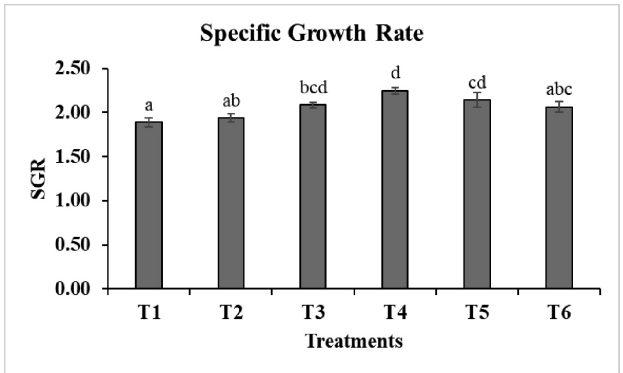

Fig. 6. Specific growth rate of GIFT juveniles reared in RAS for 60 days.

Treatments are represented as follows: T1: 40% CP and 7% lipid in the diet; T2: 38% CP and 8% lipid in the diet; T3: 36% CP and 9% lipid in the diet; T4: 34% CP and 10% lipid in the diet; T5: 32% CP and 11% lipid in the diet; T6: 30% CP and 12% lipid in the diet. Different superscripts above each bar signify statistical differences among treatments ($p<0.05$).

(Liu et al., 2023) when the fish is fed with high-lipid diets, as lipid metabolism predominantly mediated in the liver.

The present study aimed to optimize the diet of GIFT fingerlings by decreasing the protein inclusion and by increasing lipids in recirculatory aquaculture systems. A key focus of the experiment was evaluating the protein-sparing efficiency of lipids, and henceforth, reducing the ammonia load in the system. This strategy helped the fish to use protein efficiently for growth and tissue generation rather than as an energy source, directly reducing the excretion of TAN into the culture tanks. The findings of this study demonstrate that fish fed with a diet comprising 34% dietary protein and 10% dietary lipid (T4) showed better growth and prompted comparatively lower excretion of TAN, NO_2-N, and NO_3-N in the culture tanks. The activity of antioxidant enzymes was also at a comparatively optimal level in the T4 group, confirming the efficacy of this diet in RAS for optimal results.

Ethical statement

The handling and sampling of the fish were done following the guidelines issued by ICAR-CIFE, Mumbai. The harm caused to the fish during the experiment was intentional and solely for research purposes. The fishes were anaesthetised using clove oil (50 μL/L) before sampling, following the research standard protocols (Jana et al., 2021).

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References

Alebachew, S., Kebtieneh, N., Tesfahun, A., Gedefaw, T., Yilma, T., & Abiyu, A. (2022). Effects of different stocking densities on the growth performance of Nile tilapia (*Oreochromis niloticus* L) fingerlings cultured in earthen pond system. *Ethiopian Journal of Applied Science and Technology*, *13*(1), 56–70.

Badiola, M., Basurko, O. C., Piedrahita, R., Hundley, P., & Mendiola, D. (2018). Energy use in recirculating aquaculture systems (RAS): A review. *Aquacultural Engineering*, *81*, 57–70. https://doi.org/10.1016/j.aquaeng.2018.03.003.

Boyd, C. E. (2003). Guidelines for aquaculture effluent management at the farm level. *Aquaculture*, *226*(1–4), 101–112. https://doi.org/10.1016/S0044-8486(03)00471-X.

Dar, S. A., Bhat, S. U., & Dar, S. A. (2022). Wetland ecosystem monitoring through water quality perspectives. In A. K. Rathoure (Ed.), *Handbook of research on monitoring and evaluating the ecological health of wetlands* (pp. 51–70). IGI Global.

Davidson, J., Good, C., Welsh, C., & Summerfelt, S. T. (2014). Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout (*Oncorhynchus mykiss*) within water recirculating aquaculture systems. *Aquacultural Engineering*, *59*, 30–40. https://doi.org/10.1016/j.aquaeng.2014.01.003.

Deng, J., Zhang, X., Sun, Y., Zhang, L., & Mi, H. (2021). Optimal dietary lipid requirement for juvenile Asian red-tailed catfish (*Hemibagrus wyckioides*): Dietary lipid level for Hemibagrus wyckioides. *Aquaculture Reports*, *20*, Article 100666. https://doi.org/10.1016/j.aqrep.2021.100666.

El-Sayed, A. F. M. (2006). *Tilapia culture*. CABI Publishing.

Godoy-Olmos, S., Jauralde, I., Monge-Ortiz, R., Milián-Sorribes, M. C., Jover-Cerdá, M., Tomás-Vidal, A., & Martínez-Llorens, S. (2022). Influence of diet and feeding strategy on the performance of nitrifying trickling filter, oxygen consumption and ammonia excretion of gilthead sea bream (*Sparus aurata*) raised in recirculating aquaculture systems. *Aquaculture International*, *30*(2), 581–606. https://doi.org/10.1007/s10499-021-00821-3.

Gou, N., Chang, Z., Deng, W., Ji, H., & Zhou, J. (2019). Effects of dietary lipid levels on growth, fatty acid composition, antioxidant status and lipid metabolism in juvenile *Onychostoma macrolepis*. *Aquaculture Research*, *50*(11), 3369–3381. https://doi.org/10.1111/are.14295.

Gullian-Klanian, M., & Arámburu-Adame, C. (2013). Performance of Nile tilapia *Oreochromis niloticus* fingerlings in a hyper-intensive recirculating aquaculture system with low water exchange. *Latin American Journal of Aquatic Research*, *41*(1), 150–162. https://doi.org/10.3856/vol41-issue1-fulltext-12.

Huang, X., Aalto, S. L., Dalsgaard, J., & Pedersen, P. B. (2024). Can dietary C:N ratio influence water quality and microbiology in recirculating aquaculture systems? *Aquaculture International*, *32*, 7789-7805. https://doi.org/10.1007/s10499-024-01541-0.

Huang, Y., Wen, X., Li, S., Li, W., & Zhu, D. (2016). Effects of dietary lipid levels on growth, feed utilisation, body composition, fatty acid profiles and antioxidant parameters of juvenile chu's croaker *Nibea coibor*.

Aquaculture International, *24*, 1229–1245. https://doi.org/10.1007/s10499-016-9980-5.

Jana, P., Sahu, N. P., Sardar, P., Shamna, N., Varghese, T., Deo, A. D., Harikrishna, V., Paul, M., Panmei, H., Gupta, G., Nanda, C., & Krishna, G. (2021). Dietary protein requirement of white shrimp, *Penaeus vannamei* (Boone, 1931) juveniles, reared in inland ground water of medium salinity. *Aquaculture Research*, *52*(6), 2501–2517. https://doi.org/10.1111/are.15100.

Jiang, W. D., Wen, H. L., Liu, Y., Jiang, J., Wu, P., Zhao, J., Kuang, S. Y., Tang, L., Tang, W. N., Zhang, Y. A., Zhou, X. Q., & Feng, L. (2016). Enhanced muscle nutrient content and flesh quality, resulting from tryptophan, is associated with anti-oxidative damage referred to the Nrf2 and TOR signalling factors in young grass carp (*Ctenopharyngodon idella*): Avoid tryptophan deficiency or excess. *Food Chemistry*, *199*, 210–219. https://doi.org/10.1016/j.foodchem.2015.12.003.

Joseph, I., & Augustine, A. (2020). Marine biotechnology for food. In Y. S. Malik, D. Barh, V. Azevedo, & S. P. Khurana (Eds.), *Genomics and biotechnological advances in veterinary, poultry, and fisheries* (pp. 271-283). Academic Press.

Kamp, A., Høgslund, S., Risgaard-Petersen, N., & Stief, P. (2015). Nitrate storage and dissimilatory nitrate reduction by eukaryotic microbes. *Frontiers in Microbiology*, *6*, Article 1492. https://doi.org/10.3389/fmicb.2015.01492.

Kaushik, S. J., Doudet, T., Médale, F., Aguirre, P., & Blanc, D. (1995). Protein and energy needs for maintenance and growth of Nile tilapia (*Oreochromis niloticus*). *Journal of Applied Ichthyology*, *11*(3–4), 290–296. https://doi.org/10.1111/j.1439-0426.1995.tb00029.x.

Lee, S. M., Jeon, I. G., & Lee, J. Y. (2002). Effects of digestible protein and lipid levels in practical diets on growth, protein utilisation and body composition of juvenile rockfish (*Sebastes schlegeli*). *Aquaculture*, *211*(1–4), 227–239. https://doi.org/10.1016/S0044-8486(01)00880-8.

Li, W., Wen, X., Zhao, J., Li, S., & Zhu, D. (2016). Effects of dietary protein levels on growth, feed utilisation, body composition and ammonia-nitrogen excretion in juvenile *Nibea diacanthus*. *Fisheries Science*, *82*, 137–146. https://doi.org/10.1007/s12562-015-0945-9.

Li, X. F., Liu, W. B., Jiang, Y. Y., Zhu, H., & Ge, X. P. (2010). Effects of dietary protein and lipid levels in practical diets on growth performance and body composition of blunt snout bream (*Megalobrama amblycephala*) fingerlings. *Aquaculture*, *303*(1–4), 65–70. https://doi.org/10.1016/j.aquaculture.2010.03.014.

Liu, Y., Huang, E., Xie, Y., Meng, L., Liu, D., Zhang, Z., Zhou, J., Zhang, Q., & Tong, T. (2023). The effect of dietary lipid supplementation on the serum biochemistry, antioxidant responses, initial immunity, and mTOR pathway of juvenile tilapia (*Oreochromis niloticus*). *Fishes*, *8*(11), Article 535. https://doi.org/10.3390/fishes8110535.

Martins, C. I. M., Eding, E. H., Verdegem, M. C. J., Heinsbroek, L. T. N., Schneider, O., Blancheton, J. P., d'Orbcastel, E. R., & Verreth, J. A. J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering*, *43*(3), 83–93. https://doi.org/10.1016/j.aquaeng.2010.09.002.

Mihalca, G. L., Tita, O., Tita, M., & Mihalca, A. (2010). A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *International Multidisciplinary Scientific GeoConference*, *2*, 369-378.

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, *247*(10), 3170–3175. https://doi.org/10.1016/S0021-9258(19)45228-9.

Mota, V. C., Limbu, P., Martins, C. I. M., Eding, E. H., & Verreth, J. A. J. (2015). The effect of nearly closed RAS on the feed intake and growth of Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and European eel (*Anguilla anguilla*). *Aquacultural Engineering*, *68*, 1–5. https://doi.org/10.1016/j.aquaeng.2015.06.002.

National Research Council [NRC]. (2011). *Nutrient requirements of fish and shrimp*. The National Academies Press.

Orire, A. M., & Sadiku, S. O. E. (2011). Protein sparing effects of lipids in the practical diets of *Oreochromis niloticus* (Nile tilapia). *Nigerian Journal of Basic and Applied Sciences*, *19*(1). https://doi.org/10.4314/njbas.v19i1.69360.

Patel, E. A. (2021). *Evaluation of dietary nitrogen and phosphorus in recirculating aquaculture system of genetically improved farmed tilapia* (M.F.Sc. Dissertation, ICAR-Central Institute of Fisheries Education). ICAR eLibrary.

Prakash, P., Kumar, S., Sardar, P., Munilkumar, S., Sahoo, S., Satheesh, M., Reena, H., Mannur, V., & Patel, A. (2023). Optimisation of weaning strategy in the climbing perch (*Anabas testudineus*, Bloch 1792) larvae on growth, survival, digestive, metabolic and stress responses. *Fish Physiology and Biochemistry*, *49*(6), 1151–1169. https://doi.org/10.1007/s10695-023-01248-8.

Sagada, G., Chen, J., Shen, B., Huang, A., Sun, L., Jiang, J., & Jin, C. (2017). Optimising protein and lipid levels in practical diet for juvenile northern snakehead fish (*Channa argus*). *Animal Nutrition*, *3*(2), 156–163. https://doi.org/10.1016/j.aninu.2017.03.003.

Sheikhzadeh, N., Tayefi-Nasrabadi, H., Oushani, A. K., &

Enferadi, M. H. N. (2012). Effects of *Haematococcus pluvialis* supplementation on antioxidant system and metabolism in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, *38*, 413–419. https://doi.org/10.1007/s10695-011-9519-7.

Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y., & Nishimura, E. T. (1960). Hypocatalasemia: A new genetic carrier state. *The Journal of Clinical Investigation*, *39*(4), 610–619. https://doi.org/10.1172/JCI104075.

Talukdar, A., Deo, A. D., Sahu, N. P., Sardar, P., Aklakur, M. D., & Prakash, S. (2021). Effects of different suboptimal dietary protein levels on growth, nutrient utilisation and physio metabolic status of *Anabas testudineus* fingerlings in inland saline water. *Aquaculture Nutrition*, *27*(6), 2673–2689. https://doi.org/10.1111/anu.13394.

Teles, A. O., Couto, A., Enes, P., & Peres, H. (2020). Dietary protein requirements of fish – A meta analysis. *Reviews in Aquaculture*, *12*(3), 1445–1477. https://doi.org/10.1111/raq.12391.

Thirunavukkarasar, R., Kumar, P., Sardar, P., Sahu, N. P., Harikrishna, V., Singha, K. P., Shamna, N., Jacob, J., & Krishna, G. (2022). Protein-sparing effect of dietary lipid: Changes in growth, nutrient utilisation, digestion and IGF-I and IGFBP-I expression of genetically improved farmed tilapia (GIFT), reared in inland ground saline water. *Animal Feed Science and Technology*, *284*, Article 115150. https://doi.org/10.1016/j.anifeedsci.2021.115150.

TNAU Agritech Portal. (2015). *Tilapia fish culture*. Retrieved from https://agritech.tnau.ac.in/banking/nabard_pdf/Fisheries/6.GIFT_Tilapia_culture_15.pdf. (Accessed on October 22, 2024)

Wang, L., Hu, S., Lou, B., Chen, D., Zhan, W., Chen, R., Liu, F., & Xu, D. (2018). Effect of different dietary protein and lipid levels on the growth, body composition, and intestinal digestive enzyme activities of juvenile yellow drum *Nibea albiflora* (Richardson). *Journal of Ocean University of China*, *17*, 1261–1267. https://doi.org/10.1007/s11802-018-3660-1.

Zhang, W., Dan, Z., Zhuang, Y., Zheng, J., Gong, Y., Liu, Y., Mai, K., & Ai, Q. (2022). Effects of dietary lipid levels on growth, digestive enzyme activities, antioxidant capacity, and lipid metabolism in turbot (*Scophthalmus maximus* L.) at three different stages. *Aquaculture Nutrition*, *2022*(1), Article 1042263. https://doi.org/10.1155/2022/1042263.