

# Elevated rearing temperatures increase dietary lipid requirements in GIFT tilapia, *Oreochromis niloticus* (Linnaeus, 1758)

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## Abstract

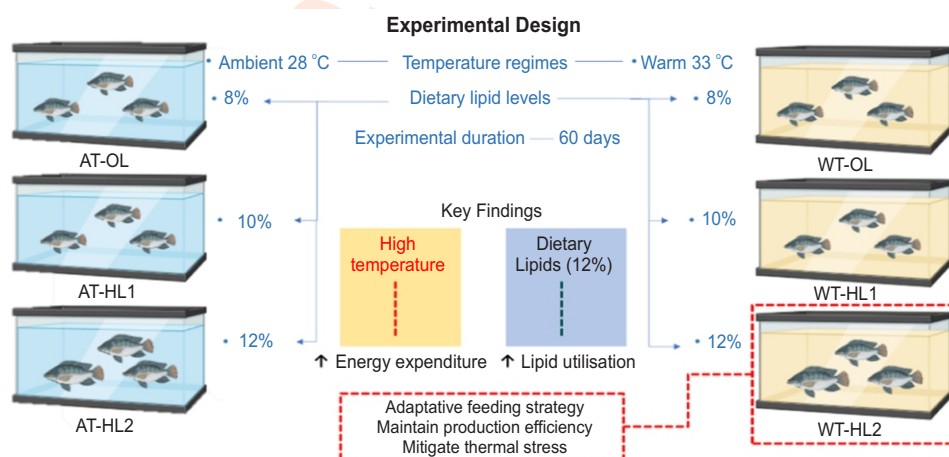
**Aim:** This study investigated the effects of dietary lipid levels and temperature on growth performance, lipid metabolism, and physiological responses in GIFT tilapia juveniles, aiming to optimise feeding strategies under climate warming scenarios.

**Methodology:** GIFT tilapia juveniles were cultured for 60 days under two temperature regimes (ambient: 28°C, warm: 33°C) and three dietary lipid levels (8%, 10%, 12%). Growth parameters, body composition, serum biochemistry, antioxidant enzymes, and liver histology were analysed. A Two-way ANOVA was used to assess the effects of temperature, dietary lipids, and their interaction.

**Results:** The 12% lipid diet yielded the highest weight gain and thermal growth coefficient at both temperatures, but growth was reduced across all diets at 33°C. Warm temperature decreased body lipids, lipase activity, and intraperitoneal fat index. Serum protein and globulin levels increased at 33°C, while glucose and cortisol levels rose with increased dietary lipids and temperature. High temperature negatively affected the antioxidant enzyme activities but was enhanced by higher dietary lipids. Liver histology revealed better regulation of hepatocyte size at 33°C with increasing dietary lipids.

**Interpretation:** The study demonstrates that higher dietary lipids could be utilised to enhance the growth of GIFT tilapia in the wake of thermal stress. The complex interactions observed between temperature and diet in regulating lipid metabolism and physiological responses suggest that tilapia farmers may need to adjust feed formulations based on water temperature. With a rise in global temperature, increasing dietary lipid levels may help meet higher energy requirements and support stress adaptation in warmer conditions. This research provides crucial insights for optimising tilapia production in the face of climate change.

**Key words:** Climate change adaptation, Dietary lipids, Liver histology, Oxidative stress, Thermal stress



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## Introduction

Climate models predict a global average temperature increase of 1.5°C to 3.2°C by 2100, altering the aquatic ecosystems and affecting wild and farmed fish populations (IPCC, 2023). Climate change is pushing marine species toward cooler polar regions, degrading coral reefs, causing ocean acidification, and thereby disrupting the food webs and marine reproduction. (Wang *et al.*, 2024). These changes threaten wild fish populations and pose significant risks to aquaculture, as many farmed species may find it increasingly difficult to thrive in the altered environmental conditions. For example, species such as shrimp and tilapia, crucial to many aquaculture industries, may experience stress due to rising temperatures and changing water chemistry, leading to reduced growth rates and higher susceptibility to diseases (Maulu *et al.*, 2021). This jeopardises the viability of aquaculture operations that depend on specific water conditions and fish stocks like open water systems, cages, etc. The intertwining effects of climate impact on both wild and farmed species may also exacerbate the struggles of communities reliant on fishing or farming for their livelihoods and nutrition.

As ectotherms, fish body temperatures fluctuate with water temperature, affecting their metabolism, enzyme activity, and overall physiology. Rising temperatures increase metabolic rates, energy demands and oxygen consumption (Das *et al.*, 2018). This affects feed intake, digestion rates, nutrient utilisation efficiency and growth rates, typically improving up to an optimal temperature before declining (Volkoff and Rønnestad, 2020). Environmental stressors affect the respiratory rate and cause significant alterations in the behaviour of fish (Varghese *et al.*, 2019). Higher temperatures also trigger stress responses, potentially compromising growth and immune function (Skevaki *et al.*, 2024). Consequently, fish may exhibit altered nutrient requirements and preferences at elevated temperatures to sustain increased metabolic demands. Optimising fish nutrition and management in response to these changes is crucial for maintaining sustainable aquaculture production in a warming climate. This study examines the impact of increased water temperature and varying dietary lipid levels on GIFT tilapia (*Oreochromis niloticus*), a globally important farmed species.

While the fish have an optimal rearing temperature of around 28°C (Ma *et al.*, 2015), they often face higher temperatures in various farming conditions and natural waters (Nivelle *et al.*, 2019). They have been selectively bred for rapid growth and adaptability (Bhosle *et al.*, 2022), making them an ideal model for studying responses to temperature changes over short periods, with direct industry relevance. Their ability to tolerate and utilise higher dietary lipid levels than many fish species makes them particularly suitable for investigating lipid-temperature interactions (He *et al.*, 2015). Additionally, their metabolic flexibility allows for dietary adjustments, which might mitigate the effect of environmental stressors (Wang *et al.*, 2022). Lipids are crucial for fish metabolism, providing concentrated energy and forming essential cell membrane components. They

influence cellular processes like nutrient uptake and hormone signalling (Anderson *et al.*, 2011). Optimal dietary lipid levels for tilapia typically range from 5-12%, varying with species, life stage and environment (FAO, 1997). Warmer waters may increase lipid requirements due to higher metabolic demands. In intensive farming, higher lipid levels can support increased growth rates and have a protein-sparing effect. This study examines how dietary lipid concentrations (8%, 10%, 12%) interact with ambient (28°C) and elevated warm (33°C) temperatures, affecting tilapia growth, metabolism and physiology. By analysing growth performance, body composition, serum biochemistry, antioxidant enzyme activity, and liver histology, the research aims to elucidate the dietary lipid requirements of GIFT tilapia at elevated environmental temperatures.

## Materials and Methods

**Ethical approval:** The fish utilized in this study is a food fish that does not necessitate approval from the animal ethics committee in India. Nevertheless, the statutory authorities of the ICAR-Central Institute of Fisheries Education in Mumbai, India, recognized under Section 3 of the University Grants Commission Act of 1956 and certified in 2008, approved the synopsis of this research as part of student research in 2021 (No: FNT-PB0-01).

**Experimental design:** GIFT (*O. niloticus*) juveniles collected from MPEDA-RGCA, Kochi, were transported to ICAR-Central Institute of Fisheries Education, Mumbai, India. Fish were acclimated for 20 days in a 1000l FRP tank filled with fresh water. They were fed twice daily at 3% body weight with a commercial diet (35% crude protein, 5% crude lipid) from Growel Feeds Pvt. Ltd., India. The study utilised a freshwater recirculating aquaculture system (RAS) with 18 identical units. Each unit consisted of a 100l rectangular tank (0.81x0.57x0.39 m<sup>3</sup>) connected to a gravel bed biofilter with regulated water recirculation. The water recirculation system was programmed to operate on a cyclical timer, engaging for 10 min followed by a 20 min cessation, ensuring optimal filtration efficiency. This process thus ran for approximately 4 hr daily, allowing sustainable operation while maintaining the desired water quality within the system (Harika *et al.*, 2023). This design represents a slight modification of a previously standardized setup in our institute, specifically tailored to enhance tilapia culture within the RAS framework (Hussain *et al.*, 2015). Water was replenished to offset evaporation. All tanks were equipped with uniform, sufficient aeration. For the trial, the tilapia juveniles were randomly distributed into the tanks (18 RAS tanks, 10 fish per tank). They were exposed to two temperatures: ambient temperature (AT) of 28.3 ± 0.022°C, serving as a control and a warm temperature (WT) of 33.3 ± 0.07°C, simulating a 5°C rise from the ambient temperature. The WT groups were gradually acclimated to the target temperature of 33°C using submersible glass automatic aquarium heaters (200W, Zhongshan RISHENG Electrical Product Co., Ltd., China), increasing the temperature by 1°C each day until the target was reached. Based on FAO (1997), recommendations for GIFT tilapia fingerlings, 34% protein and

**Table 1:** Formulation and proximate composition of experimental diets with varying lipid levels for GIFT *Tilapia juveniles* reared at ambient (28°C) and warming temperatures (33°C) for a period of 60 days

Ingredient composition (g kg <sup>-1</sup> )	Diets		
	OL	HL1	HL2
Fish meal	50	50	50
Soybean meal	250	250	250
Groundnut Oil cake	204	204	204
Mustard Oil cake	110	110	110
Wheat flour	160.6	140.6	119.6
Corn flour	50	50	50
De-oiled Rice bran	100	100	100
Soybean Oil	22	32	42.5
Fish oil	22	32	42.5
Vitamin mineral mixture	15	15	15
Carboxymethyl cellulose	15	15	15
Butylated hydroxy toluene	0.2	0.2	0.2
Choline chloride	0.9	0.9	0.9
Stay Cb	0.2	0.2	0.2
Vitamin E	0.12	0.12	0.12
Total	1000	1000	1000
Proximate composition (on dry matter basis, g kg <sup>-1</sup> )			
Dry matter	934.2	935.5	944.9
Crude protein	341.5	344.2	341.1
Ether extract	82.2	100.5	120.7
Nitrogen-free extract c	440.8	418.9	401.9
Crude fiber	61.7	58.3	55.7
Total Ash	73.8	78.1	80.6
Digestible energy (MJ kg <sup>-1</sup> )	15.9	16.3	16.7

<sup>a</sup>Composition of the Vitamin-mineral mixture (quantity kg<sup>-1</sup>): Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin E, 750 mg; Vitamin K, 1000 mg; Ascorbic acid, 2500 mg; Vitamin B2, 2000 mg; Vitamin B6, 1000 mg; Vitamin B12, 6 mg; Calcium pantothenate, 2500 mg; Nicotinamide, 10 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 mg; Selenium, 125 mg. <sup>b</sup>Stay C-L ascorbate 2-triphosphate Calcium salt, HiMedia Ltd, India. <sup>c</sup>Nitrogen free extract (g kg<sup>-1</sup>) = 1000 - (crude protein (g kg<sup>-1</sup>) + crude fat (g kg<sup>-1</sup>) + crude fibre (g kg<sup>-1</sup>) + Total ash (g kg<sup>-1</sup>).

8% lipid were used as the basal dietary requirement levels. Two additional lipid levels (10% and 12%) were tested at both temperatures. This design resulted in six treatment groups: AT-OL, AT-HL1, AT-HL2, WT-OL, WT-HL1 and WT-HL2, where AT and WT represent ambient and warm temperatures, respectively, and OL, HL1 and HL2 represent optimal lipid level of 8%, high lipid level of 10% and high lipid level of 12%, respectively. The 60-day feeding trial employed a completely randomised design (CRD).

**Diet formulation and feeding protocol:** Three isonitrogenous diets with varying lipid levels were formulated (Table 1). Heat-stable ingredients were mixed, autoclaved, and then combined with heat-labile ingredients. The mixture was pelletised (1.2 mm diameter), dried to <10% moisture, and stored at 4°C. Fish were fed these diets at 3% biomass, twice daily at 9 am and 5 pm throughout the trial.

**Sample collection and preservation:** After a 24 hr fasting period, all fish were counted and weighed. Two fish per tank were sampled for body lipid analysis. Three fish per replicate were anaesthetised with clove oil (50 µl l<sup>-1</sup>), and blood was collected via caudal venipuncture for serum analysis. The intestine and liver

from two fish were homogenised in chilled phosphate buffer, pH 7.5 in sucrose (0.25M), centrifuged, and the supernatant was stored at -20°C for enzyme assays.

**Growth performance, feed utilisation measurements and body fat index:** Growth performance and feed utilisation were assessed following the standard protocols described in NRC, (2011). Initial and final weights were measured to the nearest 0.1 g. Feed intake was recorded daily throughout the 60-day experimental period. Water temperature was monitored and recorded daily to calculate the thermal growth coefficient. The collected peritoneal fat was weighed to the nearest 0.001 g to calculate the intraperitoneal fat index.

**Serum parameters:** Serum analysis was conducted using various methods. Cortisol levels were measured using a solid phase competitive ELISA kit (Calbiotech, Inc.). Standard diagnostic kits (ERBA®, Transasia Bio-medicals Ltd., Solan, HP, India) were used to determine glucose (Trinder's method, Penncock *et al.*, 1973), total protein (Direct biuret, Gornall, 1949) and albumin levels (Bromocresol green method, Doumas *et al.*, 1972; Burtis, 1986). Globulin was calculated as the difference

between total protein and albumin levels, while the Albumin: Globulin (A: G) ratio was derived by dividing the albumin level by the globulin level.

**Enzyme assays:** Lipase activity was measured spectrophotometrically using para nitrophenyl acetate (PNPA) as a substrate, following the modified version of Gomes *et al.* (2011) protocol. Lipase activity was expressed as  $\mu$  mole of para nitrophenol (PNP) released  $\text{mg protein}^{-1} \text{min}^{-1}$ . Superoxide dismutase (SOD) activity was determined following the method of Misra and Fridovich (1972). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of epinephrine auto-oxidation. Results were expressed as units of SOD activity  $\text{mg protein}^{-1}$ . Catalase activity was measured following the method of Takahara *et al.* (1960). One unit of catalase activity was defined as the amount of enzyme required to decompose 1  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2 \text{min}^{-1}$ . The enzyme activity was expressed as unit activity  $\text{mg protein}^{-1}$ . Lowry method was employed to determine the protein content (Lowry *et al.*, 1951) of the tissues used for enzyme assay.

**Body lipid analysis:** The whole-body lipid composition of fish was determined by the ether extraction method with a Soxhlet apparatus (Socs Plus-SCS 08 AS, Pelican, India). Fish samples

were oven-dried at  $105^\circ\text{C}$  for 24 hr and ground to a fine powder. A known weight of the powdered sample was placed in a cellulose thimble and extracted with diethyl ether for 1.5 hr in the Soxhlet apparatus. After extraction, the solvent was evaporated, and the extracted lipid was weighed. The lipid content was calculated as a percentage of dry weight of fish sample (AOAC, 1995).

The lipid content was expressed as: Crude Lipid (%) =  $[\text{Weight of ether extract} \times (\text{Weight of dry sample})^{-1}] \times 100$

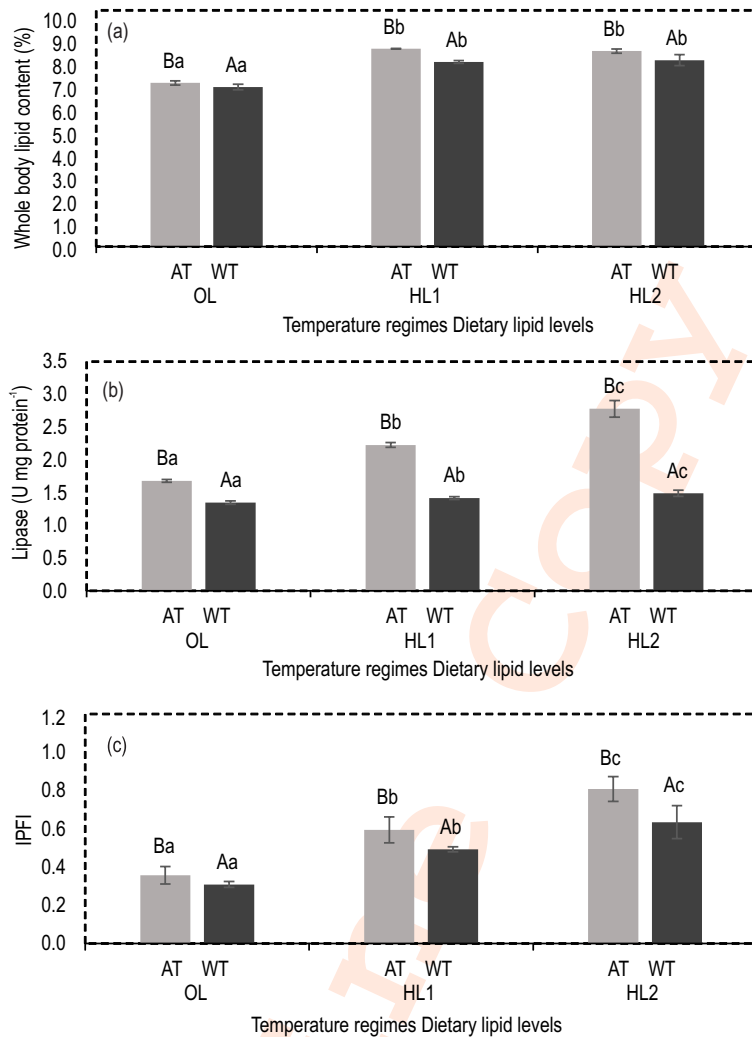
**Histological examination:** Liver tissue samples were fixed in 10% neutral buffered formalin for 24 hr (Luna, 1968). Fixed tissues were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 5  $\mu\text{m}$  thickness were cut using a rotary microtome and mounted on glass slides. Following the standard protocols, the sections were deparaffinised, rehydrated and stained with Harris' hematoxylin and eosin (HandE) (Bancroft and Gamble, 2008). Stained sections were examined under a light microscope and photographed for analysis of tissue structure and cellular morphology.

**Statistical analyses:** Statistical analysis was performed using SPSS 22.0. Two-way ANOVA was applied, followed by Duncan's Multiple Range Test for post hoc comparisons. Differences were considered significant at  $P < 0.05$ .

**Table 2:** Effects of temperature and dietary lipid levels on growth performance and feed efficiency in GIFT Tilapia juveniles reared at ambient ( $28^\circ\text{C}$ ) and warm temperatures ( $33^\circ\text{C}$ ) for a period of 60 days

One-way ANOVA							
Treatments <sup>1</sup>	IW2 (g)	FW3 (g)	WG4 (g)	WGP5 (%)	TGC6	FER7	Survival
AT-OL	8.32 $\pm$ 0.14	27.35 <sup>b</sup> $\pm$ 0.12	18.82 <sup>b</sup> $\pm$ 0.10	212.44 <sup>a</sup> $\pm$ 1.10	0.56 <sup>d</sup> $\pm$ 0.001	0.53 <sup>b</sup> $\pm$ 0.002	93.33 $\pm$ 6.67
AT-HL1	8.29 $\pm$ 0.17	32.84 <sup>e</sup> $\pm$ 0.25	24.27 <sup>e</sup> $\pm$ 0.33	283.16 <sup>c</sup> $\pm$ 2.90	0.68 <sup>e</sup> $\pm$ 0.006	0.65 <sup>d</sup> $\pm$ 0.010	90.00 $\pm$ 5.77
AT-HL2	8.37 $\pm$ 0.12	35.66 <sup>f</sup> $\pm$ 0.23	26.99 <sup>f</sup> $\pm$ 0.17	316.08 <sup>d</sup> $\pm$ 2.75	0.73 <sup>f</sup> $\pm$ 0.003	0.73 <sup>e</sup> $\pm$ 0.004	90.00 $\pm$ 5.77
WT-OL	8.62 $\pm$ 0.03	26.78 <sup>b</sup> $\pm$ 0.09	18.14 <sup>a</sup> $\pm$ 0.05	199.05 <sup>a</sup> $\pm$ 10.15	0.48 <sup>b</sup> $\pm$ 0.002	0.43 <sup>b</sup> $\pm$ 0.012	93.33 $\pm$ 6.67
WT-HL1	8.62 $\pm$ 0.03	29.42 <sup>c</sup> $\pm$ 0.08	20.89 <sup>c</sup> $\pm$ 0.12	243.33 <sup>b</sup> $\pm$ 0.97	0.52 <sup>b</sup> $\pm$ 0.002	0.57 <sup>c</sup> $\pm$ 0.003	93.33 $\pm$ 3.33
WT-HL2	8.3 $\pm$ 0.17	30.49 <sup>d</sup> $\pm$ 0.09	21.94 <sup>d</sup> $\pm$ 0.06	255.78 <sup>b</sup> $\pm$ 1.09	0.54 <sup>c</sup> $\pm$ 0.001	0.60 <sup>c</sup> $\pm$ 0.003	90.00 $\pm$ 5.77
p-value	0.234	0.000	0.000	0.000	0.000	0.000	0.990
Two-way ANOVA							
Effect of temperature variable							
AT	8.326	31.954 <sup>b</sup>	23.359 <sup>b</sup>	270.561 <sup>b</sup>	0.656 <sup>b</sup>	0.638 <sup>b</sup>	91.111
WT	8.513	28.901 <sup>a</sup>	20.320 <sup>a</sup>	232.718 <sup>a</sup>	0.514 <sup>a</sup>	0.534 <sup>a</sup>	92.22
SEM	0.097	0.094	0.097	2.608	0.002	0.004	3.333
p-value	0.091	0.000	0.000	0.000	0.000	0.000	0.818
Effect of dietary lipid levels							
OL	8.470	27.068 <sup>a</sup>	18.479 <sup>a</sup>	205.744 <sup>a</sup>	0.520 <sup>a</sup>	0.480 <sup>a</sup>	93.333
HL1	8.452	31.135 <sup>b</sup>	22.578 <sup>b</sup>	263.242 <sup>b</sup>	0.601 <sup>b</sup>	0.613 <sup>b</sup>	91.667
HL2	8.336	33.080 <sup>c</sup>	24.462 <sup>c</sup>	285.933 <sup>c</sup>	0.635 <sup>c</sup>	0.665 <sup>c</sup>	90.000
SEM	0.088	0.115	0.119	3.194	0.002	0.005	4.082
p-value	0.521	0.000	0.000	0.000	0.000	0.000	0.848
Interaction of temperature* Dietary lipids							
p-value	0.000	0.238	0.000	0.001	0.000	0.002	0.946

Statistical data presented as mean  $\pm$  standard error, with sample size of 3. Within each column, averages marked with distinct superscript letters indicate statistically significant differences ( $p < 0.05$ ) between experimental groups. 1Treatments combine two temperature regimes (AT:  $28^\circ\text{C}$ , WT:  $33^\circ\text{C}$ ) with three dietary lipid levels (OL: 8%, HL1: 10%, HL2: 12% crude lipid), yielding six experimental groups. AT represents ambient temperature, while WT simulates warming conditions. 2Initial weight, 3Final weight, 4Weight gain, 5Weight gain percentage, 6Thermal growth coefficient, 7Feed efficiency ratio



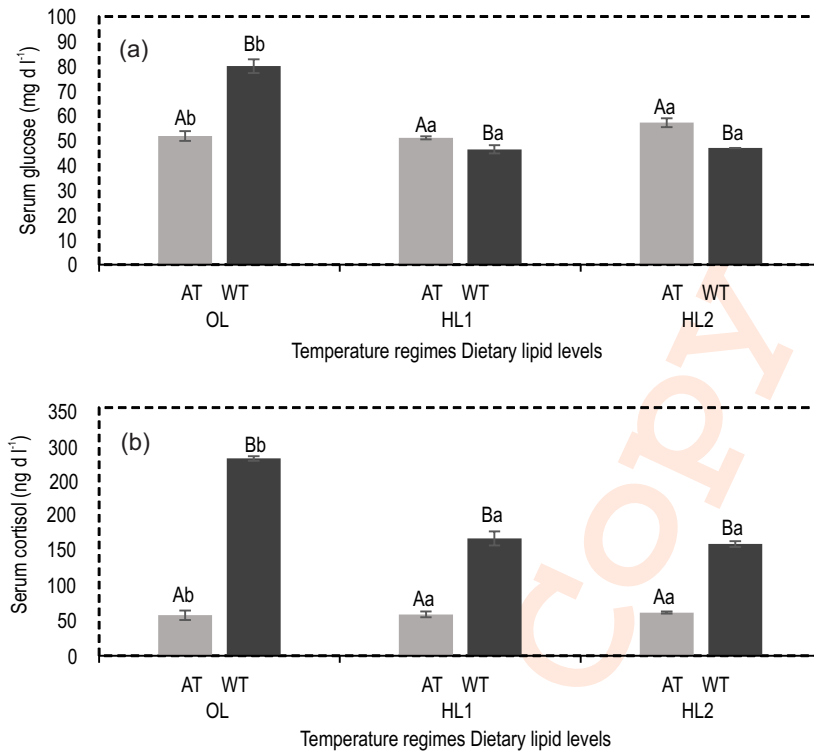
**Fig. 1:** Effect of temperature and dietary lipid levels on whole body lipid content (A), Lipase activity (B), and Intraperitoneal fat index, IPFI (C) of GIFT Tilapia juveniles reared at ambient (28°C) and warm temperatures (33°C) for a period of 60 days. Statistical data presented as mean  $\pm$  standard error, with sample size of 6. Treatments combine two temperature regimes (AT: 28°C, WT: 33°C) with three dietary lipid levels (OL: 8%, HL1: 10%, HL2: 12% crude lipid), yielding six experimental groups. Lipase activity measured in  $\mu\text{mol}$  of p-nitrophenol released per mg of protein per minute. Different superscripts in lowercase above each bar of the same group signify statistical differences in dietary lipids ( $P < 0.05$ ). Different superscripts in uppercase above each bar of the same group signify statistical significance of temperature differences ( $P < 0.05$ ).

## Results and Discussion

The temperature and dietary lipid levels independently and interactively influenced the growth performance and feed efficiency of GIFT tilapia juveniles (Table 2). Two-way ANOVA results suggested that the warm temperature groups showed significantly lower weight gain percentage, thermal growth coefficient and feed efficiency ratio than ambient temperature groups ( $p < 0.05$ ). Higher temperatures (above 32°C) negatively affected the Nile tilapia fingerlings, reducing growth, survival and feed efficiency (Pandit and Nakamura, 2010).

The increased metabolic cost at higher temperatures

probably led to reduced energy allocation for growth (Ma *et al.*, 2015; Hunt von Herbing, 2024). However, higher lipid diets (HL1 and HL2) resulted in significantly better growth performance and feed efficiency compared to the optimal lipid diet ( $p < 0.05$ ), possibly by providing more energy-dense nutrients. Tilapia can tolerate high-fat diets comparatively to other fish species (He *et al.*, 2015). Adjusting dietary lipid levels may help offset the negative impacts of high temperatures on growth, given that lipid storage and utilisation vary significantly ( $p < 0.05$ ) among fish species. Significant interaction ( $p < 0.05$ ) was observed between temperature and dietary lipids for all parameters, suggesting that both temperature and dietary lipid levels significantly influenced the growth and feed efficiency of GIFT Tilapia juveniles with

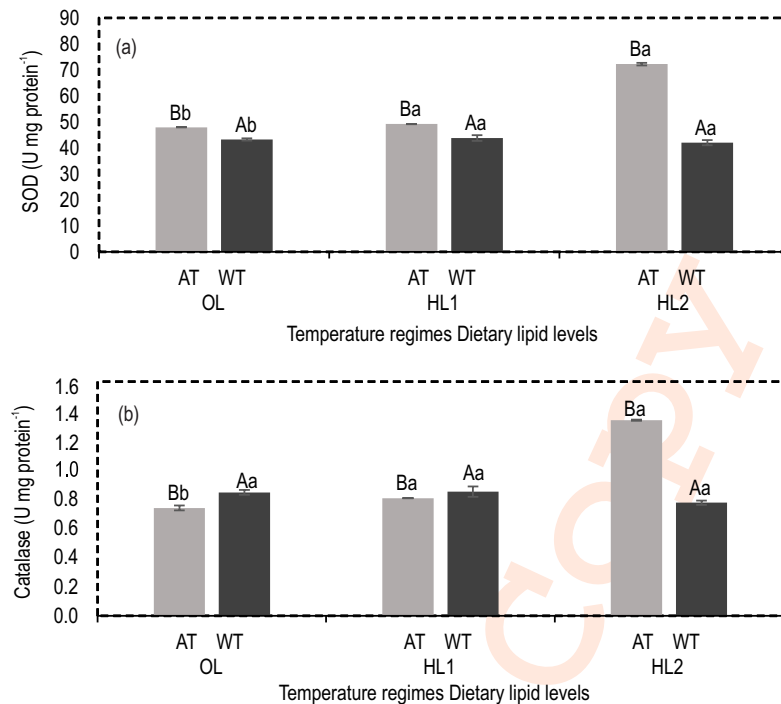


**Fig. 2:** Effect of temperature and dietary lipid levels on serum glucose (A), and cortisol (B) of GIFT Tilapia juveniles reared at ambient (28°C) and warm temperatures (33°C) for a period of 60 days. Statistical data presented as mean  $\pm$  standard error, with sample size of 6. Treatments combine two temperature regimes (AT: 28°C, WT: 33°C) with three dietary lipid levels (OL: 8%, HL1: 10%, HL2: 12% crude lipid), yielding six experimental groups. Different superscripts in lowercase above each bar of the same group signify statistical differences in dietary lipids ( $P < 0.05$ ). Different superscripts in uppercases above each bar of the same group signify statistical significance of temperature differences ( $P < 0.05$ ).

higher lipid diets (12%), better optimising the growth performance in the wake of thermal stress in this study. Fig. 1 illustrates the effects of temperature and dietary lipid levels on whole-body crude lipid (CL) content (Fig. 1A), intestinal lipase activity (Fig. 1B), and intraperitoneal fat index (IPFI) (Fig. 1C) in GIFT Tilapia juveniles. At warm temperature, the whole-body lipid was reduced ( $p < 0.05$ ). Higher lipid diets (HL1 and HL2) resulted in significantly increased values for all parameters versus the optimal lipid (OL) diet ( $p < 0.05$ ). There was no significant ( $p > 0.05$ ) interaction for CL and IPFI. This result implies that warmer temperatures might reduce the overall lipid deposition, probably due to increased energy expenditure for metabolism, leaving less energy available for fat storage. A higher rearing temperature (34°C) mediated lipid utilisation decreased fat deposition in dark barbel catfish (Qiang *et al.*, 2017).

The significant interaction between temperature and dietary lipids for lipase activity ( $p < 0.05$ ) suggests that the response of lipase activity was less pronounced at warm temperatures than ambient temperature. It was also noted that the magnitude of increase of intestinal lipase is restricted at warm temperature, suggesting a complex interplay between temperature and diet in regulating lipid metabolism enzymes

(Prabu *et al.*, 2023). Hence, the lower lipase activity, slightly lower body lipid, and lower IPFI at warm temperatures indicate that fish allocate energy away from digestive processes to cope with the metabolic demands of higher temperature. However, they still managed to increase body lipid and IPFI with higher dietary lipids, suggesting they are utilising the available lipids (Pörtner and Farrell, 2008). The results of serum glucose (Fig. 2A) and cortisol (Fig. 2B) levels in GIFT Tilapia juveniles revealed significant effects of temperature, dietary lipid levels, and their interaction ( $p < 0.05$ ). At ambient temperature, the glucose levels remained relatively stable across dietary lipid treatments, with a slight increase in the HL2 group. In contrast, the glucose levels were significantly ( $p < 0.05$ ) higher at warm temperature in the optimal lipid group but decreased markedly in the HL 1 and HL 2 groups. Cortisol levels showed a dramatic difference between temperature treatments: at ambient temperature, the cortisol remained low and stable across all lipid levels, while at warm temperature, the cortisol was significantly elevated ( $p < 0.05$ ), with the highest levels in the OL group and decreasing levels in HL1 and HL 2 groups. These results align with the reports of increased serum glucose levels (Panase *et al.*, 2019) and cortisol secretion (Musa *et al.*, 2017) in tilapia under thermal stress. The high glucose and cortisol levels in the WT-OL group indicate a



**Fig. 3:** Effect of temperature and dietary lipid levels on hepatic SOD (A), and Catalase (B) of GIFT Tilapia juveniles reared at ambient (28°C) and warm temperatures (33°C) for a period of 60 days. Statistical data presented as mean  $\pm$  standard error, with sample size of 6. Treatments combine two temperature regimes (AT: 28°C, WT: 33°C) with three dietary lipid levels (OL: 8%, HL1: 10%, HL2: 12% crude lipid), yielding six experimental groups. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of epinephrine auto-oxidation. One unit of catalase activity was defined as the amount of enzyme required to decompose 1  $\mu$ mol of H<sub>2</sub>O<sub>2</sub> per minute. Different superscripts in lowercase above each bar of the same group signify statistical differences in dietary lipids ( $P < 0.05$ ). Different superscripts in uppercase above each bar of the same group signify statistical significance of temperature differences ( $P < 0.05$ ).

pronounced stress response and potential metabolic disturbance when lipid intake is insufficient at higher temperatures.

The reduction in both glucose and cortisol with increased dietary lipids at warm temperature supports the hypothesis of an increased lipid requirement under thermal stress. In the context of global warming, increasing dietary lipid content to 12% appears to be an effective strategy to help GIFT tilapia combat thermal stress. This approach may improve their ability to maintain metabolic homeostasis and reduce stress responses, aligning with the findings from other species (Glencross, 2009; Torrecillas *et al.*, 2017). The alteration in temperature and diet lipid levels resulted in a change in the serum proteins, as illustrated in Table 3. The fish reared at a warm temperature (33°C) exhibited higher total protein and globulin levels than those reared at an ambient temperature (28°C), although the A:G ratio was significantly reduced ( $p < 0.05$ ) at high temperatures.

The effect of temperature on albumin levels was not statistically significant ( $p > 0.05$ ). Notably, no significant interactions ( $p > 0.05$ ) were observed between temperature and dietary lipid levels for any of the serum protein parameters.

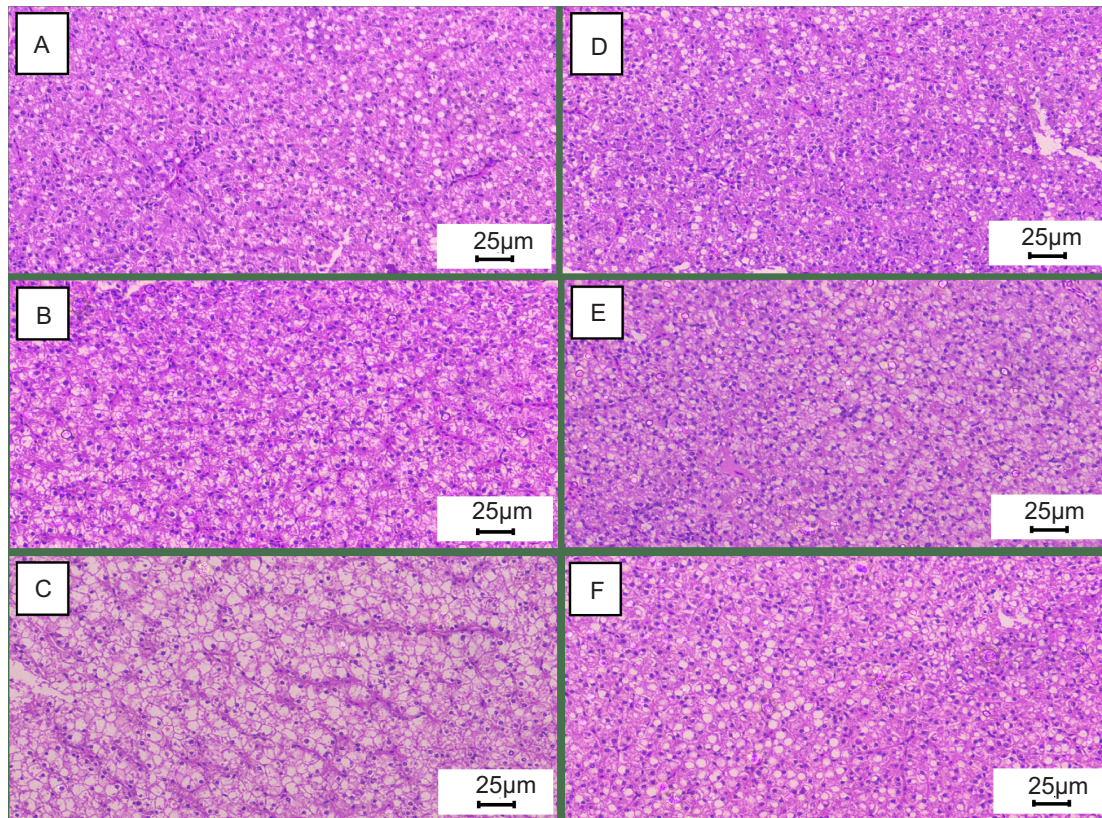
Increasing total protein and globulin at elevated temperatures may indicate an enhanced immune or stress response, as globulins are associated with immune function. A comparable response was documented in *Trachinotus blochii* juveniles reared at 34°C (Prabu *et al.*, 2023). The lower A:G ratio at warmer temperature provides further evidence to support this hypothesis, indicating a shift towards higher globulin production relative to albumin. The absence of any significant effects or interactions ( $p > 0.05$ ) between dietary lipid levels and the tested temperatures suggests that serum protein levels in GIFT tilapia are more sensitive to changes in the environmental temperature than to variations in dietary lipid content within the range tested. The observed alterations in serum protein levels at higher temperatures likely represent a complex interplay of stress responses, immune system activation and metabolic adjustments. Although these protein measurements do not reflect this directly, the increased lipid requirement and utilisation at higher temperatures could support these physiological changes by providing necessary energy for enhanced protein synthesis and other adaptive responses to thermal stress (Khieokhajokhet *et al.*, 2022).

The SOD and CAT levels can be used as indicators of

**Table 3:** Effects of temperature and dietary lipid levels on serum protein levels in GIFT Tilapia juveniles reared at ambient (28°C) and warm temperatures (33°C) for a period of 60 days

Two-way ANOVA				
Treatments <sup>1</sup>	Total protein (g dl <sup>-1</sup> )	Albumin (g dl <sup>-1</sup> )	Globulin (g dl <sup>-1</sup> )	A: G ratio
Effect of temperature variable				
AT	3.309a	1.088	2.221a	0.495b
WT	3.906b	1.059	2.847b	0.375a
SEM	0.062	0.031	0.056	0.018
p-value	0.000	0.532	0.000	0.000
Effect of dietary lipid levels				
OL	3.710	1.111	2.599	0.434
HL1	3.646	1.029	2.618	0.402
HL2	3.467	1.082	2.385	0.468
SEM	0.076	0.038	0.069	0.021
p-value	0.106	0.338	0.062	0.143
Interaction of temperature* Dietary lipids				
p-value	0.233	0.370	0.230	0.363

Statistical data presented as mean ± SEM, with sample size of 3. Within each column, averages marked with distinct superscript letters indicate statistically significant differences ( $p < 0.05$ ) between experimental groups. <sup>1</sup>Treatments combine two temperature regimes (AT: 28°C, WT: 33°C) with three dietary lipid levels (OL: 8%, HL1: 10%, HL2: 12% crude lipid), yielding six experimental groups. AT represents ambient temperature, while WT simulates warming conditions



**Fig. 4:** Liver histology sections (20x magnification) of GIFT tilapia juveniles reared at ambient (28°C) and warm temperatures (33°C) for 60 days. Statistical data is presented as mean ± S.E., with a sample size of 6. Treatments combine two temperature regimes (AT: 28°C, WT: 33°C) with three dietary lipid levels (OL: 8%, HL1: 10%, HL2: 12% crude lipid), yielding six experimental groups, viz, (A) AT-OL, (B) AT-HL1, (C) AT-HL2, (D) WT-OL, (E) WT-HL1 and (F) WT-HL2.

oxidative stress. The results for liver SOD (Fig. 3A) and CAT (Fig. 3B) activities in GIFT tilapia juveniles demonstrated significant ( $p < 0.05$ ) changes with temperature, dietary lipid levels and their interaction ( $p < 0.05$ ). At ambient temperature, both SOD and CAT activities demonstrated a tendency to increase with elevated dietary lipid levels, with the highest values observed in the HL2 group. Similarly, the liver SOD and CAT activities were observed to increase in juvenile tilapia fed a high-lipid diet (Liu *et al.*, 2023). As lipids are the primary source of oxidised metabolites in the body, the increased antioxidant enzymes observed in the higher lipid-fed groups may indicate an adaptive response to potential oxidative stress resulting from increased lipid metabolism. However, at warm temperature, both SOD and CAT activities were significantly reduced ( $p < 0.05$ ), especially at a higher degree in the HL2 group. This trend of reduction of enzyme activity could indicate that the enzymes are thermolabile, which would result in a weakened antioxidant defence at high temperature (Guo *et al.*, 2023). Alternatively, the high temperature may have been above the range required for optimum activity of antioxidant enzymes, indicating the temperature-induced inhibition of enzymatic activity.

Liver histology (Fig. 4) showed that at ambient temperature, the size of hepatocytes increased with higher dietary lipid content, with fish fed a 12% lipid diet exhibiting hypertrophic, swollen hepatocytes containing more vacuoles (Fig. 4A, B, C). In contrast, hepatocyte sizes were reduced and more regulated at warm temperatures than their ambient temperature counterparts, particularly in the 12% lipid group (Fig. 4D, E, F). This histological evidence, combined with the previously observed changes in serum glucose, cortisol and liver antioxidant enzymes, suggest enhanced lipid utilisation at warm temperature (He *et al.*, 2015). These findings indicate that higher dietary lipid levels, particularly 12%, may better regulate lipid metabolism at elevated temperatures, potentially favouring catabolism over anabolism. The reduced body lipid content indicated by a lower IPFI and reduced hepatocyte size further supports the enhanced lipid metabolism and utilisation at warm temperature. This is consistent with the previous studies showing that fish can adapt to thermal stress by modifying their lipid metabolism (Tocher *et al.*, 2003; Sissener *et al.*, 2018). The reduced hepatocyte size and more regulated appearance at warm temperature suggest that the fish more efficiently processing and utilise dietary lipids under thermal stress (Ma *et al.*, 2015). This is further supported by the lower glucose and cortisol levels observed in high-lipid diets at warm temperature, indicating improved energy metabolism and stress response.

The results of this study, emphasise the need for temperature-specific feed formulations in tilapia aquaculture. This adaptive feeding approach could be valuable for aquaculture in regions facing rising temperatures, potentially improving fish welfare and maintaining production efficiency under challenging environmental conditions. However, the optimal lipid level must be carefully determined to balance the benefits of stress reduction and metabolic stability with other physiological needs and potential long-term effects of high-lipid diets. Future research could focus on refining feed formulations and investigating the

inclusion of additives that accelerate lipid uptake and utilisation for enhancing fish production in the face of climate change.

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