# Dynamics of growth in response to varied temperature and feeding regimes in tilapia (*Oreochromis niloticus*) under high stocking density

# Short title: Effect of temperature and feed on tilapia

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**Author's contribution**

Wajeeha Komal contributed to the design and conceptualization of the research, performed the experiments, and wrote the manuscript.

Shafaq Fatima designed and conceptualized the experiments. She also analyzed the data to draw meaningful conclusions.

Qandeel Minahal assisted with the methodology, data analysis, critical review of the manuscript, and the discussion.

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**Abstract**

The study aimed to determine the optimal feeding regime for tilapia reared at high stocking densities (4.50 kg/m³) under varying temperature conditions. Tilapia were divided into groups subjected to high (29.88 ± 0.38°C) and low (21.78 ± 0.38°C) temperatures, with three feeding frequencies (2%, 3%, and 4%) within each temperature group, over 60 days. All the groups were in three replicates. Results indicated better growth at higher temperatures, with a 4% feeding frequency leading to the best growth rate and weight gain across both temperature conditions. Higher temperatures facilitated better feed utilization due to increased metabolism. The feed conversion ratio (FCR) was optimal at a 2% feed frequency for both temperature conditions, with high temperatures showing better FCR results. Variations in feeding frequency did not affect the chemical composition or digestive enzyme activity of the fish. The study concluded that

temperature fluctuations within the tolerance range did not adversely affect weight gain or nutritional quality. Similar growth patterns were observed across different feed frequencies, suggesting that a 2% feeding frequency is effective for various culture systems without compromising growth rates.

**Keywords:**
Growth efficiency; metabolism; feed conversion ratio; feeding regime

## Introduction

Aquaculture is rapidly expanding, with intensive units raising concerns about fish stress and the impact of diet and feeding methods on growth rates (Ayinla, 2007; Parma *et al*., 2020). Feeding practices are critical, accounting for 60% of production costs (Ayinla, 2007). Optimal feeding must balance energy and nutrients for maintenance and growth (Hepher, 2009). Feed quality, including palatability and digestibility, is essential for meeting fish nutritional needs (Craig and Helfrich, 2002; Omitoyin, 2007). Effective feeding regimes enhance growth, survival, size uniformity and water quality, improving production efficiency and profitability (Dwyer *et al*., 2002; Isyagi *et al*., 2009). Proper feeding strategies are vital for financial gains and competitiveness in aquaculture (Dias *et al*., 2010; Eriegha and Ekokotu, 2017).

Feed administration in aquaculture involves variables like size, rate, distribution methods, time with feeding rate being critical for maximizing feed utilization and minimizing waste and costs (Xie *et al*., 2011; Kousoulaki *et al*., 2015; Tian *et al*., 2015). Species-specific dietary habits and digestive tract structure influence how feeding schedules affect nutrient absorption (NRC, 2011; Gilannejad *et al*., 2019). Environmental conditions and management practices, including physicochemical quality of the rearing medium, impact feed consumption and fish physiological state, potentially causing stress and neuroendocrine imbalances (Wynne *et al*., 2005). Stressful conditions like hypoxia or high ammonia levels reduce feed intake (Kaushik, 2013). Fish also adjust their food intake after periods of hunger, affecting successive feed amounts (Hepher, 2009).

Feeding rate in aquaculture refers to the number of times fish are fed daily, influenced by their return to hunger and stomach capacity (Riche and Garling, 2003; Ali *et al*., 2005). Factors like temperature, physiochemical parameters, fish biomass, and feed intake affect stomach emptying rate (Davies *et al*., 2006; Ndome *et al*., 2011). Smaller fish require more frequent feeding due to higher energy demands and rapid growth, with fry and fingerlings needing up to eight feedings per day (Kaushik, 2013). Juveniles need more frequent feeding than adults, who can be fed once daily. Fish around 400 grams can be fed once daily, as their larger stomachs hold enough food for the day (Ashley-dejo *et al*., 2014). Increased feeding frequency generally improves growth rate and feed conversion ratio (Craig and Helfrich, 2002).

Fish growth relies on adequate feed that provides all essential nutrients and energy. Optimal growth is achieved by feeding fish until they are satisfied (Li *et al*., 2006; Kaushik, 2013), but reducing feed can cause size disparities within groups (Jobling *et al*., 2001). Feeding rates vary with fish size and water temperature (Riche and Garling, 2003; Craig and Helfrich, 2002), and typically decrease as fish grow larger (Kaushik, 2013). A feeding rate of about 5% of body weight is ideal for maximum growth in fingerlings and juveniles (Yuan *et al*., 2010; Ashley-dejo *et al*.,

2014). Both underfeeding and overfeeding can negatively impact feeding efficiency, growth, and the environment (McCarthy *et al*., 1992; Thorpe and Cho, 1995; Cho and Bureau, 1998; Talbot *et al*., 1999; Gaylord *et al*., 2001; Bureau *et al*., 2006; Hatlen *et al*., 2006;), with rates also influenced by time, season, and water quality (Eriegha and Ekokotu, 2017).

Water temperature significantly influences fish growth, feed consumption, feed conversion efficiency (FCE), and gastrointestinal emptying rate (Kasumyan and Doving, 2003; Handeland *et al*., 2008). There is a direct correlation between feed consumption and water temperature, with higher temperatures increasing feed intake (Martinez-Palacios *et al*., 1996; Koskela *et al*., 1997; Cho and Bureau, 1998). However, temperatures beyond a species optimal range reduce feeding behavior (Walberg, 2011; Mizanur *et al*., 2014). Warm-water fish perform best between 25-32°C (Eriegha and Ekokotu, 2017), but near the upper heat tolerance limit, feed intake decreases. Low temperatures result in reduced feed consumption and halted growth (Elliott, 1991; Jobling, 1994; Bendiksen *et al*., 2002). Metabolic processes, sensitive to temperature changes, can increase significantly with a 10°C rise (Person-Le Ruyet *et al*., 2006; Bjornsson *et al*., 2007; Willmer *et al*., 2009; Neubauer and Andersen, 2019). The relationship between water temperature and physiological processes affects the food consumption needed for energy (Weidner *et al*., 2020).

Fish meet their energy needs through food consumption, which provides chemical energy for maintenance, growth, reproduction, and reserves (Weidner *et al*., 2020). Digestive efficiency and nutrient digestibility decrease when temperatures deviate from optimal, affecting enzyme activity, as seen in brook trout (*Salvelinus fontinalis*) (Amin *et al*., 2016). Efficient nutrient breakdown relies on digestive enzyme function, with extended gut transit time enhancing enzyme activity (Volkoff and Ronnestad, 2020).

Nile tilapia (*Oreochromis niloticus*) is highly adaptable to different feeding regimes and environmental conditions, making it a popular choice in global aquaculture (Byamungu *et al*., 2001). Increased feeding frequency enhances disease resistance (Garcia and Villarroel, 2009), but feeding every two to three hours can lead to the passage of undigested feed (Riche *et al*., 2004). These fish thrive above 16°C and struggle below 10°C, yet can tolerate temperatures as high as 40-42°C (Chervinski, 1982; Philippart and Ruwet, 1982; Azaza, 2004).

The objective of this study was to investigate the ideal feeding regime for tilapia cultured under high-density conditions across two contrasting temperature extremes: low and high temperatures. Additionally, it also aimed to ascertain the optimal feeding frequencies for the feed rations employed in the study and to determine the most suitable number of feeding times to promote optimal fish growth. This study also intends to investigate whether changes in temperature and feed ration affect fish growth. It also examined the impact of feed ration on the nutritional quality of fish by analyzing their chemical composition, activity of digestive enzymes and growth hormones.

## Materials and Methods

### Experimental design

Tilapia (*Oreochromis niloticus*) (n=2,700 initial weight= 33.00 ± 0.60g) were procured from a local fish hatchery (Lahore, Pakistan) and transferred to Aquaculture facility, Lahore College for Women University. There was no mortality of fish during the transfer. This study was commenced after the

approval of Animal Ethics Committee of Department of Zoology, Lahore College for Women University (Approval #: Zoo/LCWU/932). Fish (n=2,700) were randomly distributed in 18 fiber glass tanks (water volume/tank= 1 $m^3$). Each tank has water supplied from the same water sump, treated with UV filter and biofilters. All tanks had their own water supply. Fish were acclimatized for one week before commencement of trial. Trial comprised of six groups with a stocking density of 4.50 kg/$m^3$ (150 fish/$m^3$) in each group. The trial was conducted in two phases, each corresponding to different seasons: summer (high temperature) and winter (low temperature). The first phase took place in the month of June and July, during high temperature (HT). In this phase, fish were stocked at a high density (4.50 kg/$m^3$) in three different groups, each fed according to different feeding frequencies. The first group was fed at 2% of their body weight twice a day. The second group was fed at 3% of their body weight three times a day, and the third group was fed at 4% of their body weight four times a day. The second phase occurred in the month of October and November, during low temperature (LT). In this phase, fish (95.00 ± 1.22g) were stocked at the same density (4.50 kg/$m^3$) as in the first phase in three groups. These groups were fed in the same manner as in the first phase: at 2%, 3%, and 4% of their body weight twice, thrice, and four times a day, respectively. Overall, the study comprised six groups, three at high temperature and three at low temperature, with each group replicated three times. Thus, three different feeding frequencies i.e. 2% (2F), 3% (3F) and 4% (4F) were provided to both high temperature (H2F, H3F, H4F) and low temperature (L2F, L3F, L4F) (Table 1). The difference in the initial weight of tilapia in first phase (30.00±0.60 g) and second phase (95.00±1.22 g) occurred because all the fingerlings (n=2,700) were purchased simultaneously at the end of May. After one week of acclimatization, first phase was commenced in nine cohorts in June. Meanwhile, the remaining nine cohorts were fed with 2% feed until September to achieve conditions of low water temperature for the start of second phase. The fish gained weight (from 30.00±0.60 g-95±1.22 g) over the four months (June-September) before the commencement of second phase, which resulted in the initial weight difference between the two phases. The duration for each phase of the trial was 60 days i.e. 60 days for low temperature and 60 days for high temperature. All the fishes of both phases were healthy by end of this trial. This time period for the trial was selected following Fatima *et al*., (2021), which investigated the growth of tilapia over a period of 171 days, aiming to achieve a final stocking density of 57.81 kg/$m^3$. Furthermore, 60 days is the most recommended study period to detect the effect of any feeding alteration on the growth of fish. Fish husbandry conditions and health were excellently maintained throughout the entire trial period to minimize the mortality. Random weight check of fish was performed for each replicate after every 15 days to adjust the daily ration.

**Water Quality Parameters and Survival Rate**

Water quality was very well maintained in all tanks to ensure the welfare of fish. A total 20% of water was exchanged every day from each tank. Water quality parameters were measured twice a day to ensure its standard quality levels throughout the trial period. Aeration pumps (120V/60Hz, Airmax SilentAir LR25, USA) were used to deliver air via diffuser grids. There was one rectangular diffuser grid in each tank (L ×W: 1 × 0.5 ft). Each diffuser grid was built by using the anti-microbial tubing (outer diameter: 25.4 mm; inner diameter: 12.7 mm; airflow: 2.2 $m^3$/h/meter) to generate microbubbles ensuring the good air saturation in water (>80%). All tanks were back washed on daily basis to drain solid waste settled at the bottom of tank. Water quality

parameters including water temperature, dissolved oxygen and pH were monitored twice a day by using portable meters (HI98494, Hanna, USA). Ammonia and nitrite were monitored twice a week by using commercial kits (HI733, HI93708, Hanna, USA). Fish in every tank were checked for any sign of disease, abnormal behavior and mortality twice a day. Survival rate observed was 100% due to well-maintained husbandry conditions over the study period.

**Sample analysis**

At end of the trial, five fish were randomly sampled from each replicate of all feeding frequency groups (15 fish per group of temperature). A total of 90 fish were euthanized at terminal sampling out of 2,700 fish used in the trial following the designated limit of 5% of population approved by Animal Ethics Committee for both high and low temperature. Remaining 2,610 fish were humanely released in nearby lake, administered by Department of Fisheries, Pakistan for stocking purpose (Release Approval #: DOF/27858/2022). Before sampling, fish were fasted for 24 hours. On sampling day, they were euthanized using clove oil (0.8 ml/L of water, Sigma-Aldrich, USA). This dose of clove oil is standard to euthanize fish in a very humane way which took less than ten minutes to euthanize sampled fish.

Blood was collected from the caudal vein in to the tube contained clot activator for plasma collection (ATC, Pakistan). Blood samples were centrifuged (Centurion scientific Ltd., UK) at 5,000 rpm for 15 minutes and plasma was collected in separate eppendorf tubes and stored at -20 $^{o}$C. The total body weight and total body length were measured before dissection. Fish were dissected and muscle samples were collected and stored at – 20$^{o}$C for chemical composition analysis following the guidelines of Association of Official Analytical Chemists (AOAC) (AOAC, 2005). Muscle samples were dehydrated in a hot air oven (PCSIR Laboratories, Pakistan) at 80°C until a consistent dry weight was reached. These dried samples were processed for further chemical

**Table 1:** Experimental design of feeding regime provided to high density groups according to 2%,3% and 4% body weight at both low and high temperature.

| | Group | Stocking density | No. of replicates | Water temperature → | Feed given (%) per body weight | Feed/fish/day (g) | Total feed/group/day (g) | Feed/ration/day (g) |
|---|---|---|---|---|---|---|---|---|
| Experimental design | H2F | 4.50 kg/m$^3$, n=150, Initial weight: 30 g | 3; 150/replica | high | 2% | 0.66 | 99 | 49.5 |
| | H3F | 4.50 kg/m$^3$, n=150, Initial | 3; 150/replicate | high | 3% | 0.99 | 148.5 | 49.5 |
| | H4F | 4.50 kg/m$^3$, n=150, Initial | 3; 150/replicate | high | 4% | 1.32 | 198 | 49.5 |
| | L2F | 4.50 kg/m$^3$, n=50, Initial weight: 90 g | 3; 50/replicate | low | 2% | 1.90 | 95 | 47.5 |
| | L3F | 4.50 kg/m$^3$, n=50, Initial weight: 90 g | 3; 50/replicate | low | 3% | 2.85 | 142.- | 47.5 |
| | L4F | 4.50 kg/m$^3$, n=50, Initial weight: 90 g | 3; 50/replicat | lo | 4% | 3.8 | 190 | 47.5 |

analysis. The Kjeldahl apparatus (PCSIR Laboratories, Pakistan) was used to determine the crude protein, while crude lipids were identified using the Folch method (Folch, 1957) using the Soxhlet apparatus (PCSIR Laboratories, Pakistan). The ash content in muscles was determined using muffle furnace PCSIR Laboratories, Pakistan).

The intestinal samples from midgut were weighed, rinsed with deionized water and homogenized in 0.86% sterile normal saline solution (1:9). This mixture was centrifuged at 5000 rpm for 15 minutes. Supernatant was collected and stored at -20°C. Each sample was analyzed in three replicates for all analyses. Condition factor (K), specific growth rate (%) (SGR), hepatosomatic index (HSI), viscerosomatic index (VSI), fish weight gain, survival rate, feed conversion rate (FCR) was measured by using the given formulae.

$$\text{Condition factor (\%)} = \frac{\text{Total body weight (g)}}{\text{Total body length}^3} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{Time interval in days}} \times 100$$

$$\text{Hepatosomatic index} = \frac{\text{Liver weight}}{\text{Total body weight}} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Weight of feed consumed}}{\text{Weight gain (wet weight)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of fish}}{\text{Initial stocking density}} \times 100$$

$$\text{Visceroosomatic index} = \frac{\text{Viscera weight}}{\text{Total body weight}} \times 100$$

**Digestive enzymes assay**

For digestive enzyme analyses, the supernatant of processed whole intestine samples was utilized. Commercial ELISA kits were used for the activity of lipase (U/L) (Sigma Aldrich, USA, CAT No. MAK046, EC 3.1.1.3) for amylase (U/L) (Sigma Aldrich, USA, CAT No. *MAK009A,* EC 3.2. 1.1.) at 570 nm and 405 nm wavelength respectively. The activity of protease was determined following instructions of.[49] Casein 1% w/v was used as substrate in 0.2 M phosphate buffer at pH 7.0. One unit of protease indicates the amount of enzyme that releases 1 µg/ml/min of tyrosine determined at 660 nm of wavelength.

**Hormones assay**

ELISA kits were used to assess the levels of thyroid stimulating hormone (TSH) (µIU/mL; Calbiotech, USA, CAT No. TS227T, 0.05µIU/ml sensitivity), triiodothyronine (T3) (ng/ml; Calbiotech, USA, CAT No. T3379T), thyroxine (T4) (µg/dl; Calbiotech, USA, CAT No. T4224T) and growth hormone (GH) (ng/ml; Calbiotech, USA, CAT No. HG048H) in blood plasma samples. The absorbance value was read on the ELISA reader at 450 nm.

**Statistical analysis**

For all the statistical analyses, SPSS v.29 software was used. Data were presented as Mean ± SE for all the parameters. Levene test were performed to check the homogeneity of variance of data. The effect of temperature and feeding frequencies on different parameters was determined by Two-Way ANOVA. To reject the null hypothesis, 0.05 probability level was used.

**Results**

**Water quality parameters**

Water temperature in all the feeding frequency groups were in the range of 30.34 ± 0.38-29.77 ± 0.34 at high temperature and at low temperature it was 20.98 ± 0.43-21.9 ± 0.34 (Table 2). The range of dissolved oxygen at high temperature was 4.32 ± 0.40-4.52 ± 0.32 while, at low temperature it was 4.11 ± 0.36-4.42 ± 0.30. At high temperature the pH was in the range of 8.29 ± 0.04-8.66 ± 0.02, at low temperature it was 8.00 ± 0.04-8.08 ± 0.02. Range of ammonia at high

temperature was 0.72 ± 0.01-0.95 ± 0.09 while nitrites were in the range of 0.11 ± 0.01-0.15 ± 0.04. At low temperature, ammonia and nitrites were in the range of 0.66 ± 0.01- 0.77 ± 0.08 and 0.12 ± 0.01-0.14 ± 0.03 respectively.

**Table 2:** Variations (Mean ± SE) in water quality parameters indicating temperature (oC), dissolved oxygen (mg/L), pH, ammonia (ppm) and nitrate (mg/L) at high and low temperature and also at different feeding frequencies groups

| **High temperature (H)** | | | | | |
|---|---|---|---|---|---|
| **Groups** | **Water temperature (oC)** | **Dissolved oxygen (mg/L)** | **pH** | **Ammonia (ppm)** | **Nitrites (mg/L)** |
| **H2F** | 29.77 ± 0.34 | 4.52 ± 0.32 | 8.29 ± 0.04 | 0.72 ± 0.01 | 0.11 ± 0.01 |
| **H3F** | 29.89 ± 0.37 | 4.32 ± 0.40 | 8.35 ± 0.06 | 0.83 ± 0.08 | 0.12 ± 0.03 |
| **H4F** | 30.34 ± 0.38 | 4.49 ± 0.40 | 8.66 ± 0.02 | 0.95 ± 0.09 | 0.15 ± 0.04 |
| **Low temperature (L)** | | | | | |
| **Groups** | **Water temperature (oC)** | **Dissolved oxygen (mg/L)** | **pH** | **Ammonia (ppm)** | **Nitrites (mg/L)** |
| **L2F** | 21.90 ± 0.34 | 4.11 ± 0.36 | 8.00 ± 0.04 | 0.66 ± 0.01 | 0.12 ± 0.01 |
| **L3F** | 20.98 ± 0.37 | 4.21 ± 0.40 | 8.04 ± 0.06 | 0.69 ± 0.08 | 0.14 ± 0.03 |
| **L4F** | 21.78 ± 0.38 | 4.42 ± 0.30 | 8.08 ± 0.02 | 0.77 ± 0.08 | 0.13 ± 0.05 |

**Growth**

At two different temperatures, total body length ($df_1$, F=49.41), total body weight ($df_1$, F=48.64), condition factor ($df_1$, F=43.57), specific growth rate ($df_1$, F=51.59) and hepatosomatic index ($df_1$, F=8.24) were found to be significantly different (P< 0.05) except viscerosomatic index ($df_2$, F=0.91) (P> 0.05) (Table 3). A significant variation (P< 0.05) in total body length ($df_2$, F=24.42), total body weight ($df_2$, F=24.05), condition factor ($df_2$, F=21.19), specific growth rate ($df_2$, F=25.33), hepatosomatic index ($df_2$, F=18.78) and viscerosomatic index ($df_2$, F=33.90) were observed between different feeding frequencies. Other than this, the effect of temperature*feeding frequency showed an insignificant difference (P> 0.05) on total body length ($df_2$, F=0.53), total body weight ($df_2$, F=1.41), condition factor ($df_2$, F=0.10), specific growth rate ($df_2$, F=1.50), hepatosomatic index ($df_2$, F=0.08) and viscerosomatic index ($df_2$, F=3.04). The survival rate of fish both in low and high temperature against all feeding frequencies groups were 100%.

**Chemical composition of muscles**

The moisture content ($df_1$, F=26250.00), crude protein ($df_1$, F=466.66), crude ash ($df_1$, F=11666.66) and crude fat ($df_1$, F=1050.00) was significantly different (P< 0.05) At two different temperatures (Table 4). A significant difference (P< 0.05) in the content of moisture ($df_2$, F=1866.66), crude protein ($df_2$, F=5716.66), crude ash ($df_2$, F=816.66) and crude fat ($df_2$, F=1400.00) was observed between different feeding frequencies groups. Effect of temperature*feeding frequencies calculated by Two-way ANOVA also showed significant effect (P< 0.05) on moisture ($df_2$, F=1400.00), crude protein ($df_2$, F=116.66), crude ash ($df_2$, F=1516.66) except crude fat (P> 0.05).

**Table 3:** Analysis of total body length (cm), total body weight (g), condition factor, hepatosomatic index, specific growth rate, viscerosomatic index and FCR (Mean ± SE) at two temperatures in three feeding frequencies groups (2%, 3% and 4%).

| High Temperature (H) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Groups** | **TBL (cm)** | **TBW (g)** | **SGR (%)** | **K (%)** | **HSI (%)** | **VSI (%)** | **FCR** | **Survival Rate (%)** |
| **H2F** | 7.78 ± 0.27 | 79.00 ± 1.25 | 430.92 ± 1.23 | 3.02 ± 0.23 | 1.04 ± 0.02 | 3.16 ± 0.05 | 0.86 ± 0.25 | 100.00 ± 0.02 |
| **H3F** | 8.05 ± 0.28 | 80.50 ± 1.29 | 432.84 ± 1.27 | 3.06 ± 0.25 | 1.12 ± 0.03 | 3.93 ± 0.06 | 1.25 ± 0.28 | 100.00 ± 0.03 |
| **H4F** | 8.80 ± 0.29 | 87.00 ± 1.27 | 440.45 ± 1.26 | 3.22 ± 0.26 | 1.26 ± 0.04 | 4.29 ± 0.08 | 1.47 ± 0.25 | 100.00 ± 0.04 |
| **Low Temperature (L)** | | | | | | | | |
| **Groups** | **TBL (cm)** | **TBW (g)** | **SGR (%)** | **K (%)** | **HSI (%)** | **VSI (%)** | **FCR** | **Survival Rate (%)** |
| **L2F** | 15.50 ± 0.21 | 120.00 ± 1.20 | 470.95 ± 1.28 | 3.02 ± 0.23 | 2.61 ± 0.02 | 7.53 ± 0.04 | 1.02 ± 0.28 | 100.00 ± 0.04 |
| **L3F** | 15.75± 0.24 | 122.24 ± 1.27 | 473.17 ± 1.25 | 3.14 ± 0.25 | 2.52 ± 0.05 | 6.74 ± 0.07 | 1.37 ± 0.29 | 100.00 ± 0.05 |
| **L4F** | 16.25 ± 0.23 | 124.35 ± 1.27 | 474.94 ± 1.27 | 3.32 ± 0.29 | 2.47 ± 0.06 | 6.41 ± 0.05 | 1.71 ± 0.34 | 100.00 ± 0.06 |

**Table 4**: Analysis (Mean ± SE) of chemical composition (%) of muscles at two temperatures in three feeding frequencies groups (2%, 3% and 4%).

| High Temperature (H) | | | | |
|---|---|---|---|---|
| **Groups** | **Moisture (%)** | **Crude protein (%)** | **Crude fat (%)** | **Crude ash (%)** |
| **H2F** | 74.10 ± 0.34 | 18.50 ± 0.31 | 1.70 ± 0.43 | 5.70 ± 0.55 |
| **H3F** | 74.00 ± 0.36 | 18.60 ± 0.34 | 1.50 ± 0.44 | 5.90 ± 0.56 |
| **H4F** | 73.70 ± 0.38 | 18.90 ± 0.36 | 1.60 ± 0.54 | 5.80 ± 0.65 |
| **Low Temperature (L)** | | | | |
| **Groups** | **Moisture (%)** | **Crude protein (%)** | **Crude fat (%)** | **Crude ash (%)** |
| **L2F** | 74.40 ± 0.32 | 18.40 ± 0.37 | 1.60 ± 0.48 | 5.60 ± 0.59 |
| **L3F** | 74.50 ± 0.38 | 18.60 ± 0.33 | 1.40 ± 0.39 | 5.50 ± 0.62 |
| **L4F** | 74.40 ± 0.33 | 18.80 ± 0.38 | 1.50 ± 0.42 | 5.30 ± 0.58 |

**Digestive enzymes activity**

The activity of amylase ($df_1$, F=168.00), protease ($df_1$, F=168.00) and lipase ($df_1$, F=168.00) were significantly different (P< 0.05) at two different temperatures (Table 5). Different feeding frequencies also showed a significantly variation (P< 0.05) in the activity of amylase ($df_2$, F=3416.00), lipase ($df_2$, F=504.00) and protease ($df_2$, F=6962.66). Effect of temperature*feeding frequencies showed an insignificant variation (P> 0.05) in the digestive enzyme activity.

**Table 5:** Analysis (Mean ± SE) of digestive enzymes activity including amylase, lipase, protease at two different temperatures and on different feeding frequencies (2%,3% and 4%).

| High Temperature (H) | | | |
|---|---|---|---|
| **Groups** | **Amylase (U/L)** | **Lipase (U/L)** | **Protease (U/L)** |
| **H2F** | 48.05 ± 0.18 | 28.03 ± 0.13 | 27.69 ± 0.18 |
| **H3F** | 48.07 ± 0.16 | 28.09 ± 0.17 | 27.77 ± 0.19 |
| **H4F** | 48.33 ± 0.15 | 28.15 ± 0.16 | 28.11 ± 0.18 |
| **Low Temperature (L)** | | | |
| **Groups** | **Amylase (U/L)** | **Lipase (U/L)** | **Protease (U/L)** |
| **L2F** | 48.01 ± 0.17 | 27.99 ± 0.15 | 27.65 ± 0.17 |
| **L3F** | 48.03 ± 0.19 | 28.05 ± 0.17 | 27.73 ± 0.19 |
| **L4F** | 48.29 ± 0.14 | 28.11 ± 0.18 | 28.07 ± 0.15 |

**Profile of hormones**

At two different temperatures the activity of T3 ($df_1$, F=10.14), T4 ($df_1$, F=298.66), TSH ($df_1$, F=564.66) and GH ($df_1$, F=29.16) showed a significant effect (P< 0.05) (Table 6). The activity of T3 ($df_2$, F=17.14), T4 ($df_2$, F=512.16), TSH ($df_2$, F=1135.16) and GH ($df_2$, F=32.66) showed a significant variation (P< 0.05) at different feeding frequencies. Effect of temperature*feeding frequencies on these hormones also showed a significant variation (P< 0.05) on T3 ($df_2$, F=11.05), T4 ($df_2$, F=15.16), TSH ($df_2$, F=71.16) and GH ($df_2$, F=4.66).

**Table 6:** Analysis (Mean ± SE) of hormones (T3, T4, TSH and GH) at two temperatures in three feeding frequencies groups (2%, 3% and 4%).

| High Temperature (H) | | | | |
|---|---|---|---|---|
| **Groups** | **T3 (ng/ml)** | **T4 (ug/dl)** | **TSH (uIU/ml)** | **Growth hormone (ng/ml)** |
| **H2F** | 0.86 ± 0.09 | 7.63 ± 0.13 | 2.30 ± 0.09 | 0.12 ± 0.02 |
| **H3F** | 0.87 ± 0.08 | 7.6 ± 0.14 | 2.44 ± 0.10 | 0.13 ± 0.03 |
| **H4F** | 0.88 ± 0.06 | 7.7 ± 0.15 | 2.5 ± 0.11 | 0.16 ± 0.02 |
| **Low Temperature (L)** | | | | |
| **Groups** | **T3 (ng/ml)** | **T4 (ug/dl)** | **TSH (uIU/ml)** | **Growth hormone (ng/ml)** |
| **L2F** | 0.87 ± 0.04 | 7.56 ± 0.14 | 2.27 ± 0.08 | 0.11 ± 0.04 |
| **L3F** | 0.85 ± 0.05 | 7.54 ± 0.13 | 2.32 ± 0.10 | 0.12 ± 0.05 |
| **L4F** | 0.87 ± 0.06 | 7.67 ± 0.16 | 2.43 ± 0.09 | 0.13 ± 0.06 |

**Discussion:**

This study investigated the optimal feeding regime for tilapia in high-density conditions across various temperatures. Results showed that tilapia had better growth rates at higher temperatures, with growth slowing but not ceasing at lower temperatures due to reduced feeding. Growth hormone assays confirmed higher activity at elevated temperatures. While temperatures above 32°C negatively impact survival and growth (Pandit and Nakamura, 2010), the optimal range for tilapia growth is 22-30°C (Caulton, 1982), with the best growth observed at the higher end of this spectrum (Hauser, 1977). Growth rates generally increase with temperature within this range (Larsson and Berglund, 2005), but decline at upper limits due to metabolic costs and reduced appetite (Azaza *et al*., 2008). In present study, where a slight reduction in weight gain was observed at low temperatures, feeding did not cease because the temperatures were not below than the lower thermal tolerance range. Feeding ceases at temperatures between 13°C and 18°C, feeding and growth stop (Caulton, 1982; Atwood *et al*., 2006). Low temperatures lead to reduced feed intake and biochemical changes, such as increased enzyme and lipid concentrations (Burel *et al*., 1996), contributing to weight gain despite reduced feeding (Burel *et al*., 1996).

This study aimed to determine the optimal feeding frequencies and daily feedings for enhancing tilapia growth. The best growth was observed with a 4% body weight feed ration administered four times a day. While 4% was the most effective, other feeding levels also yielded significant weight gain. The standard feeding ration for tilapia is 2% (El-Saidy and Gaber, 2005), but this study showed positive results across all tested levels, confirmed by growth hormone assays. Optimal growth was also linked to temperatures between 27°C and 32°C (Pandit and Nakamura, 2010). Similar temperature-dependent feeding behaviors are noted in various fish species, where feed intake rises with moderate temperature increases but decreases outside the optimal range. Examples include salmon, feed intake increases approximately threefold as temperatures rise from 2°C to 6°C (Brett, 1979), sockeye salmon (Brett, 1971), channel catfish (Buentello *et al*., 2000), walking catfish (*Clarias batrachus*) (Ahmad *et al*., 2014), cobia (*Rachycentron canadum*) (Nguyen *et al*., 2019), Indian major carp (*Catla catla*) (Sharma *et al*., 2017), Atlantic salmon (Folkedal *et al*., 2012; Hevrøy *et al*., 2012), goldfish (*Carassius auratus*) (Nadermann *et al*., 2019), and perch fry (*Perca fluviatilis*) (Smirnov *et al*., 2019). Fish typically lose appetite at temperatures significantly below their critical maximum (Shafland and Pestrak, 1982).

The present study investigated how temperature and feeding levels affect the chemical composition of tilapia. Results showed significant impacts on protein, ash, and fat content, with higher protein levels at a 4% feed ration and higher temperatures. Previous research indicated that feed ration significantly influences whole-body protein content, especially at 4% to 6% (Khandan *et al*., 2019). Similar trends were found in mrigal carp (*Cirrhinus mrigala*) (Khan *et al*., 2004), tilapia at 22°C required more protein than those at 28°C and 34°C (Zeng *et al*., 2021) and spotted seabass at 27°C and 33°C have protein content of 46.8% and 44.9% respectively. These findings suggest protein requirements increase outside optimal temperature ranges.

The activity of digestive enzymes is significantly influenced by water temperature. The present study found a notable impact of both temperature and feeding levels on the activity of these enzymes. Quantitatively, a slight reduction in digestive enzyme activity was observed at lower temperatures. In previous study, Watanabe *et al*. (1996) reported that 25°C is the optimal

temperature for nutrient digestibility in tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*). Tilapia can tolerate high temperatures up to 40-42°C (Philippart and Ruwet, 1982), but the optimal temperature for feeding, growth and reproduction ranges between 26 and 30°C (Hauser, 1977). Increased temperatures enhance digestive enzyme activity, which can accelerate nutrient digestion and promote better growth (Shcherbina and Kazlauskene, 1971). The characteristics of digestive enzymes vary based on habitat and climate. Genetic adaptation to temperature can cause phenotypic changes such as structural differences, altered secretion rates, and the production of isozymes, which are enzymes that perform the same function but work best at different temperatures (Gelman *et al*., 2008). For example, cold-adapted fish such as Atlantic cod (Stefansson *et al*., 2017) and Antarctic icefish (*Chionodraco hamatus*) (Krogdahl *et al*., 2011) have digestive enzymes with higher catalytic efficiencies at low and moderate temperatures compared to those in warmer environments. The effects of water temperature on digestive enzyme activity are species-specific and generally align with the temperature ranges of their natural habitats. In subtropical and tropical species, such as seabass (*Dicentrarchus labrax*), digestive enzyme activities peak at temperatures range 8–24°C (Pereira *et al*., 2018). In threespine stickleback (*Gasterosteus aculeatus*), the highest amylase and trypsin activities occur at 18°C while the optimum range was 4–20°C (Hani *et al*., 2018). In walking catfish (*Clarias batrachus*), optimum range was 10–30°C; protease activities peak at 25°C and lipase activity at 30°C (Ahmad *et al*., 2014). Similarly, in Indian major carp (*Catla catla*), digestive enzyme activities are optimized at specific temperatures at range of 18–28°C (Sharma *et al*., 2017).

The impact of feeding frequency and temperature on digestive activity in fish is closely connected to thyroid hormone activity, which adjusts to temperature changes to regulate overall metabolism. Thyroid hormones play a crucial role in the growth and development of larvae and juvenile fish. Previous research has demonstrated that thyroid activity fluctuates in response to various environmental stimuli like overcrowding of organism (Rafatnezhad *et al*., 2008). Reduced food intake has been associated with decreased thyroid hormone concentrations in fish and other vertebrates. Additionally, thyroid hormones are generally linked to increased metabolic rates and are typically reduced during periods of food deprivation to conserve energy (Suchiang and Gupta, 2011). This study investigated the activity of T3, T4, and TSH hormones, revealing a significant effect of varying temperature and different feeding levels. Although various feeding levels were employed in this study at both high and low temperatures, the minimum feed ration used was 2%, which is standard for tilapia. Consequently, the fish did not experience starvation or stress due to reduced feed, resulting in no major fluctuations in thyroid hormone activity.

**Conclusion**

The findings of the current study indicate that tilapia exhibit better growth rates at higher temperature compared to lower temperature, attributed to high metabolism and increased feed intake. Among varying feed ration levels, 4% feed ration demonstrated improved growth and weight gain outcomes. However, the best feed conversion ratio (FCR) was observed at high temperatures with a 2% feed ration, suggesting improved feed efficiency. Almost uniform growth patterns across varying feed frequencies indicate that a 2% feed frequency is suitable for diverse culture systems without adversely affecting growth rates.

**Reference:**

1. Ahmad, T., Singh, S. P., Khangembam, B. K., Sharma, J. G. and Chakrabarti, R. (2014). Food consumption and digestive enzyme activity of *Clarias batrachus* exposed to various temperatures. *Aquaculture Nutrition*, *20*(3), 265-272. https://doi.org/10.1111/anu.12072
2. Ali, M. Z., Hossain, M. A. and Mazid, M. A. (2005). Effect of mixed feeding schedules with varying dietary protein levels on the growth of sutchi catfish, *Pangasius hypophthalmus* (Sauvage) with silver carp, *Hypophthalmichthys molitrix* (Valenciennes) in ponds. *Aquaculture Research*, *36*(7), 627-634. https://doi.org/10.1111/j.1365-2109.2005.01262.x
3. AOAC. Official method of Analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC, Method 935.14 and 992.24. 2005.
4. Ashley-Dejo, S. S., Olaoye, O. J., Adelaja, O. A. and Abdulraheem, I. (2014). Effects of feeding levels on growth performance, feed utilization and body composition of African catfish (*Clarias gariepinus*, Burchell 1822). *International Journal of Biology and Biological Sciences*, *3*(2), 12-16. http://www.lrrd.org/lrrd34/9/3480ashl.html
5. Atwood, H. L., Tomasso, J. R., Webb, K. and Gatlin Iii, D. M. (2003). Low-temperature tolerance of Nile tilapia, *Oreochromis niloticus*: effects of environmental and dietary factors. *Aquaculture Research*, *34*(3), 241-251. https://doi.org/10.1046/j.1365-2109.2003.00811.x
6. Ayinla, O. A. (2007). Analysis of feeds and fertilizers for sustainable aquaculture development in Nigeria. *FAO Fisheries Technical Paper*, *497*, 453.
7. Azaza, M. S., Dhraïef, M. N. and Kraïem, M. M. (2008). Effects of water temperature on growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia. *Journal of thermal Biology*, *33*(2), 98-105. https://doi.org/10.1016/j.jtherbio.2007.05.007
8. Brett, J. R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerkd*). *American zoologist*, *11*(1), 99-113. https://doi.org/10.1093/icb/11.1.99
9. Brett, J.R. (1979) Environmental factors and growth. In: Fish Physiology, Vol. VIII (eds W.S. Hoar, D.J. Randall and J.R. Brett) Academic Press: London, pp. 599–667. https://doi.org/10.1016/S1546-5098(08)60033-3
10. Buentello, J. A., Gatlin III, D. M. and Neill, W. H. (2000). Effects of water temperature and dissolved oxygen on daily feed consumption, feed utilization and growth of channel catfish (*Ictalurus punctatus*). *Aquaculture*, *182*(3-4), 339-352. https://doi.org/10.1016/S0044-8486(99)00274-4
11. Bureau, D. P., Hua, K. and Cho, C. Y. (2006). Effect of feeding level on growth and nutrient deposition in rainbow trout (*Oncorhynchus mykiss* Walbaum) growing from 150 to 600 g. *Aquaculture research*, *37*(11), 1090-1098. https://doi.org/10.1111/j.1365-2109.2006.01532.x
12. Burel, C., Person-Le Ruyet, J., Gaumet, F., Le Roux, A., Severe, A. and Boeuf, G. (1996). Effects of temperature on growth and metabolism in juvenile turbot. *Journal of Fish Biology*, *49*(4), 678-692. https://doi.org/10.1111/j.1095-8649.1996.tb00064.x
13. Cai, L. S., Wang, L., Song, K., Lu, K. L., Zhang, C. X. and Rahimnejad, S. (2020). Evaluation of protein requirement of spotted seabass (*Lateolabrax maculatus*) under two temperatures,

and the liver transcriptome response to thermal stress. *Aquaculture*, *516*, 734615. https://doi.org/10.1016/j.aquaculture.2019.734615

14. Caulton, M.S., 1982. Feeding, metabolism and growth of tilapias: some quantitative considerations. In: Pullin, R.S.V., Lowe-McConnell, R.H. (Eds.), The Biology and Culture of tilapias. ICLARM, Manila, Philippines, pp. 157–180.
15. Cho, C. Y. and Bureau, D. P. (1998). Development of bioenergetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture. *Aquatic Living Resources*, *11*(4), 199-210. https://doi.org/10.1016/S0990-7440(98)89002-5
16. Craig, S. R., Helfrich, L. A., Kuhn, D. and Schwarz, M. H. (2017). Understanding fish nutrition, feeds, and feeding.
17. Davies, O. A., Inko-Tariah, M. B. and Amachree, D. (2006). Growth response and survival of Heterobranchus longifilis fingerlings fed at different feeding frequencies. *African Journal of Biotechnology*, *5*(9).
18. Dias, J., Yúfera, M., Valente, L. M. and Rema, P. (2010). Feed transit and apparent protein, phosphorus and energy digestibility of practical feed ingredients by Senegalese sole (*Solea senegalensis*). *Aquaculture*, *302*(1-2), 94-99. https://doi.org/10.1016/j.aquaculture.2010.02.013
19. Dwyer, K. S., Brown, J. A., Parrish, C. and Lall, S. P. (2002). Feeding frequency affects food consumption, feeding pattern and growth of juvenile yellowtail flounder (*Limanda ferruginea*). *Aquaculture*, *213*(1-4), 279-292. https://doi.org/10.1016/S0044-8486(02)00224-7
20. El-Saidy, D. M. and Gaber, M. M. (2005). Effect of dietary protein levels and feeding rates on growth performance, production traits and body composition of Nile tilapia, *Oreochromis niloticus* (L.) cultured in concrete tanks. *Aquaculture research*, *36*(2), 163-171. https://doi.org/10.1111/j.1365-2109.2004.01201.x
21. Eriegha, O. J. and Ekokotu, P. A. (2017). Factors affecting feed intake in cultured fish species: A review. *Animal Research International*, *14*(2), 2697-2709.
22. Fatima, S., Komal, W., Manzoor, F., Latif, A. A., Liaqat, R., Ameen, S. and Janjua, R. S. (2021). Analysis of the growth performance, stress, profile of fatty acids and amino acids and cortisol in Tilapia (*Oreochromis niloticus*), cultured at high stocking density using in-pond raceway system. *Saudi Journal of Biological Sciences*, *28*(12), 7422-7431. https://doi.org/10.1016/j.sjbs.2021.08.048
23. Folch J, Lees M, Sloane-Stanley GM. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. J Biol Chem. 1957; 226:497–509. https://doi.org/10.1016/S0021-9258(18)64849-5
24. Folkedal, O., Torgersen, T., Olsen, R. E., Fernö, A., Nilsson, J., Oppedal, F., ... & Fernö, A. (2012). Duration of effects of acute environmental changes on food anticipatory behaviour, feed intake, oxygen consumption, and cortisol release in Atlantic salmon parr. *Physiology & behavior*, *105*(2), 283-291. https://doi.org/10.1016/j.physbeh.2011.07.015
25. Gaylord, T. G., MacKenzie, D. S. and Gatlin, D. M. (2001). Growth performance, body composition and plasma thyroid hormone status of channel catfish (*Ictalurus punctatus*)

in response to short-term feed deprivation and refeeding. *Fish Physiology and Biochemistry*, *24*, 73-79. https://doi.org/10.1023/A:1011199518135

26. Gelman A, Kuz'mina V, Drabkin V, *et al*. Temperature adaptation of digestive enzymes in fish. In: Cyrino JEP, Bureau DP, Kapoor BGeditors. Feeding and digestive functions in fishes. Enfield, NH, USA: Science Publishers; 2008. p. 155–225.
27. Gilannejad, N., Silva, T., Martínez-Rodríguez, G. and Yúfera, M. (2019). Effect of feeding time and frequency on gut transit and feed digestibility in two fish species with different feeding behaviours, gilthead seabream and Senegalese sole. *Aquaculture*, *513*, 734438. https://doi.org/10.1016/j.aquaculture.2019.734438
28. Hani, Y. M. I., Marchand, A., Turies, C., Kerambrun, E., Palluel, O., Bado-Nilles, A., ... & Dedourge-Geffard, O. (2018). Digestive enzymes and gut morphometric parameters of threespine stickleback (*Gasterosteus aculeatus*): Influence of body size and temperature. *PLoS One*, *13*(4), e0194932. https://doi.org/10.1371/journal.pone.0194932
29. Hatlen, B., Grisdale-Helland, B. and Helland, S. J. (2006). Growth variation and fin damage in Atlantic cod (*Gadus morhua* L.) fed at graded levels of feed restriction. *Aquaculture*, *261*(4), 1212-1221. https://doi.org/10.1016/j.aquaculture.2006.09.027
30. Hauser, W.J. 1977. Temperature requirement of tilapia. Calif. Fish Game 63(4): 228-233.
31. Hepher, B. (1988). *Nutrition of pond fishes*. Cambridge university press.
32. Hevrøy, E. M., Waagbø, R., Torstensen, B. E., Takle, H., Stubhaug, I., Jørgensen, S. M., ... & Hansen, T. (2012). Ghrelin is involved in voluntary anorexia in Atlantic salmon raised at elevated sea temperatures. *General and comparative endocrinology*, *175*(1), 118-134. https://doi.org/10.1016/j.ygcen.2011.10.007
33. Isyagi, N., Atukunda, G., Aliguma, L., Ssebisubi, M., Walakira, J., Kubiriza, G. and Mbulameri, E. (2009). Assessment of national aquaculture policies and programmes in Uganda. *Assessment of national aquaculture policies and programmes in Uganda.*
34. Jamabo, N. A., Fubara, R. I. and Dienye, H. E. (2015). Feeding frequency on growth and feed conversion of *Clarias gariepinus* (Burchell, 1822) fingerlings. *International journal of fisheries and aquatic studies*, *3*(1), 353-356.
35. Jobling, M., Covès, D., Damsgård, B., Kristiansen, H. R., Koskela, J., Petursdottir, T. E., ... & Gudmundsson, O. (2001). Techniques for measuring feed intake. *Food intake in fish*, 49-87. DOI:10.1002/9780470999516
36. Kaushik, S. J. (2013). Feed management and on-farm feeding practices of temperate fish with special reference to salmonids.
37. Khan, M. A., Ahmed, I. and Abidi, S. F. (2004). Effect of ration size on growth, conversion efficiency and body composition of fingerling mrigal, *Cirrhinus mrigala* (Hamilton). *Aquaculture Nutrition*, *10*(1), 47-53. https://doi.org/10.1046/j.1365-2095.2003.00279.x
38. Khandan Barani, H., Dahmardeh, H., Miri, M. and Rigi, M. (2019). The effects of feeding rates on growth performance, conversion efficiency and body composition of juvenile snow trout, *Schizothorax zarudnyi*. *Iranian Journal of Fisheries Sciences*, *18*(3), 507-516. 10.22092/IJFS.2019.118285
39. Kousoulaki, K., Sæther, B. S., Albrektsen, S. and Noble, C. (2015). Review on European sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) nutrition and feed management: a practical

guide for optimizing feed formulation and farming protocols. *Aquaculture Nutrition*, *21*(2), 129-151. https://doi.org/10.1111/anu.12233

40. Krogdahl Å, Sundby A, Bakke AM. Gut secretion and digestion. In: Farrell AP, editor. Encyclopedia of fish physiology. San Diego: Academic Press; 2011. p. 1301–1310.
41. Larsson, S., Berglund, I., 2005. The effect of temperature on the growth energetic, growth efficiency of Aractic charr (*Salvelinus alpinus* L.) from four Swedish populations. Journal of Thermal Biology 30, 29–36. https://doi.org/10.1016/j.jtherbio.2004.06.001
42. Li, M. H., Robinson, E. H. and Hogue, C. D. (2006). *Catfish nutrition: Feeding food fish*. Mississippi State University Extension Service.
43. McCarthy, I. D., Carter, C. G. and Houlihan, D. F. (1992). The effect of feeding hierarchy on individual variability in daily feeding of rainbow trout, Oncorhynchus mykiss (Walbaum). *Journal of Fish Biology*, *41*(2), 257-263. https://doi.org/10.1111/j.1095-8649.1992.tb02655.x
44. Nadermann, N., Seward, R. K. and Volkoff, H. (2019). Effects of potential climate change-induced environmental modifications on food intake and the expression of appetite regulators in goldfish. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *235*, 138-147. https://doi.org/10.1016/j.cbpa.2019.06.001
45. National Research Council, Division on Earth, Life Studies, Committee on the Nutrient Requirements of Fish and Shrimp. (2011). *Nutrient requirements of fish and shrimp*. National academies press.
46. Ndome, C. B., Ekwu, A. O. and Ateb, A. A. (2011). Effect of feeding frequency on feed consumption, growth and feed conversion of *Clarias gariepinus x Heterobranchus longifilis* hybrids. *American-Eurasian Journal of Scientific Research*, *6*(1), 6-12.
47. Nguyen, M. V., Espe, M., Conceição, L. E., Le, H. M., Yúfera, M., Engrola, S. A., ... & Rønnestad, I. (2019). The role of dietary methionine concentrations on growth, metabolism and N-retention in cobia (*Rachycentron canadum*) at elevated water temperatures. *Aquaculture nutrition*, *25*(2), 495-507. https://doi.org/10.1111/anu.12875
48. Omitoyin, B. O. (2007). *Introduction to fish farming in Nigeria*. Ibadan university press.
49. Pandit, N. P. and Nakamura, M. (2010). Effect of high temperature on survival, growth and feed conversion ratio of Nile tilapia, *Oreochromis niloticus*. *Our Nature*, *8*(1), 219-224.
50. Parma, L., Pelusio, N. F., Gisbert, E., Esteban, M. A., D'Amico, F., Soverini, M., ... & Bonaldo, A. (2020). Effects of rearing density on growth, digestive conditions, welfare indicators and gut bacterial community of gilthead sea bream (*Sparus aurata*, L. 1758) fed different fishmeal and fish oil dietary levels. *Aquaculture*, *518*, 734854. https://doi.org/10.1016/j.aquaculture.2019.734854
51. Pereira, L. F., Peixoto, M. J., Carvalho, P., Sansuwan, K., Santos, G. A., Gonçalves, J. F. M. and Ozório, R. O. A. (2018). Cross-effects of dietary probiotic supplementation and rearing temperature on growth performance, digestive enzyme activities, cumulative mortality and innate immune response in seabass (*Dicentrarchus labrax*). *Aquaculture nutrition*, *24*(1), 453-460. https://doi.org/10.1111/anu.12578
52. Philippart, J-C. and J-C. Ruwet 1982. Ecology and distribution of tilapias. In The Biology and Culture of Tilapias (Eds. R.S.V. Pullin and R.H. Lowe-McConnell). ICLARM, Manila, Philippines. pp. 15-60.

53. Rafatnezhad, S., Falahatkar, B. and Tolouei Gilani, M. H. (2008). Effects of stocking density on haematological parameters, growth and fin erosion of great sturgeon (*Huso huso*) juveniles. *Aquaculture Research*, *39*(14), 1506-1513. https://doi.org/10.1111/j.1365-2109.2008.02020.x
54. Riche, M. and Garling, D. (2003). Feeding tilapia in intensive recirculating systems.
55. Shafland, P. L. and Pestrak, J. M. (1982). Lower lethal temperatures for fourteen non-native fishes in Florida. *Environmental biology of fishes*, *7*, 149-156. https://doi.org/10.1007/BF00001785
56. Sharma, J., Singh, S. P. and Chakrabarti, R. (2017). Effect of temperature on digestive physiology, immune-modulatory parameters, and expression level of Hsp and LDH genes in *Catla catla* (Hamilton, 1822). *Aquaculture*, *479*, 134-141. https://doi.org/10.1016/j.aquaculture.2017.05.031
57. Shcherbina, M. A. and O. P. Kazlauskene, 1971. Water temperature and digestibility of nutrient substances by carp. Hydrobiologia, 9: 40-44.
58. Smirnov, A. K. and Smirnova, E. S. (2019). Behavior of perch fry *Perca fluviatilis* (Percidae) in a heterothermal environment at different levels of food availability. *Biology Bulletin*, *46*(9), 1065-1074. https://doi.org/10.1134/S1062359019090152
59. Stefansson, B., Sandholt, G. B. and Gudmundsdottir, Á. (2017). Elucidation of different cold-adapted Atlantic cod (*Gadus morhua*) trypsin X isoenzymes. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1865*(1), 11-19. https://doi.org/10.1016/j.bbapap.2016.10.005
60. Suchiang, P. and Gupta, B. B. P. (2011). Effects of partial and full feed restriction on the plasma levels of thyroid hormones and testicular activity in the male air-breathing catfish, *Clarias gariepinus* during different phases of the breeding cycle. *International Journal of Biology*, *3*(2), 32-42. doi:10.5539/ijb.v3n2p32
61. Talbot, C., Corneillie, S. and Korsøen, Ø. (1999). Pattern of feed intake in four species of fish under commercial farming conditions: implications for feeding management. *Aquaculture Research*, *30*(7), 509-518. https://doi.org/10.1046/j.1365-2109.1999.00369.x
62. Thorpe, J. E. and Cho, C. Y. (1995). Minimising waste through bioenergetically and behaviourally based feeding strategies. *Water Science and Technology*, *31*(10), 29-40. https://doi.org/10.1016/0273-1223(95)00424-L
63. Tian, H. Y., Zhang, D. D., Li, X. F., Zhang, C. N., Qian, Y. and Liu, W. B. (2015). Optimum feeding frequency of juvenile blunt snout bream *Megalobrama amblycephala*. *Aquaculture*, *437*, 60-66. https://doi.org/10.1016/j.aquaculture.2014.11.032
64. Watanabe, T., Takeuchi, T., Satoh, S. and Kiron, V. (1996). Digestible crude protein contents in various feedstuffs determined with four freshwater fish species. *Fisheries Science*, *62*(2), 278-282. https://doi.org/10.2331/fishsci.62.278
65. Wynne, K., Stanley, S., McGowan, B. and Bloom, S. (2005). Appetite control. *Journal of Endocrinology*, *184*(2), 291-318.https://doi.org/10.1677/joe.1.05866
66. Xie, F., Ai, Q., Mai, K., Xu, W. and Ma, H. (2011). The optimal feeding frequency of large yellow croaker (*Pseudosciaena crocea*, Richardson) larvae. *Aquaculture*, *311*(1-4), 162-167. https://doi.org/10.1016/j.aquaculture.2010.12.005

67. Yuan, Y. C., Gong, S. Y., LUO, Z., Yang, H. J., Zhang, G. B. and Chu, Z. J. (2010). Effects of dietary protein to energy ratios on growth and body composition of juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture nutrition*, *16*(2), 205-212. https://doi.org/10.1111/j.1365-2095.2009.00652.x
68. Zeng, N. N., Jiang, M., Wen, H., Liu, W., Wu, F., Tian, J., ... & Guo, Z. B. (2021). Effects of water temperatures and dietary protein levels on growth, body composition and blood biochemistry of juvenile GIFT tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition*, *27*(1), 240-251. https://doi.org/10.1111/anu.13181