

## Influence of interaction between feeding schedule and long-term starvation on biological indicators, intestine histological and body biochemical of Nile tilapia (*Oreochromis niloticus*) fry

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**ABSTRACT:** A-60-day factorial trial 3x2 was conducted to evaluate the responses of Nile tilapia (*Oreochromis niloticus*) fry to compensatory growth after long-term starvation under different feeding rates. Groups were performed in duplicated as the following GI7, GI10: fry were fed every day with feeding rates of 7% and 10%. GII7, GII10: fry fed for three weeks and fasted a week with feeding rates of 7 and 10%, and groups GIII7, and GIII10 fry fed two weeks and fasted two weeks during the experimental period. 120 fry with an average initial weight of  $1.98 \pm 0.16$  g and were randomly divided into 12 plastic tanks ( $54 \times 38 \times 28$  cm: L  $\times$  W  $\times$  H) with a rate of 10 fry/tank. A commercial artificial feed (30% protein) was used. Water was exchanged at a rate of 50% of the water volume weekly. The results showed that feeding rate levels (from 7% to 10%) did not significantly affect growth or feed utilization parameters and histological of the liver and intestine. Statistical analysis of the effect of long-term feeding restriction on growth performance showed no significant differences between GI that fed every day and GII that fed 3 weeks (per month) wherein GII had better histological characteristics in their intestine but groups GIII 7, 10 that fed two weeks and fasted two weeks had the lowest performance regardless the feeding rate. In addition, interaction between feeding rate and long-term restriction on growth performance showed that GI10 was the best followed by GII7. Thus, this study suggests that GII7 fed 3 weeks and fasting a week with a feeding rate of 7% was the best in terms of feed conversion ratio and intestinal histological.

**Keywords:** *Oreochromis niloticus*; Nile tilapia; Feed restriction; Compensatory growth

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### 1. INTRODUCTION

The feeding process is generally the highest variable cost at aquaculture facilities. In intensive aquaculture, commercial feed is one of the inputs of the greatest economic impact, since it represents 30 to 60% of production costs (Guimarães et al., 2008; Borski et al., 2011). Numerous endogenous variables, such

as hormone regulation, food type and size, frequency of feeding, feeding time, the ability to absorb nutrients, environmental conditions and feed absorption capacity, have an impact on feeding efficiency (Abdel-hakim et al., 2006; Abdel-Aziz et al., 2021, 2024).

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Among these variables, feeding rate is crucial for controlling food consumed, growth, waste output, and production expenses in fish farms (Silva *et al.*, 2007; Tian *et al.*, 2015).

Nevertheless, Fish growth and food consumption must be balanced. Therefore, in order to attain economic efficiency, it is crucial to understand the optimal feeding strategies for each species of fish (Gökçek *et al.*, 2008). Feed ration management can offer growth advantages and decrease its use (Booth *et al.*, 2008; El-Dakar *et al.*, 2021).

As it is known, feeding rate is the percentage of daily feeding determined based on fish biomass. If the feeding rates are not optimized, feeding can be either insufficient or excessive for the cultivated species (Kim *et al.*, 2021).

Overfeeding leads to feed wastage, increased waste, and deterioration of water quality (Hwoan *et al.*, 2003). Poor management of feed quantity negatively affects aquaculture water quality, resulting in increased production costs for water treatment and periodic changes. This can be addressed through compensatory growth phenomena that appears in a wide range of fish species which are subjected to nutritional deprivation followed by a period of feeding and then regaining their original body weight or growth path. Compensatory growth may be used as a management tool to improve growth rates and feed efficiency and to reduce feeding costs. In various fish species, deprivation or limited-feeding strategies have been used to induce compensatory growth (Poot-López *et al.*, 2020; Xiao *et al.*, 2013).

There are some factors underlying the rapid growth during compensatory growth phases including hyperphagia and increased feed efficiency in fish after feed limitation (Boersma and Wit, 1997) such as fish size, feeding schedule, nutrient levels in the diet, and feeding regimes (Bilton and Robins 1973; Martinez *et al.*, 2002; Gaylord and Gatlin 2001; Oh *et al.*, 2011).

Therefore, this study investigated the effects of interaction between long-term starvation and the

different feeding levels on growth, histological changes of the intestinal tract, and body composition of Nile tilapia *Oreochromis niloticus* fry.

## **2. MATERIALS AND METHODS**

### **2.1. Site of work**

This research carried out at Aquaculture Nutrition Laboratory, Faculty of Aquaculture and Marine Fisheries, Arish University, Arish, North Sinai, Egypt.

### **2.2. Fish fry and Experimental design**

Fry was transported from the fish farm of Arish University to the experiment site in a plastic tank (70 liters) over a distance of 15 minutes. Fry was acclimatized to laboratory conditions for a week. After the adaptation period, fry that varied in size and were weak were excluded and randomly distributed in 12 plastic tanks (54 × 38 × 28 cm: L × W × H) at rate of 10 fry/ tank. Tanks were equipped with continuous aeration by a connected compressor with air stones. The average initial weight of fry was 1.98 ± 0.16 g. The current study was performed to be a factorial trial to inspect three different feeding protocols of long-term starvation with two levels of feeding rate and was as the follows: GI7, GI10: fry were fed every day with feeding rates of 7% and 10%. GII7, GII10: fry fed for three weeks and fasted a week with feeding rates of 7 and 10%, and groups GIII7, GIII10: fry fed two weeks and fasted two weeks during the experimental period.

### **2.3. Feed and feeding**

A basal diet was brought from SKRETTING Egypt Company and contained 30% crude protein, 6 % fat, fiber 5.22 % and gross energy 3900 kcal/kg. Diet was used as mash to suitable with fry mouth. Feeding was by hand in twice daily at 9 a.m. and 16 p.m. at the week end, tanks was cleaned by siphoned with changed water at rate of 50%. Fish were weighted every two weeks to adjust the amount of offered feed.

#### 2.4. Water measurements

The fishes were reared in brackish water with a 3-ppt salinity, temperature, and pH with multiparameter pH / ORP / °C / EC / TDS / Salinity Bench Meter, dissolved oxygen with Water Proof Portable Dissolved Oxygen and BOD Meter (HI98193), and ammonia with Hanna instruments test kits (HI4829), will be periodically measured, daily. Generally, water physiochemical indices were within optimum limits during the trial duration for tilapia growth as reported by (El-Sayed, 2006).

#### 2.5. Measurements of growth

Weight gain (WG, g) = FW-IW where: FW is final body weight and (g) IW is initial body weight(g).

Average daily gain (ADG, g), WG/days

Specific growth rate (SGR, %), (In FW-In IW/ days) x100.

Survival rate (SR, %), (Number of fishes at the end/Number of fishes at the beginning) x100.

Feed conversion ratio (FCR), feed intake g per fish/WG, g.

Hepatosomatic index (HSI) weight of the liver/ weight of the body.

#### 2.6. Sampling and examinations

Each fish in each tank was weighed individually (in grams) at the finish of the trial, and four fish from each replicate were euthanized with a deep anesthetic dose of 100 mg/l clove oil (El-Dakar et al., 2021). Subsequently, two fish were dissected for histological analysis, and the other fish was dried to assess the chemical composition of the whole-body fish.

##### 2.6.1. Histopathological examination

Liver and intestinal samples were taken, and they were preserved in 10% neutral buffered formalin. The tissues were subsequently kept in a 50% ethyl alcohol solution for 48 hours. Then the tissues passed through alcohol series, and they were stored at 65°C temperature in paraffin overnight.

To perform tissue sectioning, specimens were fixed in paraffin and cut at a thickness of 5 µm. The tissue sectioning procedure involves applying a deparaffinization process and exposing the tissue to a series of alcohol and xylene. Tissue sections were stained with hematoxylin and eosin, and fixed with Stellan and histopathologic changes were examined with a light microscope (Kurtoglu et al., 2016).

##### 2.6.2. Chemical analysis

Composition of the basal diet and whole-body fish was conducted following the methods described in AOAC (2010). The gross energy was estimated for diet using the factors 5.64, 9.44, and 4.22 Kcal for CP, EE, and carbohydrates, respectively (NRC, 1993).

#### 2.7. Statistical Analysis

Data were analyzed by Two-way analysis of variance (ANOVA). The differences among groups were determined using the Waller-Duncan test at  $P \leq 0.05$  as the significance level using SPSS Statistical Package Program v.23.

### 3. RESULTS

#### 3.1. Biological indicators

The effect of feeding rate on the growth performance of Nile tilapia fry alone was presented in Table (1). Statistical analysis indicated no significant differences ( $P \leq 0.05$ ) between all groups that fed at a feeding rate of 7% and those fed at a rate of 10% in WG, ADG, SGR, FCR, HSI.

Data in Table (1) showed the effect of long-term feeding alone on the growth performance of Nile tilapia fry. Measurements of FW, WG, ADG, SGR, and FCR did not significantly change between fish fed daily and those in GII that were fed three weeks with fasting week. But fish in group GIII that fed for two weeks with fasting for two weeks had significantly lower growth indices than the other groups.

Besides, there were significant differences among treatments as a result of interaction between the both tested factors.

Whereas, GI10 and GII7 were the highest in all growth indices followed by GI7, GIII10. While GIII7 and GIII10 were the worst.

**Table 1.** Role of feeding levels with long-term starvation on performance of Nile tilapia *Oreochromis niloticus* fry for 60 days

Groups	Measurements						
	IW, g	FW, g	WG, g	ADG,g/day	SGR, %/day	FCR	HIS, %
<i>Effect of feeding rate alone</i>							
Feeding rate 7%	3.92	14.49	10.56	0.188	2.29	2.46	2.35
Feeding rate 10%	3.81	14.79	10.98	0.196	2.37	2.37	3.21
<i>P-value</i>	0.32	0.88	0.84	0.84	0.79	0.81	0.26
SED*	0.105	2.04	2.09	3.65	0.28	0.355	0.73
<i>Effect of long-term starvation alone</i>							
GI: Feeding every day	3.79	17.44a	13.65a	0.24a	2.71a	2.14	3.74a
GII: Fasting a week/month	3.66	15.69a	11.8a	0.21a	2.48a	2.34	2.98ab
GIII: Fasting two week/month	3.94	10.8b	6.86b	0.12b	1.79b	2.77	1.62b
<i>P-value</i>	0.52	0.002	0.002	0.002	0.02	0.33	0.04
SED*	0.13	1.3	1.37	0.025	0.183	0.4	0.7
<i>Interaction between feeding rate and long-term starvation</i>							
GI7	3.93	15.55bc	11.62bc	0.21bc	2.45b	2.02	3.84a
GI10	3.65	19.33a	15.68 a	0.28a	2.98 a	2.26	3.65 a
GII7	3.91	17.31ab	13.4ab	0.24ab	2.66 ab	2.25	2.15 ab
GIII10	3.86	14.07c	10.21c	0.18c	2.3b	2.43	3.81 a
GIII7	3.94	10.61d	6.67d	0.12d	1.77 c	3.11	1.08b
GIII10	3.94	10.99d	7.05d	0.13d	1.82c	2.43	2.17ab
<i>P-value</i>	0.66	0.001	0.001	0.001	0.003	0.657	0.09
SED*	0.195	0.96	1.09	0.019	0.18	0.637	0.913

(a, b, c, d) Average in the same column having different superscripts differ significantly ( $P \leq 0.05$ )

\* SED is the standard error of difference.

### 3.2. Histological examinations

#### 3.2.1. Liver

All groups' livers under a light microscope revealed polygonal hepatocytes arranged in cords divided by blood sinusoids. Every hepatocyte exhibited a unique central nucleus that was spherical in shape and contained granular cytoplasm and nucleoli. The hepatic artery, terminal hepatic vein, and biliary duct were located in the portal area. There existed exocrine pancreatic tissue, or hepatopancreas, which was encircled by connective tissue and set apart from the liver cells by blood vessel-containing sinusoids, while both groups (7%

and 10%) which were starved for two weeks show aggregation of the cell nucleus and increase in the nucleus size than other groups and control. Additionally, groups that starved for two weeks demonstrated infiltration of blood cells as a physiological response to the starvation period. Scale bar = 500  $\mu$ m

#### 3.2.2. Intestine

Fish fed every day with 7% as feeding rate GI7 demonstrated that, the mucosal layer's typical organization and the interspersed goblet cells, which contain transparent mucous droplets, between these enterocytes. The villus's

core is made up of the lamina propria, which supports the epithelial cells. Blood arteries and immune cells were seen in this layer.

The group GII7 displayed normal mucosal layer organization, increased villus length, shrinkage in the lamina propria, and goblet cells. This layer contains blood vessels, immune cells, lymphatic vessels, and lacteal cells. The group

GIII7 exhibited abnormal mucosal layer organization, atrophy in enterocytes, atrophy and shrinkage of the lamina propria, an increase in the thickness of the muscular lamina, and a decrease in goblet cells; blood vessels, immune cells, lymphatic vessels, and lacteal cells were present in this layer.

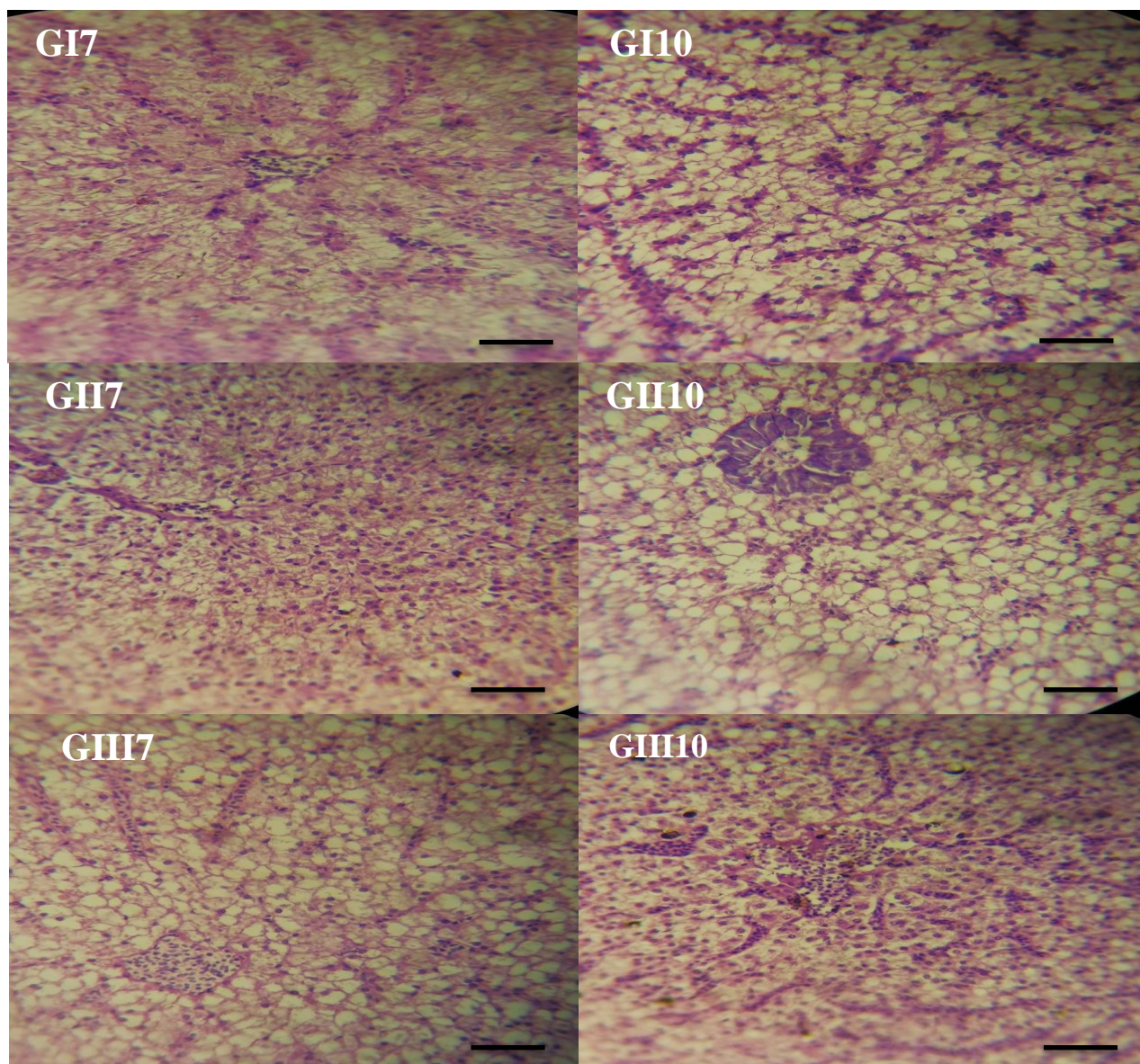


Plate 1: Show the microscopic histological structure of *Oreochromis niloticus* juvenile liver as following: For feeding rate (7%) of body weight: (GI7) control group fed every day, (GII7) fed for 3 weeks and starvation for 1 weeks, (GIII7) fed for 2 weeks and starvation for 2 weeks. For feeding rate (10%) of body weight (GI10) control group fed every day, (GII10) fed for 3 weeks and starvation for 1 weeks, (GIII10) fed for 2 weeks and starvation for 2 weeks.

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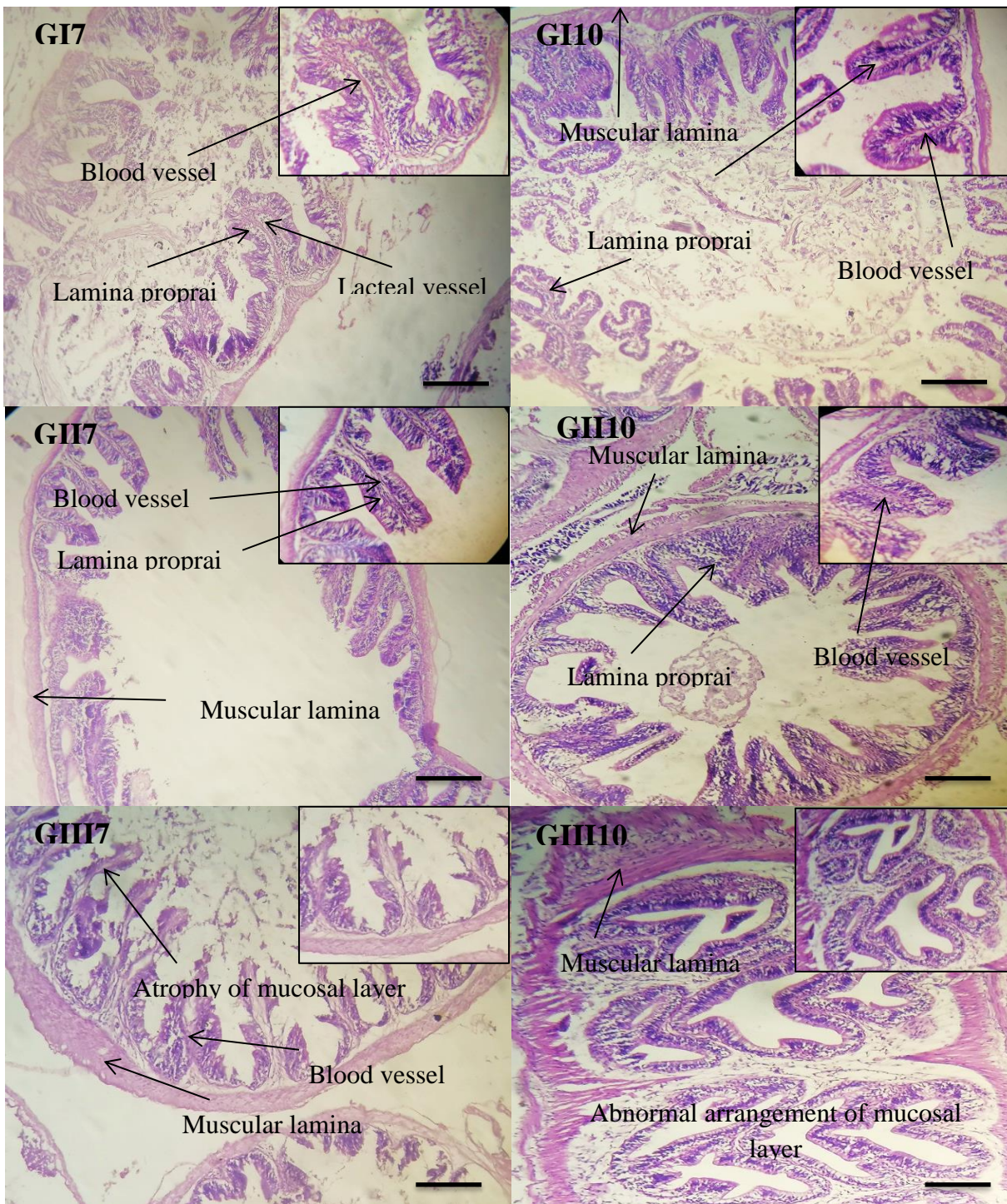


Plate 2: Show the microscopic histological structure of *Oreochromis niloticus* juvenile intestine as following: For feeding rate (7%) of body weight: (GI7) control group fed every day, (GII7) fed for 3 weeks and starvation for 1 weeks, (GIII7) fed for 2 weeks and starvation for 2 weeks. For feeding rate (10%) of body weight (GI10) control group fed every day, (GII10) fed for 3 weeks and starvation for 1 weeks, (GIII10) fed for 2 weeks and starvation for 2 weeks.

The group GI10 had normal mucosal layer organization and the interspersions of goblet cells, which contain transparent mucous droplets, between these enterocytes. The villus's core is made up of the lamina propria, which supports the epithelial cells. Blood arteries and immune cells were seen in this layer. The group GIII10 exhibited abnormal arrangement of mucosal layer and atrophy in enterocytes; additionally, atrophy and shrinkage of the lamina propria and an increase in the thickness of the lamina propria. The group GII10 displayed the following: the mucosal layer was normally organized, the villus length increased, the lamina propria shrank, an increase in the thickness of the muscular lamina, and goblet cells were noted; this layer contains immune cells, blood vessels, lymphatic vessels, and lacteal cells. muscular

lamina; a decrease in goblet cells was noted; blood vessels, immune cells, and a lymphatic vessel were present in this layer. Scale bar = 300  $\mu$ m.

### 3.3. Chemical analysis of whole-body fish

Results of chemical body composition were presented in table 2. The different rate of feeding had not significantly ( $p \leq 0.05$ ) affected on fish body composition. However, fasting a week or two weeks led to significant increase in protein and ash content than lipid. While fish fed every day had the highest lipid content.

Interaction between feeding level and feeding deprivation showed significant effect on body composition. GIII10 had the highest protein followed by GIII7, GII7, GII10 and the lowest protein was recorded by groups GI7 and GI10 which recorded the highest lipid content

**Table 2.** Role of feeding levels with long-term starvation on whole body composition of Nile tilapia *Oreochromis niloticus* fry for 60 days

Groups	Measurements			
	Moisture, %	Crude protein, %	Lipid, %	Ash, %
<i>Effect of feeding rate alone</i>				
Feeding rate 7%	71.44	60.88	24.43	14.7
Feeding rate 10%	74.32	62.34	22.34	15.31
<i>P-value</i>	0.009	0.27	0.447	0.676
SED*	1.52	2.2	4.59	2.21
<i>Effect of long-term starvation alone</i>				
GI: Feeding every day	71.3	59.12 <sup>b</sup>	28.46 <sup>a</sup>	12.41 <sup>b</sup>
GII: Fasting a week/month	73.24	62.10 <sup>a</sup>	22.60 <sup>b</sup>	15.29 <sup>a</sup>
GIII: Fasting two week/month	74.03	63.58 <sup>a</sup>	19.10 <sup>b</sup>	17.31 <sup>a</sup>
<i>P-value</i>	0.19	0.002	0.001	0.001
SED*	1.36	0.87	1.57	0.85
<i>Interaction between feeding rate and long-term starvation</i>				
GI7	69.4 <sup>d</sup>	58.65 <sup>c</sup>	29.79 <sup>a</sup>	11.55 <sup>d</sup>
GI10	73.36 <sup>b</sup>	59.59 <sup>c</sup>	27.13 <sup>b</sup>	13.27 <sup>c</sup>
GII7	72.96 <sup>b</sup>	62.08 <sup>b</sup>	21.58 <sup>d</sup>	16.33 <sup>b</sup>
GII10	73.52 <sup>b</sup>	62.11 <sup>b</sup>	23.62 <sup>c</sup>	14.26 <sup>b</sup>
GIII7	71.97 <sup>c</sup>	61.84 <sup>b</sup>	21.93 <sup>d</sup>	16.22 <sup>b</sup>
GIII10	76.10 <sup>a</sup>	65.33 <sup>a</sup>	16.26 <sup>c</sup>	18.40 <sup>a</sup>
<i>P-value</i>	20.001	20.001	20.001	20.001
SED*	0.26	0.37	0.43	0.38

(a, b, c, d) Average in the same column having different superscripts differ significantly ( $P \leq 0.05$ ).

\* SED is the standard error of difference.

#### 4. DISCUSSIONS

Tilapia farmers can surely derive more income from their aquaculture activities if the amount of money spent on feeds is reduced considerably (Limbu and Jumanne, 2014).

Feeding strategies can be adapted to minimize the environmental impact of aquaculture, production costs, or the quality of the fish (Poot-López *et al.*, 2014, El-Dakar *et al.*, 2023). Oh *et al.* (2011) stated that the optimizing feeding regimes during the growth process of fish could reduce the costs of feeding, without slowing growth, increasing quality and profits, which could ensure the future success of aquaculture development and management.

In the light of the presented results, increasing the feeding levels from 7% of biomass to 10% did not occur any changes in growth rate or FCR. A similar trend was observed by Huang *et al.* (2015) they tested different feeding rates of 2, 4, 6, 8, and 10 % of body weight on GIF tilapia fry with initial weight 0.85 g and found that a feeding rate of 6% BW/d and a feeding frequency of 2 meals/d is the most favorable protocol for the growth and physiological balance of GIFT during the fry phase. Also, this result was completely conformity with Ali *et al.* (2007) Nile tilapia (*Oreochromis niloticus*) fingerlings weighing  $1.33 \pm 0.15$  g were fed at certain ration levels (10%, 9%, 8% and 7% of their body weights per day) for 86 days and found that insignificant differences in growth among the treatments.

Accordingly, it can be recommended that increasing feeding rate than optimum range have a negative effect on growth by abetting the deterioration of water quality (Anderson and Fast, 1991; NRC 2011). It has long been recognized that overfeeding is more dangerous than underfeeding. Feed ration greater than optimum feed level would increase the waste food, increasing the feed conversion ratio.

In the same context, the quadratic model analysis also suggested that growth responses of fish reach a maximum as feeding rates increase, but then decrease when the nutrient levels increase to intolerable levels (Lee *et al.*, 2014).

Interestingly, the strategy of depriving feed for a week and then refeeding for 3 weeks achieved a growth rate that was not statistically different with fish fed continuously. Several studies confirmed that compensatory growth is significantly associated with feed-deprivation period (Gao and Lee, 2012), also they added that hyperphagia and growth efficiency play major roles in compensatory growth during the refeeding period. The mechanism of compensatory growth in different fish species may be more complex than can be explained by the lipid stability model, but compensatory growth in Nile tilapia depends on the length of the period of feeding deprivation.

Moreover, Wang *et al.* (2000) and Tian and Qin (2004) reported that hyperphagia occurs in several fish species and is the major mechanism for compensatory growth in this species. In the light of this, Ali *et al.* (2016) found that the feed intake of the restricted groups was higher than that of the control group throughout the refeeding period, although no significant differences were found. It was suggested that HSI could be a good indicator to reflect compensatory growth of channel catfish (Gaylord and Gatlin 2001). The present study noted that HSI did not significantly by the different feeding levels but significantly decreased with GIII and GII regardless feeding rate, this may be due to utilize of store energy in liver for basal metabolism and maintenance for survival even during fasting. Whereas starvation depleted the hepatic glycogen store and resulted in low HSI levels as reported by (Xiao *et al.*, 2013).

With reference to results the interaction between feeding rate and long-term starvation, the continuous feeding with feeding rate of 10



and fish fed with rate of 7% with fasting a week/month (GII7) had the higher performance and FCR did not significant differ between them in comparison with fish fed daily at rate of 7% feeding rate (GI7) and groups fed two weeks with fasting two weeks GIII7 or GIII10.

These observations confirmed statement of Tian and Qin (2004) inducing compensatory growth in fish is of considerable importance in aquaculture since it can offer advantages such as increased growth rates, a reduction in feed conversion ratio, and a consequent decrease in nitrogenous waste. Obviously, compensatory growth is typically accompanied an increase in the fish's appetite, which leads to improved feed efficiency also re-feeding after feeding deprivation increases the digestive enzymes activities in fish gut leading maximum utilization of feed intake and minimum fish wastes in cultured ponds. In the same manner, Mo *et al.* (2016) and Abdel-Aziz *et al.* (2024) they said that, a possible mechanism for reducing ammonia excretion may be associated with significant upregulation of proteolytic activities, mainly trypsin and chymotrypsin, which reduce the amount of waste nitrogenous compounds excreted by the fish as a result of applying optimal feeding protocols.

There is a belief in energy expenditure during starvation of fish can be reduced by decreased locomotor activity (Wieser *et al.* 1992). Reduced activity during refeeding could contribute to compensatory gain by increasing the proportion of the energy available to growth. However, hyperphagia is usually associated with a higher level of foraging activity, so, from a cost-benefit perspective, only a reduction of nonfeeding activity could be regarded as a beneficial effect. Juvenile roach showed a reduction in swimming activity during 3 weeks of starvation, but activity returned to control levels as soon as refeeding started (van Dijk *et al.*, 2005). In the sablefish, compensatory growth seems to be mediated by a shift in resource allocation, which results in a

reduced physiological capacity for forced swimming (Sogard and Olla, 2002; Ali *et al.*, 2003).

Responses rates to compensatory growth differed according to many factors, the most important of them, species, fish age and duration of feeding deprivation (Abdel-Aziz *et al.*, 2024). Wherein tilapia adapts well to a variety of settings and feeding regimens, including sub-optimal feeding levels, whereas fish may use a variety of behavioral and physiological strategies to meet their metabolic demands when they are starved (Lyons *et al.*, 1995). Likewise, Turano *et al.* (2008) indicate that feeding regimes that do not cause weight loss and are long enough in duration to elicit a compensatory response may have the most potential for eliciting complete compensation. However, our results showed decreasing in growth and feed efficiency when fish (GIII7 or 10) were fasted two weeks with re-feeding two weeks. Wherein Ali *et al.* (2016) recommended shortening the deprivation period, while increasing the refeeding period.

Our results of interaction between the tested factors was partially similar with Wang *et al.* (2009) and Gao *et al.* (2015) obtained compensatory growth of the feed-restricted groups during the refeeding period, although the growth of none of the restricted groups caught up with that of the control group over the experimental period.

Histomorphometry study displayed that liver of all groups did not affect by feeding deprivation and re-feeding compared to continuously feeding. However, GII7 and GIII10 that fasted for one week with re-feeding 3 weeks showed a positive effect as the length of the villi increase which increase the surface area of absorption in comparison with Groups GIII7 and GIII10 that fasted two weeks whereas their intestine had abnormal organization of mucosal layer and atrophy in enterocytes and decreased of lamina

propria. Similar observations of Naghshpour *et al.* (2021) detected that 2 to 4 days of starvation reduced the size of liver cells, but this trend was not significant compared to the control treatment. However, Rios *et al.* (2005) reported that, the hepatocyte atrophy occurred after 30 days of fasting in the neotropical traira (*Hoplias malabaricus*) and it may reflect mobilization of glycogen reserves. Generally, differing opinions on this topic may be related to feeding deprivation period. Also, vacuolar degeneration and fatty degeneration were more at higher feeding rates. Much greater liver damage was evident in Nile tilapia fry fed at 10% BW/d (Huang *et al.*, 2015).

Regarding histomorphometry of intestine, measurements of intestinal villi of fish are parameters of the integrity of the intestinal mucosa and are indicators of the ability of the fish intestine to absorb the nutrients. (Ferreira *et al.* 2018). In the same case, Zaldua and Naya, 2014) suggested that there is a progressive decrease of intestinal mass with starvation time and an increase of gut mass during refeeding, and these could involve changes in the enterocyte turnover rate and intestinal epithelial configuration. Also, Honorato *et al.* (2015) the length of the intestinal villi of Nile tilapia varied according to food availability. Our finding identical with Porto *et al.* (2024) who found the length and perimeter of intestinal villi of *Piaractus brachypomus* decreased during fasting and increased at 30 days of refeeding relative the control group.

However, may be increased the duration of starvation period led to negative effects on intestine morphological as occurred in GIII groups. Similarly, Ostaszewska *et al.* (2006) found that the reduction in length and perimeter of villi was directly related to fasting period, as the fish of the fasting group may not have developed their intestinal villi as much as the control group. Fasting causes changes that can compromise digestive activity, causing a reduction in villi height and length and reducing the area of epithelium, which, as a consequence,

decreases absorption capacity (Shaibani *et al.* 2013). Hence the positive effect of starvation and re-feeding on gut morphological depends on fasting duration. General, there are opposite trends with our findings in channel catfish, there was no deference between control and starved fish during 18-week trials (Kim and Lovell, 1995).

Growth performance did not differ between control and starved group after refeeding for 7 weeks in rainbow trout (Speare and Arsenaault, 1997). Limbu and Jumanne (2014) reported that, feed restriction did not affect mean body and final weights as well as growth performance of *O. niloticus*. Moreover, Moutou *et al.*, (1998) and Álvarez (2012) there disadvantages have been reported in its implementation, particularly effects on size heterogeneity and increases in the aggressiveness of fish when feeding deprivation or when it is abundant, due to the hyperphagia characteristic.

Regarding fish body composition, some works have indicated that feeding regime and feed restriction affects the chemical composition of the body fish due to the use of nutrients (crude protein and total lipids) as an energy source for necessary physiological processes (Cho, 2005). Our findings were completely likeness to Wang *et al.* (2000) demonstrated that tilapia body moisture and ash tended to be higher, while lipid and protein contents tended to be lower as the duration of feed deprivation increases. In the same trend, Nile tilapia fed under the restricted feeding regime showed the highest protein retention rate, but the lowest lipid retention rate (NRC, 2011).

In relation to the effects of feeding rate on body composition, El-Saidy *et al.*, (2015) and El-Dakar *et al.* (2021) said that body content of CP was not significantly affected with the different feeding rates; this result is completely agreed with our findings in Table (2).

Conversely, Ahmed (2007) affirmed that body composition is often used as an indicator of fish growth quality and is affected by several

factors, including feeding frequency and feeding rates.

## CONCLUSION

From the results mentioned above, it can be summarized that the optimum feeding rate of Nile tilapia fry within an average initial weight of 1.98 g is 7% biomass. Fry that were exposed to long-term starvation for a week and re-feeding for 3 weeks had growth rates that did not significantly vary with fry that fed continuously as a result of hyperphagia and high feed utilization when re-feeding and improvement in the histological properties of its digestive tract. However long-term starvation for 2 weeks with re-feeding for 2 weeks had negative effects on biological and intestinal histological indices of fish. On the other side, the difference in feeding rate did not significantly change body composition but feeding deprivation with re-feeding led to a significant increase in protein content in fish bodies.

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## DECLARATIONS CONFLICT OF INTEREST:

The authors declare that no conflict of interest.

## AVAILABILITY OF DATA AND MATERIAL

All required data are included in this article

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