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Research article

Short-term starvation at different feeding regimes on appetite responses, feeding utilization and physiological indices, of red hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) fingerlings reared in brackish water

Mohamed F. Abdel-Aziz [a,*], Dalia S. Hamza [b,c], Tasnim A. Elwazer [a], Ahmed S. Mohamed [a], Ashraf Y. El-Dakar [a]

[a] *Department of Aquaculture and Biotechnology, Faculty of Aquaculture and Marine Fisheries, Arish University, Arish, Egypt*
[b] *Department of Biology, College of Science, Jouf University, Sakaka 72341, Saudi Arabia*
[c] *Department of Zoology, Faculty of Science, Benha University, Benha 13518, Egypt*

A R T I C L E I N F O

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A B S T R A C T

A 42 day factorial trial (3x2) was designed to evaluate the effect of short-term starvation with different feeding frequencies on performance, feed utilization, physiological status and appetite responses of red hybrid tilapia fingerlings. Eighteen plastic tanks with a capacity of (55 L) were used to accomplish this work. Fingerlings with an average initial weight of 23 g ± 0.2 (SE) were randomly stocked at a rate of 8 fingerlings/aquarium. Six groups were designated as the following: II/ED: fish was fed twice every day; IV/ED: fish fed four times every day; II/EOD: fish fed twice every other day (alternate-day feeding or one day of feeding followed by another of fasting); IV/EOD: was fed four times every other day; II/EO3D: fish fed twice every other three days (three day of feeding followed by another of fasting) and IV/EO3D: fish fed four times every other three days. Fish were fed on commercial diets 30 % protein (4 % of biomass). Results showed insignificant differences between fish fed every other day and those fed every day in some growth indicators. In the same trend, the interaction between feed deprivation and feeding frequency cleared that fingerlings of IV/EOD did not significantly differ with those fed every day in growth indices. Moreover this treatment was the best in feed conversion efficiency and several physiological indicators.

1. Introduction

The aquaculture industry in Egypt has been expanding rapidly over the past three decades. Egypt now ranks as the top aquaculture producer in Africa (accounts for 71 % of the continent's output) and a major global aquaculture powerhouse [1,2]. Importantly, Egypt is the third largest tilapia producer globally (after China and Indonesia), with tilapia aquaculture playing a significant role for the national economy and food security [3,4], as all the national output is marketed locally [2]. Despite this success, Egyptian tilapia sector faces multiple sustainability challenges. Whereas, the tilapia aquaculture sector experiences declining profitability and production

* Corresponding author.
E-mail address: M_fathy8789@yahoo.com (M.F. Abdel-Aziz).

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efficiency due to disease outbreaks, seasonal climate, and sensitive ecosystems [5]. But the biggest challenges are inadequate feed raw material in quantity and quality, also the continuous increase in the costs of feed inputs, instability of supply, and increased competition for feed ingredients with other animal production sectors have led to a significant increase in the cost of processed aqua feeds [6]. This led to the exit of a large number of farmers and investors from this sector over the past two years. In the last year, many small fish farms in Egypt were unable to provide feed for their fish to complete the production cycle due to the double increase in the prices of feed ingredients. Therefore, new feeding management and husbandry strategies must be developed to confront such challenges and the success of culturing fish depending on maximizing cost-effective of manner production and important approach to reducing feed cost. Several studies have been reported on optimized feeding regimes such as, feeding time, and feeding frequency during the production cycle reduced the cost of feeding without slowing growth with increasing the profit [7,8,and9]]. Furthermore, some feeding restriction protocol intermittent feeding schedule or re-feeding lowers feed cost without a net reduction in fish weight gain. Re-feeding regime has been applied to induce compensatory growth in fish. As it is known, compensatory growth is characterized by a phase of accelerated growth in terms of fish weight gain following a period of restricted feeding [9]. Tian and Qin [10] confirmed that, compensatory growth can offer advantages like an increase growth rates, reducing feed intake, and maintaining the water quality. Likewise, restriction feeds in short or long term duration led to reduce diseases, degradation of water quality, and improve feed efficiency [11]. However, there are group of factors that control inducing the compensatory growth such as, response of fish species to re-feeding, long or short feeding restriction, fish size and age, rearing practices, and dietary protein. Additionally, Karpluse [12] suggested that intermittent feeding schedule is implemented particularly effects of size heterogeneity and increases in the aggressiveness of fish when feed is limited. It is believed that feeding frequency as a rearing strategy may be a solution to overcome the problems of deprivation and re-feeding. Tian et al. [13] and Abdel-Aziz et al. [8] mentioned that feeding frequency plays an important role in regulating the feed intake, waste output, production cost, dominance and size variation between individuals.

Surprisingly, the effect of deprivation and re-feeding has been not extensively investigated of growing the fish species where compensatory growth was not compared under long with short feeding restriction also, compensatory growth was not assessed under different feeding frequency. Therefore, the current study is considered the first scientific paper to investigate short-feeding restrictions as a stimulus to compensatory growth under different feeding frequency and their effect on growth performance, physiological indices and function of digestive system of red hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) fingerlings.

2. Materials and methods

2.1. Location of experiment

This work was performed at the Aquatics Laboratory, Faculty of Aquaculture and Marine Fisheries, Arish University, Arish, North Sinai, Egypt.

2.2. Experimental fish and rearing system

Trial fish were procured from the fish farm, Arish University, Arish, North Sinai, Egypt. Fingerlings were transferred to the experimental site in plastic tanks (100 L) supplied with oxygen and they were acclimatized to laboratory conditions for one week.

A 18 plastic tank were used with dimensions of 52 L x 38 W × 28 H, and continuously aerated using a 6 air pump (220 v, 50 Hz, 5 w, pump can produce around 4 L of oxygen/minute) with submerged air diffusers. Hybrid tilapia fingerlings had an average body weight of 23 g ± 0.2 (SE) and they were randomly divided with a stocking rate at 8 fish per tank. The fish were reared in water salinity of 8 ppm (brackish water) with a water change rate of 30 % of the water volume daily after the second meal to remove uneaten feed residues and fish waste from the bottom of the tank using a small suction pump.

2.3. Treatments and feeding

A 3x 2 factorial rearing attempt was accomplished to assess the short term starvation with different feeding frequency on growth, physiological status and feeding cost of red hybrid tilapia, *(Oreochromis mossambicus* × *Oreochromis niloticus)* fingerlings for 42 days. The experiment protocol was designed to include the following: II/ED: fish was fed twice every day; IV/ED: fish fed four times every day; II/EOD: fish fed twice every other day (alternate-day feeding or one day of feeding followed by another of fasting); IV/EOD: was fed four times every other day; II/EO3D: fish fed twice every other three days (three day of feeding followed by another of fasting), and IV/EO3D: fish fed four times every other three days. Each treatment was triplicated, and a basal floating diet (with diameter 2 mm) from SKRETTING Egypt Company contained 30 % protein, 6 % fat, fiber<5.22, and gross energy 3900 kcal/kg was used with a feeding rate (4 % biomass). Treatments were hand-fed at 9:00 a.m. and 5:00 p.m. (twice) or four times at 9:00 a.m., 12 p.m., 3 p.m., and 5 p.m. (depending on feeding frequency).

2.4. Water physicochemical analysis

Water temperature, dissolved oxygen, and pH were periodically measured using a multipara meter water quality Bench meter (HI2550-02); they were fall between 25.4 and 27.2 °C, 6.85–7 mg/l and 7.50–8.40 respectively. Total ammonia was measured every two weeks by chemical methods [14] and had an average of 0.17–0.21 mg/l. The measured parameters of water quality were within the optimal ranges favorable for the cultivation of tilapia species [15].

2.5. Calculation indicates

$$\text{Weight gain (WG, g)} = \text{Final body weight, g } (F_W) - \text{initial body weight, g } (I_W)$$

$$\text{Average daily gain (ADG, g)} = \frac{WG, g}{days}$$

$$\text{Specific growth rate (SGR, \%)} = \frac{\ln F_W - \ln I_W}{\text{days}} \times 100$$

$$\text{Survival rate (SR, \%)} = \frac{\text{Number of fish at the end}}{\text{Number of fish at the beginning of trial period}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake, g / fish}}{\text{WG, g}}$$

2.6. Data collection and sampling

Fishes were weighed every two weeks to adjust the feeding amount according to the new body weight and all aquaria were drained and cleaned.

At the end of the trial, Weight (g) and length (cm) of all fishes in each tank were individually measured followed by taking a random sample of 4 fish per treatment to evaluate the chemical composition of the whole-body fish. Also a random sample of 6 fish/treatment was taken and placed in a tank and anesthetized with MS-222 (0.1 gL^{-1} tricaine methanesulfonate; Sigma-Aldrich) to reduce the handling stress during the drawing blood samples.

2.6.1. Analysis of fish body chemical composition

A proximate composition of whole body fish were analyzed using standard AOAC methods [16]. Fish were dried by an oven to a constant weight at 105 °C, the Kjeldahl method was used to analyze crude protein (CP, %) (N × 6.25), Soxhlet method with petroleum ether extraction (60–80C) was used to analyze ether extracts (EE, %), the ash by incineration at 550 °C till constant weight, Gross energy was estimated for diets using the factors 5.65, 9.45, and 4.2 Kcal/g for CP, EE, and carbohydrates, respectively [17].

2.6.2. Blood collection

Blood samples were randomly collected from the caudal vein of the fish using 3-mL syringes and emptied into two tubes, of which one contained ethylenediamine tetra acetic acid (anticoagulant 10 %) to prevent coagulation for estimating the hematological assessment and the other tube did not contain ethylenediaminetetraacetic acid to measure the serum parameters. Thereafter, the sample tubes were immediately transported to a hematological laboratory. Hematological indices such as white blood cells, Red blood

Table (1)
Effect of short-term starvation, re-feeding with different daily feeding frequencies on performance indices of Red hybrid tilapia fingerlings for 42 days.

Treatments	I_W, g	F_W, g	WG, g	ADG, g	SGR, %/day	FCR, %	SR %
Effect of short-term starvation alone							
Every day feeding (ED)	23.625	41.507	17.88a	0.426a	1.34a	1.214	87.50
Every other day feeding (EOD)	22.810	37.420	14.61b	0.348b	1.17a	1.091	92.50
Every other three day feeding (EO3D)	23.405	37.100	12.94b	0.308b	1.095b	1.163	87.50
P-value	0.440	0.083	0.022	0.023	0.073	0.602	0.101
Effect of feeding frequency alone							
II/daily	23.13	38.10	15.80	.3448	1.19	1.12	90.00
IV/daily	23.43	39.24	15.8100	.3765	1.22	1.19	88.30
P-value	0.563	0.564	0.442	0.441	0.690	0.432	0.170
Interaction of short-term starvation with feeding frequencies							
II/ED	23.50	41.09^{ab}	17.59^{a}	.4188a	1.33a	1.19b	90.00a
IV/ED	23.75	41.925^{a}	18.17^{a}	.4327a	1.35a	1.23 ab	85.00a
II/EOD	22.12	34.1c	11.98^{b}	.2852b	1.03b	1.21b	95.00a
IV/EOD	23.50	40.74^{ab}	17.24^{a}	.4105a	1.31a	1.07c	90.00a
II/EO3D	23.7500	39.125^{b}	13.87^{b}	.3304b	1.18 ab	1.04c	85.00a
IV/EO3D	23.0600	35.075^{c}	12.01^{b}	.2863b	1.01b	1.37a	90.00a
P-value	0.492	0.00	0.002	0.002	0.032	0.003	0.005
PSE[a]	0.254	0.937	0.816	0.194	0.046	0.047	4.13

Averages of treatments with different superscript letters in the same column indicate significant differences at level $P \leq 0.05$.
II/ED: fish was fed twice every day; IV/ED: fish fed four times every day; II/EOD: fish fed twice every other day (one day of feeding followed by another of fasting); IV/EOD: was fed four times every other day; II/EO3D: fish fed twice every other three days (one day of feeding followed by another of fasting), and IV/EO3D: fish fed four times every other three days.

[a] Pooled standard error.

cells, hemoglobin, and hematocrit were measured by fully automatic veterinary hematological analyzer model: BK-5000VET, Brand: Biobase. While blood samples that were obtained to determine serum paramaters, centerfuged at 3000 rpm/10 min. Serum paramaters (glocuse, cortisol, Aspartate aminotransferase (AST) and Alanin aminotransferase (ALT), and Urea were measured spectrophotometerically using commercial test kits (Bio-diagnostic, Egypt). Digestive enzymes activities also were analyzed using colorimetric methods according to Refs. [18,19].

2.7. Statistical analysis

Statistical Package Program version17 was used to analyze the obtained data through two-way analysis of variance (ANOVA). The differences among groups were compared using Waller–Duncan test at $P \leq 0.05$ as the significance level.

3. Results

Table 1 displayed the effects of short-term starvation, feeding frequency, and their interactions on growth index's performance. Only WG and SGR were significantly affected by short-term starvation. Whereas SGR did not change between fish fed every day (ED) and every other day (one day of feeding followed by another of fasting, EOD), fish fed every day (ED) had the highest WG. Fish fed every other three days (EO3D) was the lowest in these parameters. The other parameters of FCR or SR did not significantly change with all treatments. Feed frequency regardless feed deprivation had not significantly effects on fish growth rate or SR..

Regarding, interaction between short-term starvation with feeding frequency significantly affected on all parameters of growth except SR. Treatments of II/ED, IV/ED, and IV/EOD did not significantly vary among them in WG, ADG, and SGR, but they were the highest in these parameters compared to II/EO3D, II/EOD, and IV/EO3D respectively. Also FCR was significantly changed through interaction of short feeding starvation and feeding frequency, whereas IV/EOD and II/EO3D recorded the best FCR followed by a II/ED, II/EOD, and IV/ED while IV/EO3D had the worst FCR.

Hematological parameters of RBCs, WBCs, Hb, and Hct were not significantly affected by short term starvation alone or feeding frequency alone, but the interaction between them appeared significant differences among treatments as shown in Table 2. Fish fed every day had the highest hematological indicators followed by IV/EOD, and II/EO3D then IV/EO3D, while II/EOD had the lowest values.

It can be seen from the results in Tables 2 and 3 serum biochemical indices were significantly affected by short-term starvation regardless feed frequency except glucose and cortisol levels. Fish fed each different three days and fasting three days (EO3D) had the highest ALT, Urea, glucose and cortisol, Urea and ALT of groups ED and EOD were not significantly varied. Fish of EOD recorded the lowest value of AST, glucose, and cortisol. Also indices of AST, ALT, glucose, and cortisol were significantly affected with interaction between the tested feeding strategies. AST was significant similar in groups of II/EO3D, II/ED, and IV/ED and these groups had significantly higher than IV/EO3D and II/EOD, while IV/EOD recorded the lowest level. Treatments of II/EO3D and IV/EO3D achieved the highest ALT followed by, IV/EOD, IV/ED II/ED, and II/EOD respectively. The lowest glucose and cortisol were recorded with IV/

Table (2)
Effect of short-term starvation, re-feeding with different daily feeding frequencies on hematological indicators, liver and kidney functions of Red hybrid tilapia fingerlings after 42 days.

Treatments	RBCs $10^6/\mu l$	WBCs $10^3/\mu l$	Hb g/dl	Hct, %	AST, U/l	ALT U/l	Urea mg/dl
Effect of short-term starvation alone							
Every day feeding (ED)	1.44	25.87	6.66	30.23	216.00^{b}	35.05^{a}	13.05^{b}
Every other day feeding (EOD)	1.10	14.11	4.15	19.25	228.52^{b}	27.50^{b}	16.00^{ab}
Every other three day feeding (EO3D)	1.20	19.08	5.175	22.45	258.55^{a}	34.52^{a}	17.02^{a}
P-value	0.419	0.25	0.175	0.475	0.035	0.088	0.081
Effect of feeding frequency alone							
II/daily	1.22	17.86	5.20	22.33	253.30	33.83	16.01
IV/daily	1.28	21.52	5.45	25.62	233.31	30.86	14.66
P-value	0.784	0.572	0.854	0.671	0.921	0.449	0.492
Interaction of short-term starvation with feeding frequencies							
II/ED	1.44^{a}	25.60^{a}	6.52^{a}	29.60^{ab}	217^{d}	35.50^{a}	13.00
IV/ED	1.45^{a}	26.15^{a}	6.80^{a}	30.86^{a}	215^{d}	34.62^{a}	13.01
II/EOD	0.86^{cd}	7.44^{d}	3.20^{d}	10.40^{d}	222^{d}	29.00^{c}	17.05
IV/EOD	1.34^{ab}	20.79^{b}	5.10^{b}	28.10^{ab}	235^{c}	26.03d	15.03
II/EO3D	1.35^{ab}	20.54^{b}	5.90^{b}	27.00^{b}	267^{a}	37.01^{a}	18.01
IV/EO3D	1.05^{bc}	17.62^{c}	4.45^{c}	17.90^{c}	250^{b}	32.10^{b}	16.10
P-value	0.003	0.004	<0.001	<0.001	<0.001	<0.001	0.110
PSE[a]	0.094	2.22	0.455	2.49	5.81	1.17	0.60

Averages of treatments with different superscript letters in the same column indicate significant differences at level $P \leq 0.05$.
II/ED: fish was fed twice every day; IV/ED: fish fed four times every day; II/EOD: fish fed twice every other day (one day of feeding followed by another of fasting); IV/EOD: was fed four times every other day; II/EO3D: fish fed twice every other three days (one day of feeding followed by another of fasting), and IV/EO3D: fish fed four times every other three days.

[a] Pooled standard error.

Table (3)
Effect of short-term starvation, re-feeding with different daily feeding frequencies on blood glucose, cortisol, and digestive enzymes activities of Red hybrid tilapia fingerlings after 42 days.

Treatments	Cortisol, nmol/l	Glucose, mg/dl	Amylase, U/l	Lipase, U/l	Trypsin, U/mg
Effect of short-term starvation alone					
Every day feeding (ED)	93.00	73.23	10.90	99.25	2.48
Every other day feeding (EOD)	64.50	67.02	25.10	77.50	2.30
Every other three day feeding (EO3D)	99.50	92.50	23.03	50.05	2.03
P-value	0.37	0.31	0.129	0.102	0.13
Effect of feeding frequency alone					
II/daily	84.66	74.33	21.00	85.60	2.20
IV/daily	86.66	80.66	18.26	65.50	2.34
P-value	0.931	0.684	0.719	0.38	0.52
Interaction of short-term starvation with feeding frequencies					
II/ED	95.02^{b}	75.10^{b}	11.00^{c}	105.00^{a}	2.37^{b}
IV/ED	91.07^{b}	71.07^{b}	10.80^{c}	93.50^{ab}	2.60^{a}
II/EOD	81.00^{c}	72.05^{b}	23.00^{b}	88.00^{b}	2.15^{c}
IV/EOD	48.13^{d}	62.12^{c}	27.00^{a}	67.00^{c}	2.45^{ab}
II/EO3D	78.02^{c}	$76.08^{.b}$	29.00^{a}	64.00^{c}	2.07^{c}
IV/EO3D	121.02^{a}	109.00^{a}	17.00^{c}	36.00^{d}	1.98^{c}
P-value	0.003	0.005	0.000	0.002	0.001
PSE[a]	6.62	4.49	0.91	6.94	0.067

Averages of treatments with different superscript letters in the same column indicate significant differences at level $P \leq 0.05$.
II/ED: fish was fed twice every day; IV/ED: fish fed four times every day; II/EOD: fish fed twice every other day (one day of feeding followed by another of fasting); IV/EOD: was fed four times every other day; II/EO3D: fish fed twice every other three days (one day of feeding followed by another of fasting), and IV/EO3D: fish fed four times every other three days.
[a] Pooled standard error.

EOD, while IV/EO3D had the highest in glucose and cortisol.

It was also clear from Table (3) that the activity of digestive enzymes in the blood was not significantly affected by short-term starvation alone or feeding frequency alone, but statistical analysis of the interaction between short term starvation and different feeding frequencies showed a significant difference in these enzymes. The highest level of amylase was recorded by II/EO3D and IV/EOD followed by II/EOD, IV/EO3D, II/ED and then IV/ED. Fish feed every day had the highest value of lipase followed by groups of EOD, while EO3D was the lowest. Trypsin enzyme in blood did not significant varied among groups of II/EOD, II/EO3D, and II/EO3D these groups had significantly lower trypsin levels than other groups, whereas fish fed every day or every other day in four times had the highest blood trypsin.

The results obtained in Table 4 showed the chemical analysis of the fish body composition, and there were no significant differences in dry matter (DM), crude protein (CP), ether extract (EE), and ash between the treatments affected by short-term starvation alone or

Table (4)
Effect of short-term starvation, re-feeding with different daily feeding frequencies on whole body composition of Red hybrid tilapia fingerlings after 42 days on basis dry matter.

Treatments	Dry Matter, %	Crude Protein, %	Ether extract, %	Ash, %
Effect of short-term starvation alone				
Every day feeding (ED)	77.11	65.43	13.28	21.30
Every other day feeding (EOD)	75.58	65.19	12.33	22.72
Every other three day feeding (EO3D)	74.53	64.42	14.46	21.15
P-value	0.1	0.286	0.197	0.2
Effect of feeding frequency alone				
II/daily	75.7233	64.67	13.78	21.55
IV/daily	75.7533	65.36	12.93	21.89
P-value	0.981	0.208	0.438	0.708
Interaction of short-term starvation with feeding frequencies				
II/ED	77.6	65.31	13.26^{ab}	21.37
IV/ED	76.6	65.56	13.30^{ab}	21.23
II/EOD	75.82	64.72	13.39^{ab}	21.88
IV/EOD	75.35	65.66	11.28^{b}	23.56
II/EO3D	73.75	63.98	14.70^{a}	21.41
IV/EO3D	75.31	64.86	14.23^{a}	20.90
P-value	0.223	0.962	0.018	0.623
PSE[a]	0.387	0.447	0.35	0.424

Averages of treatments with different superscript letters in the same column indicate significant differences at level $P \leq 0.05$.
II/ED: fish was fed twice every day; IV/ED: fish fed four times every day; II/EOD: fish fed twice every other day (one day of feeding followed by another of fasting); IV/EOD: was fed four times every other day; II/EO3D: fish fed twice every other three days (one day of feeding followed by another of fasting), and IV/EO3D: fish fed four times every other three days.
[a] Pooled standard error.

frequency of feeding alone. Also, interaction between the attempted feeding systems did not appear any significant variations in fish body composition except EE, which significantly differed. Whereas IV/EOD recorded the lowest EE content.

4. Discussion

Noteworthy, the quantity and quality of feeds a major factor in the fish production cycle and directly influence conversion rate and economic efficiency, feed costs in aquaculture projects may exceed 70 % of production costs [20]. Therefore limiting feeding strategies in the short or long term may be a tool to overcome many sudden and emergency problems such as water shortage from irrigation sources, degradation of water quality, increasing feed prices, and disease outbreaks [11]. Our current work examined short-term starvation under different feeding frequencies daily. In the light of the obtained results on feeding every other day (EOD) which had better fish performance than those fed every three other days (EO3D). Additionally, short-term starvation can lead to fast growth and greater feed efficiency thereby reducing feed costs as recommended by Ref. [21]. Also [22] observed that short term starvation may be part of a strategy of feeding to break problems of water quality and uneaten feeds. Several lines of substantiation suggested that some feeding restriction protocols decreased feed costs without a net reduction product. In this trend, our results affirmed that fish that were fed every other day did not differ from those fed every day in SGR, but they had the best FCR. Data from many previous study stated that alternated-day feeding is a vital tool to improve growth rate of fish as a result of reduced feed waste either through more complete consumption or improved nutrient absorption [23]. Confirmation of our observations, Bolivar et al. [24] found that fish fed daily had higher growth but did not significantly differ with those fed alternate-day feeding (EOD), they reported that a significantly better FCR was obtained for fish fed EOD or on alternate days than for fish fed daily. In the same trend [25], found that, alternate-day feeding (EOD) group consumed significantly less feeding (27 %) compared with group that fed every day, while [26] pronounced that fish fed alternate-days solely had half of the days of feeding in contrast fish fed constantly and then FCR was once the exceptional with alternate-day feeding (EOD). Similar observations have been mentioned by Ref. [27] investigated the effect of two days fasting and one day feeding repeated for 89 days and in comparison with continuously feeding of Longfin Yellowtail, they found that SGR, feed efficiency have been not statistically differed between two days fasted and constantly feeding. Moreover [28], found Nile tilapia fingerlings that fed EOD had a best FCR than those fed every day. There are a number of hypotheses that might account for this outcome. For example, during short-term famine, the intestinal feeding efficiency is naturally increased, which results in a more effective absorption of nutrients following re-feeding utilizing a strategy of (EOD). Additionally, as shown by Refs. [29,30] short-term fasting results in complete compensatory growth, which leads to the same growth of fish fed every day. Also as was noted by Ref. [31], compensatory growth is typically accompanied with hyperphagia, or an increase in the fish's appetite, which leads to improved feed efficiency. Furthermore, 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 [32]. On the other hand, our results did not agree with the suggestions of Zheng et al. [33] illustrated that feed deprivation causes the degradation of endogenous sources of energy to maintain the fish's physiological homeostasis leading to weight loss. Accordingly, it can be found that responses to compensatory growth varied according to species, fish age, rearing conditions, dietary protein, and duration of feeding deprivation. The present study is designed to maximize fish response to take advantage of the compensatory growth phenomenon by following optimal feeding frequency which frequency plays a greater role in regulating feed intake, growth, waste output [34,35]. The interplay between short-term hunger and nutrition frequency has significantly affected growth and FCR. Feeding four times every other day (IV/EOD) gives the best growth results and FCR where increasing short periods between meals reduces overload on the digestive tract and allows enough time to make gastrointestinal enzymes, also short periods lead to the restoration of fish appetite resulting in increased feed consumed by fish.

Regarding, hematological indicators like White blood cells (WBCs), Red blood cells (RBCs), Hemoglobin (Hb) and Hematocrit (Hct). WBCs is considered a primary indicator of the disturbance in homeostatic defenses capabilities and determined non-specific immune responses of fish [36]. Indices of RBCs, Hb, and Hct reflected the increase of oxygen carrying capacity of blood, and the more active fish tend to have higher Hb and Hct than sedentary ones [37,38]. Our finding showed that fish fed every day either twice daily or four times daily had the highest hematological indicators followed by fish of IV/EOD. This finding conflicts with the statement of Sakyi et al. [19] increasing WBCs, which can be as a result of starvation stress and RBCs, Hb, Hct showed a robust increase in response to stress and immune response.

Increasing WBCs in the blood of fish fed every day may be due to consume more amount of protein than fasting groups [39].

The hematological indicators of short-term starvation alone or feeding frequency alone did not change among groups and this observation is in agreement with [40,41,and42]] they found that fasting and re-feeding had not notably affects the hematology parameters of *Oreochromis niloticus, Acipenser baerii, and Siniperca scherzeri.*

AST and ALT are two routinely evaluated enzymes or sensitive markers of hepatocellular damage with regard to serum biochemical indices [43]. Elevated serum levels of these enzymes may be connected to the severity of liver injury [44]. Also Urea in blood can indicate whether the kidney is probably function, increasing urea is a sign to deteriorate kidney functions [45]. On the other hand, rising glucose and cortisol levels in fish plasma indicate that the fish are under a lot of stress. According to Mohapatra et al. [46], when fish are subjected to stressful situations, their level of gluconeogenesis rises, which increase blood sugar. Also starvation decreases the antioxidant stores, oxidative stress and an increase in the generation of oxygen free radical mainly in the liver; this depends on duration of starvation or re-feeding [47].

Our findings of serum assay appeared fish fed every other day (EOD) in special (IV/EOD) had the best physiological status and the lowest levels of serum AST, ALT, glucose, and cortisol compared to those of (ED) or (EO3D). Sakyi et al. [19,48] found that plasma glucose was affected by starvation and the re-feeding period and stable glucose level indicated that glycolytic activity thereby enhancing protein synthesis.

In the opposite trend [38,49], found that serum AST and cortisol were increased by fasting or feed deprivation and decreased rapidly with re-feeding. While [48] reported that blood cortisol decreased in the fasted fish. Generally length of starvation-term control levels of serum indicators. As it is well knowledged, digestive enzymes are important for the breakdown of proteins, carbohydrates, and lipases, which helps in nutrient absorption [50]. Our finding cleared that fish fed ED had the highest levels of blood lipase followed by fish fed EOD and then EO3D also trypsin activity was more affected by feeding frequency and was in the same trend as lipase, whereas fish fed ED and IV/EOD had the highest trypsin levels. Accordingly, many of several previous observations were in agreement with our findings, Thongprajukaew et al. [51] showed that increasing activity of chymotrypsin or trypsin in association with growth but decreased lipase activity indicated insufficient nutrients for growth. Also [52] mentioned that the digestive enzymes of protein increased with re-feeding and they added that protein as a main source of energy during re-feeding and main nutrition store. In the same, this observation, after fasting may also be associated with the enhancement of digestive enzyme activity or increasing the dietary nutrient digestibility [53]. Amylase activity was increased with fish fed EOD or EO3D than those fed ED this may be attributed hyperphagia phenomenon which leads to the increase of the fish's appetite after re-feeding. On the contrary, amylase was unaffected by different feeding schedule indicating that *Hemibagrus nemurus* fingerlings had similar abilities for carbohydrate digestion in all tested groups.

In view of the results, body composition was found to have effect on short-term starvation alone or feeding frequency did not change the body contents of protein, ether extract, and ash this result agrees with many works which affirmed that feeding regime or schedule had not any effect on body composition. Zhu et al. [51,54] body composition in gible carp or yellow mystus was not differed between continuous feeding and restriction feeding. Besides, lipid content only was affected with interaction between short term starvation period and feeding frequency leading to fish of IV/EOD had the lowest lipid content signifying the responses these fish to compensatory growth as reported by Ref. [55] the decreased lipid content is an indicator of predictive of compensatory growth responses based on the lipostatic model. Conversely, Abdel-Hakim et al. [56] administrated that slight changes in tilapia carcass analysis in fish subjected to cycled restricted feeding also Okomod et al. [57] chemical composition of *Clarias garirpinus* was significantly affected by feeding frequency.

5. Conclusion

Finally, it can be summarized that successful nutritional restriction to reduce spent feed without any adverse effects on fish growth rates depends on several factors, such as fish species, the duration of hunger or nutrition, breeding conditions and nutritional protein. Interaction with other diets, such as daily feeding frequency and nutrition restriction, is instrumental in improving nutrition efficiency. Our findings from the short-term starvation (one-day feeding and another fasting EOD) resulted in full compensation in growth, especially by dividing meals into four daytime batches (IV/EOD) which did not differ significantly from those fed every day in growth rates. Also, this treatment had the best in FCR, physiological status and body composition.

Ethical statement

The proposal of this was carried out with the strict recommendations and approval of the Institutional Animal Care and Use Committee of Agricultural Research Cente, Egypt (ARC-IACUC) with the application number [ARC-FAMFA-83-23].

Data availability statement

Data will be available upon request from the corresponding author upon reasonable requests as we are not considering making the raw data public.

CRediT authorship contribution statement

Mohamed F. Abdel-Aziz: Writing – original draft, Supervision, Software, Investigation, Conceptualization. **Dalia S. Hamza:** Writing – review & editing, Resources, Formal analysis. **Tasnim A. Elwazer:** Validation, Supervision, Project administration, Conceptualization. **Ahmed S. Mohamed:** Visualization, Methodology, Formal analysis, Data curation. **Ashraf Y. El-Dakar:** Writing – review & editing, Supervision, Resources, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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