

Impact of Short-Term Cycled Starvation and Refeeding on Growth Performance and Physiological Indices of Orange-Spotted Grouper *Epinephelus coioides*

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Abstract

An 8-week experiment was carried out to evaluate the impact of short term cycled starvation and refeeding on growth and physiological indices of orange spotted grouper *Epinephelus coioides*. The experimental fish body weight was 55.9 g average. The treatments employed in this experiment include repeating cycles of one day starvation followed by two and three days refeeding alternately (T1); repeating cycles of one day and two days starvation alternately followed by two days re-feeding (T2); repeating cycles of one week starvation followed by one week feeding (T3); and feeding daily as control (C). Each treatment was assigned to five net cages according to completely randomized design. Twenty-five individuals were stocked in each cage. The fish were fed with trash fish at noon at 5% of bodyweight. Water quality parameters including pH, temperature and salinity were monitored daily, while dissolved oxygen was measured weekly. The results show that the final body weight of fish in T1 was not significantly different from that in control ($P>0.05$). However, the final body weight of fish in T2 and T3 was significantly lower than control ($P<0.05$). Short term cycled starvation and refeeding affects compensatory growth and body condition indices of orange spotted grouper. These findings suggest that repeating cycles of one day starvation followed by two and three days refeeding alternately could be applied for rearing orange spotted grouper. Such a feeding schedule scheme provides economic and ecological benefits through a reduction in operational costs and waste from fish.

Introduction

Grouper fish is very popular in China and Southeast Asia and is widely traded in Asian markets (Ybañez Jr., and Gonzales, 2023). The fish including orange spotted grouper *Epinephelus coioides* is a high economic value fish which has long been cultivated in Indonesia (Ismi and Darmawan, 2022). Production continues to increase but aquaculture faces several challenges such as slow fish growth. Slow growth rates result in longer production cycles, which can increase operational costs such as feed, labor, and maintenance (Burgess et al,

2020, Ybañez Jr., and Gonzales, 2023). The growth of fish in the culture system is influenced by the quantity and quality of feed and feeding strategies, so that the management of feed is very important in the success of aquaculture (Yuwono et al. 2005; Aslan, et al. 2015; Au et al, 2023). Due to the high protein content of fish diet, ineffective feeding not only causes high feed costs but also negatively affects pond water conditions (Md Noor and Harun, 2022). Organic pollution from uneaten feed and metabolic waste will worsen pond water quality (Al-Hejuje et al, 2017). The poor water quality adversely affects the survival and growth of fish (Mansour et al,

2017). Therefore, the right feeding arrangement becomes very important to improve feed efficiency and maintain the pond water quality so that it will enhance fish production. Reducing the amount of feed will reduce organic pollution and waste in aquaculture ponds, so that it will have a positive impact on survival and growth. This can be achieved by employing a cycle of starvation and refeeding (Squalli, 2020, Abdel-Aziz et al, 2024).

Cycled short-term starvation and refeeding resulted in compensatory growth, i.e. a period of rapid growth exhibited by fish receiving a period of starvation followed by normal re-feeding (Ali et al., 2003), in various cultured fish species such as channel catfish, *Ictalurus punctatus* (Gaylord and Gatlin 2000, 2001); gibel carp, *Carassius auratus gibelio* (Xie et al. 2001); olive flounder *Paralichthys olivaceus* (Cho, 2005; Cho et al., 2006); monosex tilapia *Oreochromis niloticus* (Islam et al, 2022); and Indian pompano *Trachinotus mookalee* juveniles (Xavier et al, 2023). Such a feeding regime resulted in equivalent final body weight with fish fed normally and could be promising means to reduce feed input in fish production. However, response to starvation and re-feeding is inconsistent in various species. The fish might display complete, over, partial or no compensation as response to different feeding regime. Complete compensatory growth was found among others in Asean sea bass, *Lates calcarifer* (Tian and Qin, 2003; Azodi et al, 2016), humpback grouper *Cromileptes altivelis* (Yuwono et al, 2007, 2009), Hybrid Tilapia *Oreochromis mossambicus x Oreochromis niloticus* (Gabriel et al, 2017), Abdel-Aziz et al, 2024), Sobaity Fish, *Sparidentex hasta* (Molayemraftar et al, 2019), Mandarin Fish *Siniperca scherzeri* juvenile (Kim et al, 2022). Over-compensatory growth was reported in several fish species including hybrid sunfish, *Lepomis cyanellus x Lepomis macrochirus* (Hayward et al., 1997), partial compensation in hybrid tilapia *Oreochromis mossambicus x Oreochromis niloticus* (Wang et al., 2000), European Sea Bass *Dicentrarchus labrax* (Adakh and Tasbozan, 2015), juvenile yellow mystus *Hemibagrus nemurus* (Thongprajukaew and Rodjaroen, 2017), and Indian pompano *Trachinotus mookalee* juveniles (Xavier et al, 2023). Some other species, such as *Oreochromis niloticus* (Yang et al., 2015), and juvenile red tilapia *Oreochromis* spp. (Robisalmi et al, 2021) demonstrated no compensatory growth as response to starvation.

Rapid growth is related to increased feed efficiency, for instance in channel catfish, *Ictalurus punctatus* (Li et al., 2006), humpback grouper *Cromileptes altivelis* (Yuwono et al, 2007), Hybrid Tilapia *Oreochromis mossambicus x Oreochromis niloticus* (Gabriel et al, 2017). Feeding restriction was effective in reducing the amount of feed intake and the release of metabolic waste into the water (Rodde et al, 2021). Thus, the implementation of feed restrictions in aquaculture will provide economic and ecological benefits (Gabriel et al, 2023). Implementing short-term

eating periods and fasting can be an effective strategy to optimize growth performance. However, feeding schedules and feed amounts that can minimize waste and improve feed conversion efficiency that have an impact on grouper growth performance and have not been studied. The present study aims to determine the effect of short term cycled starvation and refeeding on growth performance and physiological indices of orange spotted grouper *Epinephelus coioides*. This will also produce information about feeding strategies which might improve economic and ecological benefits.

Material and Method

The experimental unit was floating net cage measuring 1.5x2x1 m². The fish used in the experiment was purchased from fishermen in Cilacap, Central Java, Indonesia and they were acclimated during 2 weeks in the net cages prior to their use in the experiment. During acclimation, stress indicators of the experimental fish that include changes in swimming patterns, eating behavior, and social interaction are monitored. The fish body weight used in the experiment was 55.9 g on average.

Feeding treatments employed in these experiment include the following regimes: fish received repeating cycles of one day starvation followed by two and three days re-feeding alternately, the fish were not fed every Monday and Thursday (T1), fish received repeating cycles of one day and two days starvation alternately followed by two days re-feeding, the fish were not fed every Sunday and Monday and Thursday (T2); fish received repeating cycles of one week starvation followed by one week feeding (T3); and fish fed daily as control (C). Each feeding treatment was assigned to five net cages according to completely randomized design. Twenty-five individuals were stocked in each net cage. During the experiment the fishes were fed with trash fish which contains 56.15±0.15% protein, 14.5±0.05% lipid, 4.05±0.15% ash and 8.75±0.05% moisture, at noon at 5% of bodyweight. The amount of food was adjusted weekly.

Water quality parameters including pH, temperature and salinity were monitored daily, while dissolved oxygen was measured once a week. The water quality was pH 7.8-8, salinity 28–30 ppt, temperature 28–29°C, and dissolved oxygen content 5–6.5 mg/L. The stocking density of the fish has been adjusted to maintain a stable pH and dissolve oxygen. The submersible cage is covered by a para net to reduce exposure to direct sunlight to maintain a stable water temperature. Regular water exchange is ensured to maintain consistent salinity level. Parameters measured include bodyweight gain, absolute growth rate, hepatosomatic index and viscerosomatic index. All fish were weighed at the beginning of the experiment. The sample of 3 fish was collected randomly from each net cage once a week and weighed to calculate weight gain. All fish was weighed to obtain final weight gain at the

end of the experiment. Absolute growth rate (AGR); hepatosomatic index (HSI) and viscerosomatic index (VSI) were calculated as follow:

$$AGR = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{number of feeding day}} \text{ (Chatakondi and Yant, 2001).}$$

$$HSI, (\%) = \frac{\text{wet weight of liver (g)}}{\text{body wet weight of fish (g)}} \times 100 \text{ (Adaklı. \& Tasbozan, 2015).}$$

$$VSI, (\%) = \frac{\text{wet weight of viscera (g)}}{\text{body wet weight of fish (g)}} \times 100 \text{ (Yang et al, 2024).}$$

The moisture, ash and protein contents of the experimental fish were determined according to the standard method of AOAC (2005). Mean final body weight, was analysed using one-way ANOVA. The normality test and the homogeneity check of the variance was carried out before the analysis. Hepatosomatic index and Viscerosomatic Index were analysed using ANCOVA. Tukey’s multiple range test was used to determine significant differences between treatment means at the 95% confidence level.

Results

Results of the experiment using orange spotted grouper are presented in Table 1. Short term cycled starvation and refeeding affected growth of orange spotted grouper *Epinephelus coioides*. Final body weight (FBW) of fish T2 and T3 was significantly lower than control (P<0.05). However, FBW of fish starved two days a week every Monday and Thursday (T1) was not significantly different from that fed continuously (C) as control (P<0.05).

As shown in Table 1, at the end of the experiment, the fish on the T1 treatment, which received a short-term cycled starvation and re-feeding for eight weeks, reached the same size as the control in terms of body weight (P>0.05). Reducing feeding by two days per week, where the fish were not fed every Monday and Thursday, thus reducing feeding by 29% did not significantly decrease the final body weight of the experimental fish from T1 (P>0.05). However, fish at T2 starved three days per week, namely on Sunday, Monday and Thursday, and thus reducing feeding by 43%, resulting in significantly lower FBW than T1 and the

control (P<0.05). Furthermore, one week starvation followed by one week refeeding, thereby reducing feeding to 59% in fish from T3 resulted in significantly lower FBW compared to T1 and control (P<0.05), but not significantly different from T2 (P>0.05). The average amount of feed consumed per fish during 8 weeks of experiment was 403 g, 288 g, 229 g, 167g in C, T1, T2 and T3 respectively. The fish subjected to a short-term cycle of starvation and re-feeding in the experimental groups showed compensatory growth, where complete compensatory growth occurs in fish from T1, while partial compensatory growth occurs in fish from T2 and T3.

As presented in Table 1, Absolute Growth Rate (AGR) of all fish that experience a short-term cycled starvation and refeeding; (T1, T2 and T3) was significantly higher than control (P<0.05). The AGR of T1 was not significantly different from that of T3 (P>0.05), but the AGR of both groups T1 and T3 was significantly higher than T2 (P<0.05). The AGR of T2 was significantly higher than that of the control (P<0.05). The higher AGR explains the mechanism by which fish from T1 go through rapid growth to achieve the same BWG as the control. Meanwhile, high AGR in fish from T3 only allows the fish to take up rapid growth to reach a BWG that is equal to that of fish from T2.

Figure 1 shows the body weight of fish subjected to a short-term cycled starvation and re-feeding in groups T2 and T3 was always lower than control throughout the study period. However, fish that subjected to a short-term cycled starvation and re-feeding in group T1 reached body weights comparable to controls at week 6 and their final weight at week 8 is similar to control (C). Normal growth is disrupted by feed restriction (week 1-4 of T1, T2 and T3), which results in a decline in the growth trajectory and a size disparity compared to fish fed daily (C). When fish acclimating to feeding regime, hyperphagia and increased growth axis lead a hyperanabolic phase (week 4 - 6) marked by a steeper growth curve than that of fish fed daily. The compensatory growth response potentially allows stunted fish to fully (T1) and partially (T2, T3) compensate for lost growth and re-converge in size with constantly fed controls before the growth rate returns to normal. growth rate in all groups.

Table 2 shows that the hepatosomatic index (HSI) of the fish subjected to a short term cycled starvation

Table 1. Growth and survival rate of orange spotted grouper *Epinephelus coioides* receiving short term cycled starvation and refeeding. Fish in T1 consumed 29% lower feed but achieved the same final body weight as those fed daily in C as a control.

PARAMETER	TREATMENT			
	C	T1	T2	T3
Initial Body Weight (IBW), g	55,9a	56,1a	55,8a	55,8a
Final Body Weight (FBW), g	215,3a	216,1a	165,8b	155,2b
Body Weight Gain (BWG), g	159,4a	160,0a	110,0b	99,4b
Number of Feeding Day	56	40	32	23
Feed reduction, %	-	29	43	59
Absolute Growth Rate (AGR)	2,8b	4,0a	3,4a	4,3a

*Means followed by the same letter in the row are not significantly different (P>0.05).

and refeeding was significantly different from that of fish fed daily as control ($P < 0.05$). The HSI of fish in the T3 treatment was significantly lower than that of T1, T2 and control. Fish in T3 with the lowest HSI experienced the longest starvation period compared to fish in T1 and T2. The HSI of T2 fish was significantly lower than that of T1 and control (C), but significantly higher than that of T3 ($P < 0.05$). The HSI of T2 fish that experienced starvation three days a week was significantly lower than those of T1 that experienced starvation two days a week ($P < 0.05$). It seems that glycogen reserves stored during the feeding period are mobilized as an energy source when the fish are starved. The longer the starvation period, as in T2 and T3, the greater the glycogen reserves are degraded to produce energy. Consequently, the HSI decreases since the liver reduces in size due to energy reserves are mobilized to support vital functions.

The viscera somatic index (VSI) of experimental fish that received short-term cycled starvation and refeeding from T1, T2, and T3 was significantly different

from fish that were fed daily as a control ($P < 0.05$). However, there was no significant difference in VSI between fish from groups T1, T2, and T3 ($P > 0.05$). This phenomenon suggested that the fat storage probably used simultaneously with glycogen during starvation as source of energy requirement. The length of the starvation period experienced by the experimental fish groups T1, T2 and T3 probably had no effect on the amount of fat storage mobilized to produce energy during starvation in the fish of the three experimental groups.

Short term cycled starvation and refeeding is likely affect chemical body content of orange spotted grouper. The protein content of the three groups of experimental fish that received short-term starvation and refeeding cycles of T1, T2, and T3 was higher, while their lipid and fiber content was lower than that of fish fed daily as a control (Table 3). It is possible that lipids in fish receiving cycled short-term starvation and refeeding are catabolized to produce energy during starvation.

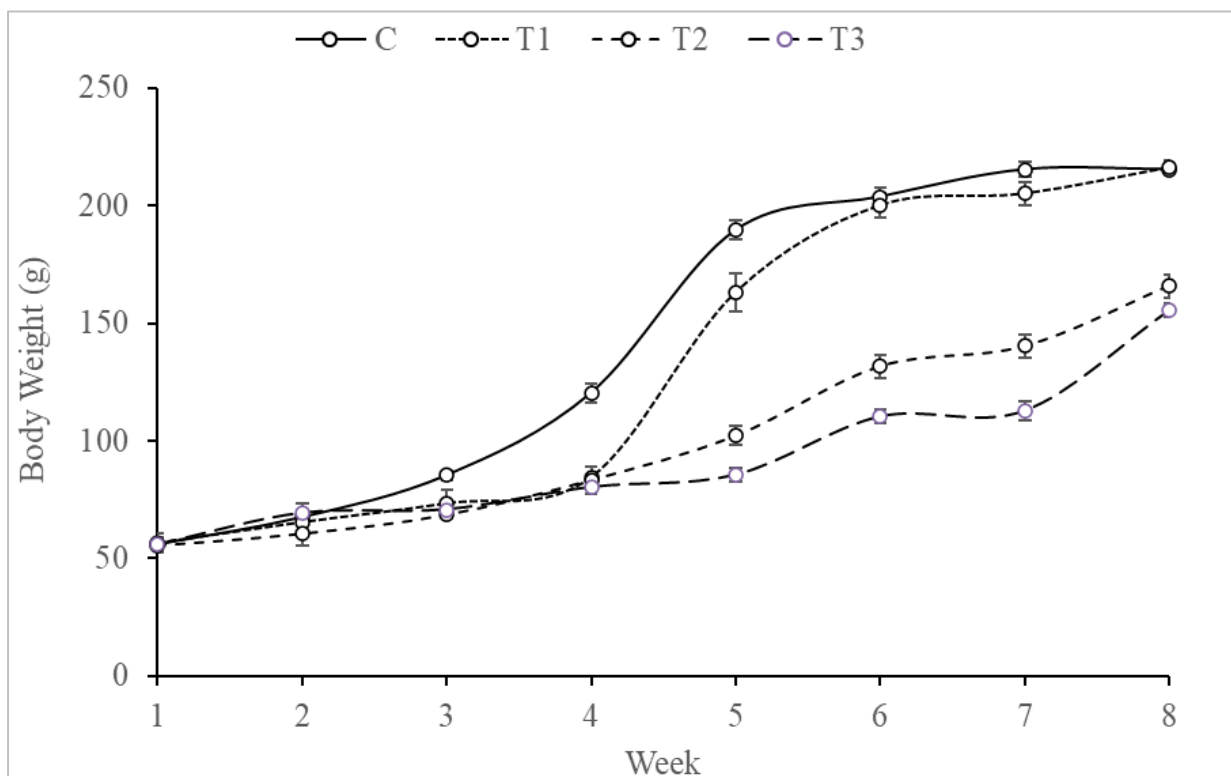


Figure 1. Body weight (means±SD) of orange spotted grouper *Epinephelus coioides* receiving short term cycled starvation and refeeding measured weekly during the experiment.

Table 2. Hepatosomatic index (HSI) and Viscerosomatic index (VSI) of orange spotted grouper *Epinephelus coioides* subjected to cycled starvation

Treatments	Hepatosomatic index (HSI)	Viscerosomatic index (VSI)
C	0,96±0,03a	0,042±0,001a
T1	0,86±0,03b	0,028±0,002b
T2	0,69±0,09c	0,030±0,006b
T3	0,62±0,04d	0,029±0,003b

*Means±SD followed by the same letter in the column is not significantly different ($P > 0.05$)

Discussion

The present study showed that orange spotted grouper, *Epinephelus coioides* that experienced unfavorable conditions i.e. a short term cycled non-fed and fed showed reduced growth and when the fish had undergone cycles of feed deprivation and refeeding, they displayed full or partial compensation. Previous studies have also shown that different fasting and refeeding regimes induce a rapid growth rate of beluga *Huso huso* (Naghshpour et al, 2021), and Nile tilapia *Oreochromis niloticus* L (Elbially et al, 2022) to reach a body weight equivalent to that fed continuously. that experienced starvation followed by normal feeding. During the fasting period, fish mobilize their endogenous energy reserves to maintain basic metabolic functions. After refeeding, the fish show hyperphagia, consuming more food than usual to replenish depleted energy stores, and the efficiency with which food is converted into body mass increases, allowing for rapid recovery of growth (Ali et al, 2003). Fish such as Golden pompano *Trachinotus ovatus* (Liu et al, 2022) and Nile Tilapia *Oreochromis niloticus* (Mishra et al, 2025), then showed metabolic adjustments and increased activity of digestive enzymes stimulated by starvation, thereby improving the ability of fish to utilize nutrients efficiently after refeeding (Won and Borski, 2013; Gabriel et al, 2017), and the biochemical composition of fish was restored (Karatas, 2019; Liu et al, 2022). Growth hormone levels increase to stimulate appetite and protein-saving lipolysis (Won & Borski, 2013) and promoting protein synthesis and growth (Wu et al, 2021). Immune system recovery occurs after refeeding so that fish can maintain health and resist disease during the growth recovery phase (Yang et al, 2024).

The present experiment showed that *Epinephelus coioides* subjected to short-term starvation, for two days per week on Monday and Thursday, and re-feeding showed no significant different final body weights to fish fed daily as a control ($P>0.05$). Meanwhile, fish starved 3 and 7 days per week, respectively, and refeeding achieved significantly lower final body weight than control ($P<0.05$). Fish starved for 2 days per week that reach the same final body weight as the control, probably experienced a phase of rapid growth greater than control growth rates associated with adequate refeeding of fish following a period of weight loss caused by starvation. According to Ali et al (2003) the rapid

growth of fish receiving a starvation and refeeding cycle which allows them to reach the same final body weight as fish fed daily was defined as complete compensatory. Such compensation has also been observed in humpback grouper *Cromileptes altivelis* kept in a recirculation system receiving short-term feed deprivation and refeeding cycle (Yuwono et al., 2007). The results of the present study also confirm previous findings that the starvation cycle and re-feeding resulted in complete compensation in several fish species, including Asian sea bass, *Lates calcarifer* (Azodi et al, 2014); Hybrid Tilapia, *Oreochromis mossambicus* x *O. niloticus* (Gabriel et al, 2017); rock bream, *Oplegnathus fasciatus* [Oh and Park, 2019], larvae and juveniles *Pyrrhulina brevis* (Abe et al., 2021); European Seabass, *Dicentrarchus labrax* (Ntantali et al., 2023). The present finding is the first to show that complete and partial compensatory growth occurs in orange spotted grouper *Epinephelus coioides* reared in net cages subjected to short term cycled starvation and re-feeding. It seems that during starvation the fish uses energy reserve efficiently and during re-feeding cycle the protein from the feed is used for compensatory growth. Although compensatory growth has been observed in many fish species, physiological mechanisms during this process are poorly understood and need to be further studied in the future.

Compensatory growth evoked by cycle of starvation followed by refeeding has been inconsistent in different fish species (Table 4). The present study found that cycled short term starvation and refeeding elicited complete compensation and partial compensation. The final body weight of fish that experienced a one-week starvation cycle and a one-week refeeding was lower than that fed daily. This suggests that prolonged starvation can inhibit the recovery of rapid growth capacity after refeeding. Meanwhile, the fish subjected to cycled of short-term starvation and prolonged refeeding achieved final weight greater than that of the fish fed daily. It is very likely that the underlying mechanisms and responses to a feeding regime may vary due to species-specific factors and genetics.

Compensatory growth in tropical fish such as in tilapia (Wang et al., 2000) and barramundi (Tian & Qin, 2003) had also been studied. More recently Persian sturgeon, *Acipenser persicus* has been reported to elicit compensatory growth (Yarmohammadi et al, 2013). In these previous studies the fish was subjected to single

Table 3. Body chemical contents of orange spotted grouper *Epinephelus coioides* reared in net cage receiving short term cycled starvation and refeeding

Treatment	Moisture %	Dry weight %	% Dry weight			
			Protein	Lipid	Fiber	Ash
C	10.19	89.80	49.91	21.71	13.20	13.63
T1	9.15	90.84	55.86	17.81	12.79	13.1
T2	8.31	91.68	54.02	18.48	12.53	13.34
T3	6.05	93.94	57.97	17.37	12.02	12.52

feed deprivation, while in present experiment the fish was receiving repeating cycle of feed deprivation followed by fixed period of refeeding. The present result confirms previous findings that cycled short-term starvation and refeeding promoted compensatory growth in fish. Such compensatory growth is an adaptive response to food scarcity by activating adaptive physiological processes for survival (Won and Borski, 2013; Mishra et al, 2025). In the early phase of starvation, the liver increases the breakdown of glycogen, which might be reflected by a decrease in the Hepatosomatic Index (HSI).

It has been reported that HSI of Persian sturgeon *Acipenser persicus* significantly decrease after 1-3 weeks starvation (Yarmohammadi et al, 2013). Red tilapia *Oreochromis* spp starved for 7, 24 and 21 days followed by feeding for 21, 14 and 7 days, respectively, also showed significant decrease in HSI (Robisalimi et al, 2021). A decrease in HSI is particularly likely to occur during starvation where fish shift from using carbohydrates to lipids as their main source of energy. This shift is necessary because glycogen stores in the liver are quickly depleted. Lipids, stored in various tissues, become the main energy source. The liver metabolizes stored lipids into fatty acids and glycerol, which are then used for energy production. This process

reduces the lipid content in the liver, contributing to the decrease in HSI (Weil et al, 2013). However, after re-feeding HSI values of the fish had rebounded to reach similar levels to that of controls. The decrease of HSI, most likely due to mobilization of glycogen storage in liver during starvation. As an instance, the level of glycogen in the liver decreased in channel catfish *Ictalurus punctatus* subjected to 14 days feed deprivation (Gaylord and Gatlin 2000). The present study demonstrated that orange spotted grouper *Epinephelus coioides* subjected to one week starvation and one-week refeeding cycle show significant decrease of HSI. This is not in agreement with previous finding in that food deprivation and re-feeding had not effect on glycogen content of liver of sobaity fish (Molayemraftar et al, 2019). Turano et al. (2007) stated that the decrease of HSI may be an indicator for potential compensatory growth response. Complete compensatory growth was observed in orange spotted grouper receiving repeating unfed–fed cycle and the HSI measured at the end of the experiment were lower than those of the control. The short term, two, three and seven days, cycled starvation and refeeding probably bring about physiological stress which affects such massive mobilization of energy reserves as to lose liver weight.

Table 4. Compensatory growth in different fish species experiencing short-term starvation and refeeding cycles

Compensatory Growth	Fish species; feeding schedule
Overcompensation: Fish experience short-term starvation and refeeding cycles reach a final body weight greater than that of fed daily.	<ul style="list-style-type: none"> • ♀ <i>Lepomis cyanellus</i> X ♂ <i>Lepomis macrochirus</i>; cycles of either 2 or 14 days no feeding and refeeding for as long as hyperphagia existed (Hayward et al., 1997). • <i>Ictalurus punctatus</i>; cycles of starvation for either 1, 2, or 3 days and feeding for either 4, 5 or 6 days (Chatakondi and Yant, 2001) • <i>Oncorhynchus mykiss</i>; cycles of no feeding on Monday and Thursday, and refeeding on other days (Guzei and Arvas, 2011).
Complete compensation: Fish that experience short-term starvation and feeding cycles again reach a final body weight equivalent to that fed daily.	<ul style="list-style-type: none"> • <i>Cromileptes altivelis</i>, non-feeding/feeding cycle of 1/2 days, cycles of no feeding on Monday and Thursday, and refeeding on other days; non feeding/feeding cycle of 1/3 day (Yuwono et al, 2007). • <i>Epinephelus coioides</i>, cycles of unfed every Monday and Thursday and fed on Tuesday and Wednesday, then on Friday, Saturday and Sunday; fish unfed every Sunday, Monday and Thursday and fed on Tuesday and Wednesday, then on Friday and Saturday (Yuwono and Sukardi, 2009). • <i>Lates calceferus</i>, cycles of starved for 4 day and refed for 16 days (Azodi et al, 2016). • <i>Oreochromis mossambicus</i> x <i>O. niloticus</i>, cycles of 2 days feed restriction/4 days refeeding (Gabriel et al., 2017) • <i>Piaractus mesopotamicus</i> x <i>Colossoma macropomum</i> cycles of 1 day feed deprivation and 6 days refeeding (Paz et al., 2018) • <i>Pyrrhulina brevis</i>; cycles of 1 day feed deprivation and 6 days refeeding (Abe et al., 2021) • <i>Huso huso</i>; cycle of 2-day feed deprivation and 8 days refeeding (Naghshpour et al., 2021) • <i>Dicentrarchus labrax</i>; cycle of 2-day feed deprivation and 8 days refeeding (Ntantali et al., 2023)
Partial compensation: Fish that experience short-term starvation and re-feeding cycles reach a lower final body weight than those fed daily.	<ul style="list-style-type: none"> • <i>Cirrhinus mrigala</i>; 5- and 10-days cyclic feeding (Iqbal et al, 2006) • <i>Dicentrarchus labrax</i>; cycles of either 2- or 5-days starvation and 8- or 20-days satiation respectively (Adakli & Tasbozan) • <i>Hemibagrus nemurus</i>; starved for 3 days per week and otherwise fed ad libitum (Thongprajukaew & Rodjaroen, 2017) • <i>Trachinotus mookalee</i>; starved for 2 days and re-fed for 5 days in a week (2S) showed partial compensation throughout the experimental period (Xavier et al., 2023)

The present experiment indicated that VSI was significantly affected by short term feed deprivation followed by feeding. The VSI of treatment groups was lower than control. This phenomenon is in accordance with previous finding in orange spotted grouper starved for a prolonged period (Yang et al, 2024). Fat storage is probably used simultaneously with glycogen during starvation as source of energy requirement. It seems that short term cycled starvation and refeeding affects fish metabolism leading to changes in the viscerosomatic index.

Body chemical contents of orange spotted grouper *Epinephelus coioides* was significantly affected by short term cycled starvation and refeeding ($P < 0.05$). At the end of this study, the crude protein content of orange spotted grouper which experienced unfed and fed cycles increased, while lipid content decreased. The same phenomenon was also found in the juvenile orange spotted grouper *Epinephelus coioides* which experienced prolonged starvation followed by normal feeding (Yang et al, 2024). Short-term starvation followed by refeeding significantly also affect the proximate composition of juvenile Sea Bass, *Dicentrarchus labrax*, with variations depending on the duration of starvation and refeeding periods (Adakli & Tasbozan, 2015). However, such feeding schedule did not significantly affect protein, lipid, and ash contents in Saifin Molly *Poecilia latipinna* (Morshedi et al, 2013) and Beluga, *Huso huso* (Naghshpour et al, 2021). It seems that fish has the metabolic regulation capability to tolerate short periods of starvation without significant changes in body composition. One week deprivation followed by seven weeks feeding did not affect the proximate composition of the Juvenile Olive Flounder *Paralichthys olivaceus* (Cho, 2014) and yellow catfish *Pelteobagrus fulvidraco* (Ruan et al., 2015). It seems that this difference is related to the number of days of starvation where in this study the fish were not fed during the experiment for a total of more than 14 days, whereas the flounder and catfish in the previous experiments were not fed for only 14 days or less. The impact of prolonged starvation on carcass chemical composition also occurred in the milkfish *Chanos chanos* (Lingam et al., 2019). However, short-term starvation does not appear to have an impact on the mobilization of energy reserves because there is a supply of macromolecules for producing energy through refeeding.

Similar with the results of the present experiment, starvation significantly affected body protein content of snapper *Lutjanus guttatus* (Hernandez & Hurtado-Oliva, 2019). During starvation liver glycogen reserve is probably mobilized, followed by the use of muscle protein to meet basal metabolic energy requirement. It appears that the duration of starvation during the experiment had an impact on compensatory growth, utilization of energy reserves and body chemical composition. So, it is necessary to investigate further how long the fish are starved and how long the

refeeding takes to find a fed-unfed cycle that has a positive impact on compensatory growth, feed efficiency and carcass quality, especially in farmed fish species. The present research showed that a 1-day starvation cycle followed by 2 days, then 3 days of alternate refeeding optimize growth and feed efficiency in orange spotted grouper. It seems that by implementing short-term periods of starvation and refeeding, overall feed consumption is reduced, leading to lower feed costs. Fish are not constantly exposed to high feed levels which can improve overall health and reduce mortality rates. Reduced feed inputs lead to lower waste production, which improves water quality and reduces the frequency of its replacement, further lowering operational costs. By balancing feed costs with growth performance, aquaculture operations can achieve higher profitability. To conclude, cycled short term starvation and refeeding could be a promising feeding strategy which will probably provide economic and ecological benefits in fish aquaculture.

Ethical Statement

The study was conducted in accordance with regulation of the Indonesian Institute of Sciences of The Republic of Indonesia Number 19 Of 2019 Concerning the Research Ethics Clearance.

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Author Contribution

Edy Yuwono: conceptualization, investigation, methodology, writing -original draft. Isdy Sulisty: investigation, methodology, data analysis writing draft. Purnama Sukardi: investigation, writing - review and editing the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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