Effects of Periodic Starvation and Refeeding on Growth Performance, Antioxidant Indices, Digestive Enzyme Activity, Amino Acid Composition, and Fatty Acid Composition in Juvenile Sepia pharaonis (Postprint)

Authors: Li Chenchen, Zhu Tingting, Lu You, Luo Jiaxiang, Jin Min, Yuan Ye, Zhou Qicun

Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of periodic starvation and refeeding on the growth performance, antioxidant indices, digestive enzyme activities, amino acid composition, and fatty acid composition of juvenile pharaoh cuttlefish (Sepia pharaonis). A total of 360 juvenile cuttlefish with an initial body weight of (6.83$±$0.01) g were randomly divided into 4 groups, with 3 replicates per group and 30 individuals per replicate. Four feeding regimes were implemented: periodic starvation for 1 day followed by refeeding for 6 days (S1F6 group), periodic starvation for 2 days followed by refeeding for 5 days (S2F5 group), periodic starvation for 3 days followed by refeeding for 4 days (S3F4 group), and continuous feeding (control group), in a 14-day feeding trial. The results showed: 1) Different starvation durations had significant effects on weight gain rate, survival rate, and specific growth rate ($P<0.05$). These three parameters in the S3F4 group were significantly lower than those in the control group ($P<0.05$), while no significant differences were observed among the S1F6 group, S2F5 group, and control group ($P>0.05$). 2) The contents of muscle moisture, crude fat, and crude protein showed no significant differences among all groups ($P>0.05$). 3) Different starvation durations had a significant effect on liver superoxide dismutase activity ($P<0.05$), which reached its maximum value in the S2F5 group; however, no significant effects were observed on liver malondialdehyde and reduced glutathione contents or glutathione peroxidase activity ($P>0.05$). 4) Different starvation durations had significant effects on liver amylase and lipase activities ($P<0.05$). With the extension of starvation duration, liver amylase activity first decreased and then increased, reaching its minimum value in the S1F6 group, while liver lipase activity first increased, then decreased, and then increased again, reaching its minimum value in the S2F5 group. 5) A total of 17 amino acids were detected in the muscle tissue of juvenile cuttlefish, and the contents of 10 essential amino acids, 7 non-essential amino acids, total amino acids (TAA), total essential amino acids (TEAA), and the TEAA/TAA ratio showed no significant differences among all groups ($P>0.05$). 6) No significant differences were observed among all groups in saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids in liver and muscle ($P>0.05$). The contents of C20:5n-3 (EPA), C22:5n-3 (DPA), and C22:6n-3 (DHA) in muscle, as well as DPA and DHA contents in liver, also showed no significant differences among all groups ($P>0.05$). However, liver EPA and n-3 polyunsaturated fatty acid contents in the S3F4 group were significantly lower than those in the control group ($P<0.05$). In conclusion, juvenile cuttlefish in both the S1F6 and S2F5 groups exhibited compensatory growth, with no significant changes in routine muscle nutrient composition. Taking into account cost savings, pollution reduction, and compensatory growth, it is recommended to adopt the feeding regime of periodic starvation for 2 days followed by refeeding for 5 days in the culture of juvenile cuttlefish.

Full Text

Abstract

A 14-day feeding trial was conducted to evaluate the effects of periodical starvation-refeeding on growth performance, antioxidant indices, digestive enzyme activities, amino acid composition, and fatty acid composition of juvenile *Sepia pharaonis*. A total of 360 juvenile cuttlefish with an initial body weight of (6.83$±$0.01) g were randomly divided into four groups with three replicates per group and 30 individuals per replicate. Four different repetitive cycles of starvation-refeeding were implemented: starvation for 1 day followed by refeeding for 6 days (S1F6), starvation for 2 days followed by refeeding for 5 days (S2F5), starvation for 3 days followed by refeeding for 4 days (S3F4), and continuous feeding (control group). The results showed: (1) Different starvation durations significantly affected weight gain rate (WGR), survival rate, and specific growth rate (SGR) ($P<0.05$). These three indicators in the S3F4 group were significantly lower than in the control group ($P<0.05$), while no significant differences were observed among the control, S1F6, and S2F5 groups ($P>0.05$). (2) No significant differences were found in muscle moisture, crude lipid, or crude protein contents among all groups ($P>0.05$). (3) Hepatic superoxide dismutase (SOD) activity was significantly affected by different starvation durations ($P<0.05$), reaching its maximum value in the S2F5 group. However, hepatic malondialdehyde (MDA) and reduced glutathione (GSH) contents, as well as glutathione peroxidase (GSH-Px) activity, were not significantly affected ($P>0.05$). (4) Hepatic amylase and lipase activities were significantly influenced by starvation duration ($P<0.05$). As starvation time extended, hepatic amylase activity first decreased then increased, with the minimum value observed in the S1F6 group, while hepatic lipase activity first increased, then decreased, and then increased again, with the minimum value in the S2F5 group. (5) Seventeen amino acids were detected in the muscle tissue of juvenile *Sepia pharaonis*, including 10 essential amino acids and 7 non-essential amino acids. No significant differences were found among groups in the contents of these amino acids, total amino acids (TAA), total essential amino acids (TEAA), or the TEAA/TAA ratio ($P>0.05$). (6) No significant differences were observed among groups in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA) in either liver or muscle tissues ($P>0.05$). Additionally, muscle C20:5n-3 (EPA), C22:5n-3 (DPA), C22:6n-3 (DHA) contents, and hepatic DPA and DHA contents showed no significant differences among groups ($P>0.05$). However, hepatic EPA and n-3 PUFA contents in the S3F4 group were significantly lower than in the control group ($P<0.05$). In conclusion, compensatory growth was observed in both S1F6 and S2F5 groups, with no significant changes in muscle conventional nutrient composition. Considering cost savings, pollution reduction, and compensatory growth, we recommend adopting the periodical starvation-refeeding pattern of 2 days starvation followed by 5 days refeeding for juvenile *Sepia pharaonis* culture.

Keywords: juvenile *Sepia pharaonis*; starvation-refeeding; conventional nutrients; digestive enzyme activities; amino acids; fatty acids

Introduction

Sepia pharaonis (pharaoh cuttlefish) belongs to the phylum Mollusca, class Cephalopoda, order Sepiida, family Sepiidae, and genus *Sepia*. This species is large-bodied, delicious, nutritious, and has high nutritional value, representing a promising cephalopod for aquaculture. In natural environments, animals frequently face starvation stress due to seasonal changes, temperature fluctuations, water quality variations, and uneven spatial distribution of food resources. Under starvation conditions, many animals reduce their metabolic rate and mobilize stored tissue substances to cope with the stress [1]. Animals actively utilize stored substances by regulating various enzyme activities to sustain life [2]. During starvation, the utilization sequence of amino acids and fatty acids also differs.

Compensatory growth refers to the phenomenon where animals exhibit growth rates exceeding normal levels after experiencing starvation or nutritional deprivation and then returning to normal feeding [3]. The degree of compensatory growth in aquatic animals varies significantly depending on species, developmental stage, starvation duration, and refeeding period [4-6]. Based on changes in specific growth rate and body mass during the recovery period, compensatory growth in aquatic animals can be classified into four categories: hyper-compensatory, complete compensatory, partial compensatory, and non-compensatory growth [7]. Research has shown that compensatory growth can promote growth, improve feed utilization, reduce labor costs [8-10], and decrease nitrogen emissions, thereby reducing water pollution [11]. Current studies have primarily focused on fish [12-14], crustaceans [15], and bivalves [16], with limited reports on cephalopods [17].

Different starvation and refeeding protocols yield varying results in compensatory growth studies [18]. Cyclic starvation is an effective approach to achieve desirable compensatory growth [19], as it reduces labor intensity, saves labor costs, and shortens starvation duration. Le et al. [3] investigated compensatory growth in newly hatched *Sepia pharaonis* larvae, but in-depth research on compensatory growth after multiple cycles of starvation stress in juvenile *Sepia pharaonis* is lacking. Therefore, this study examined the effects of different periodical starvation-refeeding patterns on growth performance, muscle conventional nutrients, antioxidant indices, digestive enzyme activities, amino acid composition, and fatty acid composition of juvenile *Sepia pharaonis*. The objective was to explore the physiological response mechanisms under starvation stress and provide a scientific basis for developing efficient feeding strategies for juvenile *Sepia pharaonis*.

1.1 Experimental Design

Juvenile *Sepia pharaonis* were obtained from Xiangshan Laifa Aquatic Seedling Farm, and the experiment was conducted at the Marine Science and Technology Innovation Base in Ningbo, Zhejiang Province. Prior to the experiment, the cuttlefish were acclimated with fresh shrimp for one week and fasted for 24 hours before grouping. A total of 360 healthy individuals of uniform size [initial body weight (6.83$±$0.01) g] were randomly divided into four groups: one control group and three periodical starvation-refeeding groups (S1F6, S2F5, and S3F4). Each group had three replicates with 30 individuals per replicate, stocked in 300 L tanks. Water was exchanged twice daily (morning and afternoon) at approximately 50% each time. Natural seawater after dark sedimentation and sand filtration was used, with salinity ranging from 21.2‰ to 26.7‰, temperature from 23.8 to 27.4 °C, and pH from 7.5 to 8.0. Natural lighting and micro-flow aeration were provided. The diet consisted of small fresh shrimp (proximate composition, amino acid composition, and fatty acid composition of the fresh shrimp are shown in Table 1), fed twice daily at 07:30 and 15:30. Feeding behavior was observed within 1 hour after feeding.

1.2 Feeding Protocols

Control group: continuous feeding for 14 days; S1F6 group: starvation for 1 day followed by refeeding for 6 days, repeated for 2 cycles; S2F5 group: starvation for 2 days followed by refeeding for 5 days, repeated for 2 cycles; S3F4 group: starvation for 3 days followed by refeeding for 4 days, repeated for 2 cycles. The experimental period lasted 14 days.

1.3 Sample Collection and Analysis Methods

At the end of the feeding trial, all cuttlefish were fasted for 24 hours. Before sampling, each tank was weighed and counted individually to calculate weight gain rate, specific growth rate, and survival rate. Four cuttlefish were randomly selected from each tank. Muscle tissue was dissected for analysis of conventional muscle composition, amino acid composition, and fatty acid composition, while liver tissue was dissected for determination of antioxidant indices, digestive enzyme activities, and fatty acid composition. All operations were performed on ice.

Proximate composition analysis of diet and muscle: Moisture content was determined by oven drying at 105 °C; crude ash content was measured by incineration in a muffle furnace at 550 °C; crude protein content was analyzed using a protein analyzer (LECO FP-528); and crude lipid content was determined using a fat analyzer (SX360).

Amino acid composition determination: Samples were hydrolyzed with 6 mol/L HCl in a sand bath for 24 hours, then diluted to 50 mL in a volumetric flask. One milliliter of the solution was rotary evaporated, and the residue was dissolved in 0.02 mol/L HCl before analysis using a high-speed automatic amino acid analyzer (L-8900, HITACHI, Japan).

Fatty acid composition determination: Diet, liver, and muscle samples were freeze-dried for 48 hours. Lipids were extracted using HCl-methanol and KOH-methanol solutions for pretreatment, then sent to the Analysis Center of Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences for determination by gas chromatography-mass spectrometry (GCMS-QP2010 Plus, SHIMADZU, Japan).

Antioxidant indices determination: A precise amount of tissue was weighed and homogenized with physiological saline at a ratio of 1:9 (weight:volume) under ice-water bath conditions. The homogenate was centrifuged at 2,500 r/min for 10 minutes, and the supernatant was stored at -80 °C until analysis. Hepatic superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, as well as reduced glutathione (GSH) and malondialdehyde (MDA) contents, were measured using commercial kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

Digestive enzyme activity determination: Tissue pretreatment followed the same procedure as for antioxidant indices. Hepatic lipase and amylase activities were measured using commercial kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.4 Calculation Formulas

Weight gain rate (%) = 100 × (final mean weight - initial mean weight) / initial mean weight
Specific growth rate (%/d) = 100 × (ln final body weight - ln initial body weight)

/ feeding days
Survival rate (%) = 100 × (final number - initial number) / initial number

1.5 Data Processing and Analysis

All data are expressed as mean ± standard error (mean±SE). One-way ANOVA was performed using SPSS 19.0 software. When significant differences were detected among groups, Tukey' s multiple comparison test was conducted, with $P<0.05$ as the significance threshold.

2.1 Effects of Periodical Starvation-Refeeding on Growth Performance

As shown in Table 2 , starvation duration significantly affected weight gain rate, specific growth rate, and survival rate ($P<0.05$). The weight gain rate, specific growth rate, and survival rate of the S3F4 group were significantly lower than those of the control group ($P<0.05$), while no significant differences were observed between the S1F6 group, S2F5 group, and control group ($P>0.05$).

2.2 Effects of Periodical Starvation-Refeeding on Muscle Conventional Nutrients

Table 3 shows that no significant differences were found in moisture, crude protein, or crude lipid contents of juvenile *Sepia pharaonis* muscle among the three periodical starvation-refeeding groups and the control group ($P>0.05$).

2.3 Effects of Periodical Starvation-Refeeding on Hepatic Antioxidant Indices

As shown in Table 4 , different starvation durations significantly affected hepatic superoxide dismutase (SOD) activity ($P<0.05$). With prolonged starvation time, hepatic SOD activity first decreased, then increased, and then decreased again, reaching its maximum value in the S2F5 group. However, no significant effects were observed on hepatic malondialdehyde (MDA) and reduced glutathione (GSH) contents or glutathione peroxidase (GSH-Px) activity ($P>0.05$).

2.4 Effects of Periodical Starvation-Refeeding on Hepatic Digestive Enzyme Activities

Table 5 indicates that different starvation durations significantly affected hepatic amylase (AMS) and lipase (LPS) activities ($P<0.05$). As starvation time extended, hepatic AMS activity first decreased then increased, with the minimum value in the S1F6 group and the maximum value in the S3F4 group, and these two groups differed significantly ($P<0.05$). Hepatic LPS activity first increased, then decreased, and then increased again, with the minimum value in the S2F5 group, which was significantly lower than all other groups ($P<0.05$), and the maximum value in the S1F6 group, which was significantly higher than both the S2F5 and S3F4 groups ($P<0.05$).

2.5 Effects of Periodical Starvation-Refeeding on Muscle Amino Acid Composition

As shown in Table 6 , 17 amino acids were detected in the muscle tissue of juvenile *Sepia pharaonis*, including 10 essential amino acids and 7 non-essential amino acids. No significant differences were found among groups in the contents of these 10 essential and 7 non-essential amino acids, nor in total amino acids (TAA), total essential amino acids (TEAA), or the TEAA/TAA ratio ($P>0.05$).

2.6 Effects of Periodical Starvation-Refeeding on Fatty Acid Composition

Table 7 shows that 24 fatty acids were detected in the liver tissue of juvenile *Sepia pharaonis*, with carbon chain lengths ranging from 12 to 24, including 7 saturated fatty acids (SFA), 5 monounsaturated fatty acids (MUFA), and 12 polyunsaturated fatty acids (PUFA). No significant differences were observed among groups in hepatic MUFA, PUFA, SFA, or n-6 PUFA contents ($P>0.05$). Hepatic C22:5n-3 (DPA) and C22:6n-3 (DHA) contents also showed no significant differences among groups ($P>0.05$). However, hepatic C20:5n-3 (EPA) content in the three periodical starvation-refeeding groups decreased to varying degrees compared with the control group. While the differences were not significant in the S1F6 and S2F5 groups ($P>0.05$), the S3F4 group showed significantly lower EPA content than the control group ($P<0.05$). Similarly, hepatic n-3 PUFA content decreased in all three periodical starvation-refeeding groups compared with the control, with the S3F4 group showing a significant difference ($P<0.05$).

Table 8 shows that 18 fatty acids were detected in the muscle tissue of juvenile *Sepia pharaonis*, with carbon chain lengths ranging from 12 to 22, including 4 SFAs, 4 MUFAs, and 10 PUFAs. No significant differences were found among groups in muscle SFA, MUFA, PUFA, or n-3 PUFA contents ($P>0.05$). Muscle EPA, DPA, and DHA contents also showed no significant differences among groups ($P>0.05$). Although muscle n-6 PUFA content in the three periodical starvation-refeeding groups did not differ significantly from the control group ($P>0.05$), the S1F6 group was significantly higher than the S2F5 group ($P<0.05$).

3 Discussion

Starvation affects a series of physiological changes in aquatic animals, including behavior, survival, growth, and metabolism. After starvation stress, aquatic animals exhibit various compensatory growth responses, including non-compensatory, partial compensatory, complete compensatory, and hyper-compensatory growth [20]. Ribeiro et al. [21] suggested that feeding rate, feed conversion efficiency, protein synthesis rate, and energy reserves are key indicators for evaluating compensatory growth. Zhang et al. [22] considered weight gain rate and survival rate as evaluation metrics for *Sepiella japonica* due to cannibalism during starvation stress. A et al. [23] used body mass change rate and specific growth rate as evaluation criteria for compensatory growth in turbot. Based on previous studies, this experiment adopted weight gain rate, specific growth rate, and survival rate as evaluation criteria. The results showed that under equal total starvation and feeding time, different starvation durations significantly affected weight gain rate, survival rate, and specific growth rate. The S1F6 and S2F5 groups showed no significant differences from the control group, indicating compensatory growth occurred, similar to findings in turbot juveniles [23]. The S3F4 group exhibited significantly lower weight gain rate, survival rate, and specific growth rate compared with the S1F6 group, S2F5 group, and control group, suggesting that the 3-day starvation and 4-day refeeding pattern failed to induce compensatory growth. This indicates that excessively long starvation periods may prevent compensatory growth and are detrimental to industrial aquaculture of *Sepia pharaonis*. In this experiment, survival rates were relatively low across all groups, particularly in the S3F4 group, which showed significantly reduced survival compared with the control group. This may be attributed to cannibalism induced by starvation stress during culture. Considering labor cost savings, improved feed utilization, and reduced water pollution, the optimal feeding pattern for juvenile *Sepia pharaonis* appears to be periodical starvation for 2 days followed by refeeding for 5 days.

Lipids, carbohydrates, and proteins are the primary energy storage substances for aquatic animals. During starvation, aquatic animals consume these stored substances to maintain dynamic equilibrium. Different aquatic animals utilize stored substances differently during starvation [24]. Lin et al. [25] found that *Litopenaeus vannamei* primarily utilizes lipids as an energy source. Shen et al. [26] reported that grass carp can effectively use carbohydrates as an energy source. Mehner et al. [27] discovered that perch mainly utilizes protein as an energy source. In this study, no significant differences were observed in muscle moisture, crude protein, or crude lipid contents among the three periodical starvation-refeeding groups and the control group, indicating that after compensatory growth, moisture, crude protein, and crude lipid could recover to control levels within a short period. Thus, periodical starvation-refeeding does not affect the nutritional quality of juvenile *Sepia pharaonis*.

Oxygen free radicals are produced during stress responses and metabolism. The antioxidant system composed of antioxidant enzymes and antioxidants continuously scavenges these free radicals, forming a dynamic equilibrium [28]. When aquatic animals experience stress, the activities of these antioxidant enzymes change accordingly [29]. Superoxide dismutase is a key enzyme in the biological defense system that catalyzes the dismutation of superoxide anion radicals ($O_2^{-}\cdot$), reducing free radicals while producing hydrogen peroxide (H_2O_2).

In this experiment, different starvation durations significantly affected hepatic SOD activity, which showed a trend of first decreasing, then increasing, and then decreasing again with prolonged starvation time, reaching its maximum in the S2F5 group and significantly higher than in the S1F6 group. This suggests that juvenile *Sepia pharaonis* had stronger antioxidant stress capacity at this point, possibly because starvation stress induced superoxide anion radical generation, which in turn stimulated massive expression of SOD genes. Glutathione peroxidase reduces hydrogen peroxide and organic peroxides, while reduced glutathione both resists reactive oxygen species (ROS) oxidative damage and serves as a substrate for other antioxidant enzymes [30]. Malondialdehyde is a toxic product of lipid peroxidation caused by free radicals [31], and its content reflects the degree of lipid peroxidation and cell damage [32]. In this study, no significant differences were found in hepatic MDA and GSH contents or GSH-Px activity among the three periodical starvation-refeeding groups and the control group, indicating that periodical starvation-refeeding does not negatively affect these antioxidant parameters in juvenile *Sepia pharaonis*.

Under starvation stress, aquatic animals actively utilize stored energy substances by regulating digestive enzyme activities to sustain life [33-35]. Current research suggests that the primary reason for decreased digestive enzyme activities during starvation is the lack of food stimuli (olfactory, visual, etc.) that normally influence central nervous system control of digestive gland secretion [36-37]. Starvation also causes substantive changes in digestive organs, such as hepatopancreas atrophy, which reduces digestive enzyme secretion [38]. This study showed that hepatic amylase and lipase activities in juvenile *Sepia pharaonis* were affected by starvation duration. In the early starvation stage, due to lipid depletion, hepatic lipase secretion decreased to enhance adaptability. After 3 days of starvation, juvenile *Sepia pharaonis* developed some adaptability to starvation stress, and hepatic lipase activity increased again. This pattern aligns with the trend of muscle crude lipid content first increasing then decreasing with prolonged starvation time. *Sepia pharaonis* is a carnivorous species with low carbohydrate utilization efficiency, resulting in low amylase activity. In the early starvation stage, hepatic amylase activity decreased due to food scarcity, but increased in the S3F4 group, possibly because the liver enhanced utilization of muscle glycogen and hepatic glycogen to meet metabolic demands.

Amino acids play important roles in aquatic animal growth. Under starvation stress, organisms can convert amino acids to glucose for energy, leading to decreased essential amino acids and total amino acids [39]. Different aquatic animals utilize amino acids in different sequences during starvation. Channel catfish (*Ictalurus punctatus*) preferentially utilizes non-essential amino acids as an energy source during starvation [40], while *Mystus macropterus* preferentially utilizes essential amino acids [41], and starvation does not affect amino acid content in *Sepiella japonica* [22]. This study found no significant differences in the contents of 10 essential amino acids, 7 non-essential amino acids, total amino acids, total essential amino acids, or the TEAA/TAA ratio among groups, indicating that periodical starvation-refeeding does not significantly affect amino acid content in juvenile *Sepia pharaonis*, similar to findings in *Sepiella japonica* [22].

During starvation stress, aquatic animals utilize different types of fatty acids in a specific order: first saturated fatty acids, followed by low-unsaturated fatty acids, and finally high-unsaturated fatty acids [42]. This study investigated the effects of periodical starvation-refeeding on fatty acid composition in liver and muscle of juvenile *Sepia pharaonis*. The results showed no significant differences in SFA, MUFA, and PUFA contents in liver and muscle among the three periodical starvation-refeeding groups and the control group. Overall, in muscle tissue, SFAs were utilized first, followed by alternating utilization of MUFAs and PUFAs as starvation time extended. In liver tissue, PUFAs were utilized first, followed by alternating utilization of SFAs and MUFAs. This indicates that the utilization sequence of different fatty acid types varies between tissues in juvenile *Sepia pharaonis*.

Generally, EPA and DHA contents are higher in marine fish than in freshwater fish [43]. EPA, DHA, and DPA belong to n-3 polyunsaturated fatty acids and are important nutritional components in marine organisms. EPA plays a crucial role in immune and inflammatory responses, DHA is essential for neurological function and normal retinal development [43], and DPA strongly inhibits platelet aggregation and promotes bovine aortic endothelial cell migration [44-45], with potential applications in treating hypertriglyceridemia [46]. Under the three periodical starvation-refeeding patterns, muscle EPA, DHA, and DPA contents in juvenile *Sepia pharaonis* showed no significant differences compared with the control group. However, in the liver, EPA content in the S1F6 and S2F5 groups did not differ significantly from the control, while the S3F4 group showed significantly lower EPA content. Hepatic DHA and DPA contents showed no significant differences among the three periodical starvation-refeeding groups and the control group. These results indicate that starvation stress under the 1-day starvation/6-day refeeding and 2-day starvation/5-day refeeding patterns did not significantly affect important nutritional components in juvenile *Sepia pharaonis*, but the 3-day starvation/4-day refeeding pattern caused nutritional deficiencies.

4 Conclusion

1. Compensatory growth was observed in both S1F6 and S2F5 groups of juvenile *Sepia pharaonis*, with no significant changes in muscle conventional nutrient composition.
2. Considering cost savings, pollution reduction, and compensatory growth, we recommend adopting the periodical starvation-refeeding pattern of 2 days starvation followed by 5 days refeeding for juvenile *Sepia pharaonis* culture.

References

[1] Lin XQ. Effects of starvation and refeeding on physiological parameters of rainbow trout (*Oncorhynchus mykiss*) [J]. Journal of Shantou University (Natural Science Edition), 1998, 13(2): 51-57.

[2] Ou YJ, Liu ZW. Effects of starvation and refeeding on digestive enzyme activities in juvenile mangrove red snapper (*Lutjanus argentimaculatus*) [J]. Acta Oceanologica Sinica (Chinese Edition), 2007, 29(1): 86-91.

[3] Le KX, Wang Y, Peng RB, et al. Effects of starvation and refeeding on survival, growth, and digestive enzyme activities of juvenile *Sepia pharaonis* [J]. Chinese Journal of Applied Ecology, 2016, 27(6): 2002-2008.

[4] Gao LJ, Chen LQ, Song B. Effects of starvation and compensatory growth on feeding, growth, and body composition of juvenile Amur sturgeon (*Acipenser schrenckii*) [J]. Journal of Fisheries of China, 2004, 28(3): 279-284.

[5] Wang Y, Li C, Qin JG, et al. Cyclical feed deprivation and refeeding fails to enhance compensatory growth in Nile tilapia, *Oreochromis niloticus* L. [J]. Aquaculture Research, 2009, 40(2): 204-210.

[6] Fang JH, Tian XL, Jiang HB, et al. Effects of different cyclic feeding patterns on growth, body composition, metabolism, and energy budget of half-smooth tongue sole (*Cynoglossus semilaevis*) [J]. Journal of Fisheries of China, 2011, 35(7): 1090-1097.

[7] Zheng SM, Wang YN, Nie YX, et al. Study on compensatory growth and amylase activity in tiger shark after starvation [J]. Journal of Huazhong Agricultural University, 2003, 22(5): 483-487.

[8] Huang G, Wei L, Zhang X, et al. Compensatory growth of juvenile brown flounder *Paralichthys olivaceus* (Temminck & Schlegel) following thermal manipulation [J]. Journal of Fish Biology, 2008, 72(10): 2534-2542.

[9] Eroldoğan OrhanTufan, Taşbozan O, Tabakoğlu S. Effects of restricted feeding regimes on growth and feed utilization of juvenile gilthead sea bream, *Sparus aurata* [J]. Journal of the World Aquaculture Society, 2008, 39(2): 267-274.

[10] MacLean A, Metcalfe NB. Social status, access to food, and compensatory growth in juvenile Atlantic salmon [J]. Journal of Fish Biology, 2001, 58(5): 1331-1346.

[11] Blanquet I, Oliva-Teles A. Effect of feed restriction on the growth performance of turbot (*Scophthalmus maximus*) juveniles under commercial rearing conditions [J]. Aquaculture Research, 2010, 41(8): 1255-1260.

[12] Bolasina S, Pérez A, Yamashita Y. Digestive enzymes activity during ontogenetic development and effect of starvation in Japanese flounder, *Paralichthys olivaceus* [J]. Aquaculture, 2006, 252(2/3/4): 503-515.

[13] Li Y, Zhu ZQ, Ortegón O, et al. Effects of short-term starvation and refeeding on survival, growth, RNA/DNA and RNA/protein ratios in larval rock carp (*Procypris rabaudi*) [J]. Acta Hydrobiologica Sinica, 2012, 36(4): 674-681.

[14] Zeng LQ, Peng HLY, Wang JW, et al. Effects of starvation on individual variation and repeatability of swimming performance in juvenile southern catfish (*Silurus meridionalis*) [J]. Acta Hydrobiologica Sinica, 2014, 38(5): 883-890.

[15] Liu L, Wu LX, Zhang WG, et al. Effects of starvation and refeeding on carbohydrate metabolism in *Marsupenaeus japonicus* [J]. Chinese Journal of Applied Ecology, 2007, 18(3): 697-700.

[16] Yan XW, Yao T, Zhang YH, et al. Effects of winter starvation and refeeding on growth, survival, and biochemical composition of the Philippine clam (*Ruditapes philippinarum*) [J]. Chinese Journal of Applied Ecology, 2009, 20(12): 3063-3069.

[17] Fan F, Yin F, Peng SM, et al. Effects of starvation stress on juvenile *Sepiella japonica* [J]. Chinese Journal of Ecology, 2011, 30(10): 2262-2268.

[18] Ali M, Nicieza A, Wootton RJ. Compensatory growth in fishes: a response to growth depression [J]. Fish and Fisheries, 2003, 4(2): 147-190.

[19] Turano MJ, Borski RJ, Daniels HV. Effects of cyclic feeding on compensatory growth of hybrid striped bass (*Morone chrysops* × *M. saxitilis*) foodfish and water quality in production ponds [J]. Aquaculture Research, 2010, 39(14): 1514-1523.

[20] Liu B, He QG, Tang YK, et al. Effects of starvation stress on growth and physiological-biochemical indices of genetically improved farmed tilapia (*Oreochromis niloticus*) [J]. Journal of Fishery Sciences of China, 2009, 16(2): 230-237.

[21] Ribeiro FF, Tsuzuki MY. Compensatory growth responses in juvenile fat snook, *Centropomus parallelus* Poey, following feed deprivation [J]. Aquaculture Research, 2010, 41(9): e226-e233.

[22] Zhang T, Ping HL, Shi HL, et al. Effects of periodical starvation-refeeding on growth, body composition, and amino acid and fatty acid profiles of juvenile *Sepiella japonica* [J]. Oceanologia et Limnologia Sinica, 2017, 48(1): 190-197.

[23] A R, Wu LX, Jiang ZQ, et al. Effects of periodical starvation-refeeding on growth, biochemical composition, and energy budget of juvenile turbot (*Scophthalmus maximus*) [J]. Journal of Dalian Ocean University, 2013, 28(5): 462-467.

[24] Kim MK, Lovell RT. Effect of restricted feeding regimens on compensatory weight gain and tissue changes in channel catfish *Ictalurus punctatus* in ponds [J]. Aquaculture, 1995, 135(4): 285-293.

[25] Lin XT, Zhou XZ, Yu HN, et al. Effects of starvation on biochemical composition and compensatory growth of Pacific white shrimp (*Litopenaeus vannamei*)

[J]. Journal of Fisheries of China, 2004, 28(1): 47-53.

[26] Shen WY, Lin HR, Zhang WM. Effects of starvation and refeeding on biochemical composition of grass carp (*Ctenopharyngodon idellus*) fingerlings [J]. Acta Zoologica Sinica, 1999, 45(4): 404-412.

[27] Mehner T, Wieser W. Energetics and metabolic correlates of starvation in juvenile perch (*Perca fluviatilis*) [J]. Journal of Fish Biology, 2010, 45(2): 325-333.

[28] Fang YZ, Yang S, Wu GY. Free radical homeostasis [J]. Progress in Physiological Sciences, 2004, 35(3): 199-204.

[29] Jiang M, Huang SL, Lun FX, et al. Stress effects of *Alexandrium minutum* on antioxidant enzymes and ATPase activity in black seabream (*Spondyliosoma cantharus*) larvae [J]. Marine Science Bulletin, 2010, 29(4): 427-431.

[30] Meister A. Glutathione metabolism and its selective modification [J]. Journal of Biological Chemistry, 1988, 263(33): 17205-17208.

[31] Fu JJ, Huang YH, Cao JM, et al. Effects of five plant protein sources replacing fish meal on serum biochemical indices, transaminase activities, and antioxidant stress parameters of Japanese seabass (*Lateolabrax japonicus*) [J]. Hubei Agricultural Sciences, 2015, 54(20): 5087-5091, 5095.

[32] Zhou XQ, Liang HM. Changes in liver lipid peroxide content and antioxidant enzyme activities in mice under crowding stress [J]. Zoological Research, 2003, 24(3): 238-240.

[33] Qiao QS. Effects of periodical starvation-refeeding on growth performance, body composition, digestive enzymes, and antioxidant enzymes in blunt snout bream (*Megalobrama amblycephala*) and Jian carp (*Cyprinus carpio* var. Jian) [D]. Master' s thesis. Nanjing: Nanjing Agricultural University, 2011: 34-35.

[34] Li ZH, Xie S, Wang JX, et al. Effects of intermittent starvation on growth and several digestive enzymes in the oriental river prawn (*Macrobrachium nipponense*) [J]. Journal of Fisheries of China, 2007, 31(4): 456-462.

[35] Xue M, Ke CH, Wei YJ. Effects of starvation on biochemical composition and digestive enzyme activities in juvenile *Babylonia areolata* [J]. Journal of Tropical Oceanography, 2010, 29(3): 120-125.

[36] Qian GY. Effects of different domestication methods on digestive enzyme activities in mandarin fish (*Siniperca chuatsi*) [J]. Journal of Zhejiang University (Agriculture and Life Sciences), 1998, 24(2): 207-210.

[37] Qian YX. Effects of starvation on protease activity in cultured perch (*Lateolabrax japonicus*) [J]. Fisheries Science, 2002, 21(3): 6-7.

[38] Li X, Jiang ZQ, Tan XZ, et al. Effects of starvation and refeeding on histology of digestive organs in American red drum (*Sciaenops ocellatus*) [J]. Journal of Fishery Sciences of China, 2002, 9(3): 211-214, 294.

[39] Liu MH, Luo HZ, Fu RB, et al. Effects of short-term starvation stress on biochemical composition, fatty acid, and amino acid profiles of *Miichthys miiuy* [J]. Acta Hydrobiologica Sinica, 2009, 33(2): 230-235.

[40] Tan XY, Luo Z, Wang WM, et al. Effects of starvation on body weight and biochemical composition of small-sized channel catfish (*Ictalurus punctatus*) [J]. Acta Hydrobiologica Sinica, 2009, 33(1): 39-45.

[41] Ma S, Jiang HB, Yao JJ. Effects of short-term starvation on biochemical composition, amino acid, and fatty acid profiles of *Mystus macropterus* [J]. Journal of Hydroecology, 2010, 3(2): 61-65.

[42] Xie XJ, Deng L, Zhang B. Research progress on physiological and ecological effects of starvation in fish [J]. Acta Hydrobiologica Sinica, 1998, 22(2): 181-188.

[43] Li Y, Wang J, Li QL, et al. Recent research progress on EPA and DHA [J]. Academic Periodical of Farm Products Processing, 2013(2): 6-13.

[44] Akiba S, Murata T, Kitatani K, et al. Involvement of lipoxygenase pathway in docosapentaenoic acid-induced inhibition of platelet aggregation [J]. Biological & Pharmaceutical Bulletin, 2000, 23(11): 1293-1297.

[45] Tsuji M, Murota SI, Morita I. Docosapentaenoic acid (22:5,n-3) suppressed tube-forming activity in endothelial cells induced by vascular endothelial growth factor [J]. Prostaglandins, Leukotrienes and Essential Fatty Acids, 2003, 68(5): 337-342.

[46] Byelashov OA, Sinclair AJ, Kaur G. Dietary sources, current intakes, and nutritional role of omega-3 docosapentaenoic acid [J]. Lipid Technology, 2015, 27(4): 79-82.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.