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# Effect of feeding frequency on growth performance and immune response on Golden pompano *Trachinotus ovatus*

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Keywords: *Trachinotus ovatus*, feeding frequency, growth performance, biochemical indices, muscle composition

**Abstract**

Golden pompano (*Trachinotus ovatus*) is a commercially important marine fish and is widely cultured in the coastal area of South China. The aims of this study are to investigate the effect of feeding frequency on growth performance, biochemical indices, muscle composition of *Trachinotus ovatus*, 6 groups of feeding frequency were designed (1, 2, 3, 4, 5 and 6TD) for 10 weeks to analyze the survival rate of *T. ovatus*. The results showed that final body weight, weight gain rate, specific growth rate, viscerosomatic index and hepatosomatic index of the 4TD group were significantly higher than of the other groups ($P < 0.05$). However, there was no difference in survival rate and feed conversion ratio in the group ($P > 0.05$). The crude fat and crude protein content were higher in the 4TD group, but there was no difference in moisture and ash among the groups ($P > 0.05$). Total protein, total cholesterol, low-density lipoprotein and high-density lipoprotein content was lowest in the 4TD group. Superoxide dismutase, catalase and total antioxidant capacity were significantly higher in the 3 and 4TD groups than that of the other groups ($P < 0.05$), while no significant difference was found among the other groups ($P > 0.05$). Malondialdehyde was significantly higher in the 4TD group than that of the 1, 2 and 5TD groups ($P < 0.05$), but there was no significant difference among the other groups ($P>0.05$). Lysozyme was significantly lower in the 1 and 6TD groups than other groups ($P < 0.05$), while there was no significant difference among the other groups ($P > 0.05$). Feeding frequency of 4TD for the *T. ovatus* seems suitable since the fish of this treatment group showed a fast and a healthy growth while muscle quality was maintained.

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## Introduction

Fish growth is largely governed by many factors, including feeding frequency, feeding rate, feed intake and ability to absorb nutrients (Xie et al., 2011). Among these, feeding frequency should be one of key factors that have an influence on fish growth performance (Du et al., 2006; Guo et al., 2018). The optimal feeding frequency is very important to ensure optimum growth, survival, improved immunity and stress resistance (Cho et al., 2003; Imsland et al., 2019). The lower feeding frequency leads to reduced fish growth and survival; however, higher feeding frequency not only raises the cost of aquatic animals, but also accumulates waste that adversely affects the water quality (Tian et al., 2015, Thongprajukaew et al., 2017). Optimal feeding frequencies may increase growth by improving the food intake (Riche et al., 2004). Lee and co-workers (2016) pointed out that the determination of optimum feeding frequency is a critical component for aquaculture operation success.

The physiological condition of the fish is one of the critical factors that determine their health status (Imsland et al., 2019). To date, the effects of nutritional factors on the physiology and health of fish have been extensively investigated, but there is relatively little information on how feeding frequency could affect the antioxidant ability and immunity of fish. Previous studies reported that the inadequacy feeding frequency not only causes oxidative damage but also resulted in immunosuppression (Ding et al., 2017; Guo et al., 2018). Studies reported that optimal feeding frequency could enhance the growth and immunity of blunt snout bream *Megalobrama amblycephala* (Li et al., 2014). However, the mechanism that feeding frequency could affect the antioxidant ability and immune response of fish is still unknown.

Golden pompano, *Trachinotus ovatus* belongs to the Carangidae family. Golden pompano is distributed in tropical and subtropical areas of Southeast Asia and the Mediterranean Sea (Liu et al., 2019a; Liu et al., 2019b). Because of its delicious taste and rapid growth, golden pompano is one of the most important marine fish that are commercially cultured in South China (Sun et al., 2013; Guo et al., 2014; Tan et al., 2016). Currently, salinity fluctuations hinder the farming efficiency of this species since most pompano are cultured in outdoor ponds and floating sea cages in China where heavy rainfall can reduce the local salinity by 30-50% during the nursery phase (Ma et al., 2016; Liu et al., 2019b). However, to our knowledge, no studies have reported the available concerning the optimum feeding frequency of golden pompano. Therefore, the current study aimed to compare growth performance, biochemical indices, muscle composition and resistance to salinity stress challenge of golden pompano fed at different feeding frequencies, in order to develop an optimal feeding strategy to maintain healthy golden pompano populations and to provide valuable information for the intensive culture of golden pompano.

## Materials and Methods

*Fish, experimental design and experimental conditions.*

Experimental fish were obtained from the Tropical Fisheries Research and Development Centre, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Lingshui (Hainan, China). Golden pompano were acclimated for seven days feeding on commercial diet. After the acclimation, 810 fish of similar sizes (average weight: 25.81 ± 0.18 g) were randomly distributed into 18 floating net cages (1.5 m × 1.5 m × 1.0 m) with 45 fish per cage. The feeding trial was carried out with six feeding frequencies of one (1TD), two (2TD), three (3TD), four (4TD), five (5TD), and six (6TD) times daily by feeding the same commercial diet. The feeding frequency and schedule followed in different treatments were presented in Table 1. Fish were fed 5% of their body weight per day and all the feed was eaten. Feed intake was recorded daily. During the ten weeks, salinity was maintained to 32.0 ± 1.2 ‰, seawater temperature was maintained at 27-31$^{o}$C, pH was maintained approximately 7.5-8.2, and the dissolved oxygen was maintained at a level higher than 5 mg/L.

At the end of the feeding trial, fish were fasted for 24h before sampling and were anesthetized with 100 mg/L Eugenol (Shanghai Medical Instruments Co., Ltd., Shanghai, China). Blood samples were obtained from the caudal vein of three fish from each cage, using a 2.5 ml syringe without heparin. After centrifugation (3000×g, 4$^{o}$C, 10 min), serum was separated from plasma and stored at -80$^{o}$C. Finally, six fish were randomly selected from each cage to analyze the proximate composition of muscle and then stored at -20$^{o}$C for subsequent analysis. All experiments in this study were approved by the Animal Care and Use Committee of South China Sea fisheries Research Institute, Chinese Academy of Fishery Sciences (no. SCSFRI96-253) and performed according to the regulations and guidelines established by this committee.

**Table 1** Feeding frequency and feeding time for golden pompano in each group

| Feeding frequency | Feeding time | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7:00 | 9:00 | 9:30 | 10:00 | 11:00 | 12:00 | 13:00 | 14:00 | 14:30 | 15:00 | 17:00 |
| 1 | √ | | | | | | | | | | |
| 2 | √ | | | | | | | | | | √ |
| 3 | √ | | | | | √ | | | | | √ |
| 4 | √ | | | √ | | | | √ | | | √ |
| 5 | √ | | √ | | | √ | | | √ | | √ |
| 6 | √ | √ | | | √ | | √ | | | √ | √ |

*Growth*

At the end of the experiment, the total numbers and mean body weight of fish in each tank were determined. The survival rate (SR), weight gain rate (WGR), specific growth rate (SGR), and feed conversion ratio (FCR) of each tank were calculated. Hepatosomatic index (HSI) and viscerosomatic index (VSI) were determined from three individual-fish each cage by obtaining tissues (viscera and liver) and expressing ratios as a percent of body weight.

The parameters were calculated as per the following formulae:

Weight gain rate (WGR, %) = 100 × (final body weight-initial body weight) / initial body weight;

Specific growth rate (SGR, % day$^{-1}$) = 100 × (Ln finial individual weight–Ln initial individual weight) / number of days;

Feed conversion ratio (FCR, %) = 100 × dry diet feed (g)/wet weight gain (g);

Survival rate (SR, %) = 100 × (finial number of fish) / (initial number of fish);

Hepatosomatic index (HSI, %) = 100 × (liver weight, g) / (whole body weight, g);

Viscerosomatic index (VSI, %) =100 × (viscera weight, g) / (whole body weight, g).

*Proximate chemical composition*

The moisture content was determined by drying the sample at 105°C until constant weight. Protein was determined by the Kjeldahl method, and crude lipid was determined using a Soxhlet extraction method and estimated by multiplying nitrogen by 6.25. Ash content was determined using a muffle furnace at 550°C for 8-h.

*Serum parameters*

Blood samples were collected immediately from the caudal veins of three fish per cage. Following centrifugation (3000×g, 4°C, 10min), serum was separated for analysis of the serum biochemical indices. The remaining serum was stored at -80°C for analysis of the serum biochemical indices. Triglyceride (TG), cholesterol (CHO), total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured with a Mindray BS-420 automatic biochemical instrument (Shenzhen Mindray Biological Medical Electronics Co., Ltd, China).

*Immune parameter*

The levels of immune parameters were measured with commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the instructions of the manufacturer. Superoxide dismutase (SOD) activity was determined following the methods (Beauchamp and Fridovich, 1971). Catalase (CAT) activity was determined by measuring the decrease in $H_2O_2$ concentration (Aebi, 1984). $Fe3^+$ can be restored to $Fe2^+$, the latter with the Philippine-solid substances to form complexes by colorimetric assay can measure total antioxidant capacity (T-AOC; Sun et al., 2009). Malondialdehyde (MDA) level was determined following the methods (Buege and Aust, 1978). Lysozyme (LZM) activity was determined through the turbidimetric method (Hultmark et al., 1980).

*Statistical analysis*

Data are expressed as the means ± standard error of the mean (SEM). The mean values among salinity treatments were compared using a one-way analysis of variance (ANOVA), followed by Tukey's test. The significance level adopted was 95% ($p < 0.05$). All statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, USA).

## Results

*Effects of feeding frequency on growth performance of golden pompano*

Growth performance of golden pompano subjected to different feeding frequency are shown in Table 2. The experimental results showed that the final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR) and hepatosomatic index (HSI) of the fed 3- and 4TD group were significantly higher than other groups ($P < 0.05$). The viscerosomatic index (VSI) was highest at 4TD group. The survival rate (SR) and the feed conversion ratio (FCR) were not significantly different, but the survival rate was the highest and the feed conversion ratio was the lowest in the 4TD group.

**Table 2** Effects of feeding frequency on growth performance of golden pompano. Initial body weights (IBW), final body weights (FBW), survival rate (SR), weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), hepatosomatic index (HSI) and viscerosomatic index (VSI) of golden pompano reared at different frequencies for 10 weeks. Data are expressed as mean ± SE (N = 9). Different letters in the same line indicate significant different mean values among salinity treatments ($P < 0.05$).

| | Feeding frequency | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| IBW (g) | 25.71±0.85$^a$ | 25.63±0.45$^a$ | 25.88±0.44$^a$ | 25.96±0.42$^a$ | 25.71±0.75$^a$ | 25.96±0.55$^a$ |
| FBW (g) | 70.70±0.58$^d$ | 89.11±1.69$^c$ | 95.63±1.00$^b$ | 105.68±1.29$^a$ | 89.93±0.81$^c$ | 86.23±0.89$^c$ |
| SR (%) | 90.83±2.20$^a$ | 89.16±0.83$^a$ | 90.00±1.44$^a$ | 93.33±3.63$^a$ | 89.16±5.07$^a$ | 91.66±3.63$^a$ |
| WGR (%) | 174.95±2.25$^d$ | 247.70±6.62$^c$ | 269.52±3.88$^b$ | 307.10±4.99$^a$ | 249.79±3.19$^c$ | 232.17±3.46$^c$ |
| SGR (%/d) | 1.44±0.01$^d$ | 1.77±0.03$^c$ | 1.86±0.02$^b$ | 2.01±0.02$^a$ | 1.78±0.01$^c$ | 1.71±0.01$^c$ |
| FCR (%) | 1.95±0.25$^a$ | 2.08±0.18$^a$ | 2.05±0.21$^a$ | 1.82±0.24$^a$ | 2.05±0.24$^a$ | 1.90±0.16$^a$ |
| VSI (%) | 6.32±0.07$^c$ | 6.84±0.18$^b$ | 6.78±0.11$^b$ | 7.85±0.18$^a$ | 6.07±0.09$^c$ | 5.82±0.08$^c$ |
| HSI (%) | 1.56±0.03$^d$ | 1.79±0.03$^c$ | 1.90±0.05$^b$ | 2.21±0.04$^a$ | 1.70±0.05$^d$ | 1.68±0.04$^d$ |

*Composition of golden pompano muscles at different feeding frequencies*

As can be seen from Table 3, the crude protein of the fish fed 4TD group was significantly higher than the other groups ($P < 0.05$), while the other groups were not significantly different from each other. The crude lipid content in the fed 4TD group was the highest when compared with the other groups, while in the 1TD group it was significantly the lowest ($P < 0.05$). However, there was no significant difference between the other groups ($P > 0.05$). The moisture and ash content were not significantly different among all the experimental groups ($P > 0.05$).

**Table 3** Composition of golden pompano muscles at different feeding frequencies

| Feeding frequency | Indices | | | |
|---|---|---|---|---|
| | Mositure | Ash | Crude lipid | Crude protein |
| 1 | 74.83±0.03$^{a}$ | 1.50±0.00$^{a}$ | 2.40±0.15$^{c}$ | 20.43±0.67$^{b}$ |
| 2 | 73.57±0.43$^{a}$ | 1.43±0.03$^{a}$ | 2.67±0.23$^{bc}$ | 20.50±0.06$^{b}$ |
| 3 | 74.77±0.39$^{a}$ | 1.47±0.03$^{a}$ | 3.30±0.21$^{ab}$ | 20.60±0.21$^{b}$ |
| 4 | 73.77±0.25$^{a}$ | 1.47±0.03$^{a}$ | 3.57±0.09$^{a}$ | 21.30±0.20$^{a}$ |
| 5 | 74.90±0.87$^{a}$ | 1.47±0.03$^{a}$ | 3.03±0.41$^{abc}$ | 20.17±0.27$^{b}$ |
| 6 | 74.23±0.30$^{a}$ | 1.47±0.03$^{a}$ | 3.20±0.31$^{abc}$ | 20.67±0.09$^{b}$ |

*Effects of feeding frequency on serum biochemical indices of golden pompano*

Serum constituents of golden pompano subjected to different feeding frequencies were presented in Table 4. The total protein (TP) content of the fed 4TD group was lower than the other groups ($P < 0.05$). Glucose (GLU) content of the fed 4TD group was significantly higher than that of the 5TD ($P < 0.05$), but no significant difference was found in the 3TD and 4TD groups ($P > 0.05$). Cholesterol (CHO) content of the 3TD and 5TD groups was significantly higher than that of the 4TD group ($P < 0.05$). The feeding frequency had no significant effect on the triglyceride (TG) content ($P > 0.05$). Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels of the 4TD group were significantly lower than of the fed 5TD group ($P < 0.05$). Alanine aminotransferase (ALT) showed lower in the 3TD and 4TD groups ($P < 0.05$). Aspartate aminotransferase (AST) increased with increasing feeding frequency from 1TD to 6TD group and then significantly increased.

**Table 4** Effects of feeding frequency on serum biochemical indices of golden pompano. Total serum protein (TP), Glucose (GLU), Total cholesterol (TC), Triglyceride (TG), Low-density lipoprotein (LDL), High density lipoprotein (HDL), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in the golden pompano acclimated to different frequencies for 10 weeks. Data are expressed as the mean ± SE (N = 9). Bars marked with different letters are significantly different from each other ($P < 0.05$).

| Indices | Feeding frequency | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| TP（g/L） | 25.16±0.74$^{a}$ | 24.97±1.20$^{ab}$ | 25.60±1.44$^{a}$ | 20.12±0.42$^{b}$ | 26.69±1.48$^{a}$ | 22.99±0.50$^{ab}$ |
| GLU（mmol/L） | 7.39±0.59$^{a}$ | 5.77±0.11$^{b}$ | 6.91±0.13$^{a}$ | 6.66±0.05$^{a}$ | 5.31±0.11$^{b}$ | 7.30±0.12$^{a}$ |
| TC（mmol/L） | 1.60±0.04$^{b}$ | 2.36±0.15$^{a}$ | 2.45±0.20$^{a}$ | 1.74±0.05$^{b}$ | 2.55±0.11$^{a}$ | 1.12±0.01$^{c}$ |
| TG（mmol/L） | 1.14±0.25 | 1.42±0.32 | 1.15±0.13 | 1.07±0.07 | 1.51±0.33 | 1.01±0.08 |
| LDL（mmol/L） | 0.14±0.02$^{c}$ | 0.61±0.04$^{a}$ | 0.36±0.01$^{b}$ | 0.32±0.03$^{b}$ | 0.52±0.02$^{a}$ | 0.12±0.01$^{c}$ |
| HDL（mmol/L） | 0.89±0.05$^{c}$ | 1.63±0.07$^{a}$ | 1.34±0.06$^{ab}$ | 1.04±0.04$^{bc}$ | 1.44±0.27$^{a}$ | 0.66±0.05$^{c}$ |
| ALT（U/L） | 26.80±0.68$^{a}$ | 18.14±1.11$^{b}$ | 13.16±0.92$^{c}$ | 15.08±0.92$^{bc}$ | 16.32±0.27$^{bc}$ | 18.08±1.10$^{b}$ |
| AST（U/L） | 164.27±3.05$^{c}$ | 187.51±8.17$^{c}$ | 238.70±6.11$^{b}$ | 239.42±5.57$^{b}$ | 253.84±17.95$^{b}$ | 299.75±10.77$^{a}$ |

*Effects of feeding frequency on serum immune indices of golden pompano*

The superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (T-AOC) of the 3TD and 4TD groups were significantly higher than that of the other groups ($P < 0.05$). There was no significant difference among other feeding groups ($P > 0.05$). However, SOD, CAT, and T-AOC were the highest in the 4TD groups. Malondialdehyde (MDA) of the fed 4TD group was significantly higher than of the 1TD, 2TD and 5TD groups ($P < 0.05$), while there was no significant difference among the other groups ($P > 0.05$). Lysozyme (LZM) of the fed 1TD and 6TD groups was significantly lower than that of the fed 4TD group ($P < 0.05$), and there was no significant difference among the other feeding groups ($P > 0.05$) (Table 5).

**Table 5** Effects of feeding frequency on serum immune indices of golden pompano. Superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (T-AOC), malondialdehyde (MDA), lysozyme (LZM) in the golden pompano acclimated to different frequencies for 10 weeks. Data are expressed as mean ± SE (N = 9). Different letters in the same line indicate significant different mean values among salinity treatments ($P < 0.05$).

| | Feeding frequency | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| SOD | 46.31±0.37$^{b}$ | 47.58±0.84$^{b}$ | 54.93±1.46$^{a}$ | 57.34±1.08$^{a}$ | 45.75±1.86$^{b}$ | 46.31±1.51$^{b}$ |
| CAT | 56.55±1.90$^{b}$ | 56.17±0.96$^{b}$ | 67.67±1.53$^{a}$ | 68.84±0.63$^{a}$ | 58.23±0.94$^{b}$ | 54.14±1.01$^{b}$ |
| T-AOC | 9.56±0.15$^{c}$ | 10.18±0.13$^{c}$ | 12.29±0.19$^{b}$ | 14.61±0.27$^{a}$ | 10.85±0.57$^{c}$ | 10.31±0.23$^{c}$ |
| MDA | 4.03±0.06$^{c}$ | 3.81±0.07$^{c}$ | 5.02±0.17$^{ab}$ | 5.71±0.03$^{a}$ | 5.41±0.17$^{b}$ | 5.94±0.39$^{a}$ |
| LZM | 256.85±3.86$^{c}$ | 311.82±5.24$^{a}$ | 325.72±2.07$^{a}$ | 322.12±1.20$^{a}$ | 315.11±2.16$^{a}$ | 294.75±1.04$^{b}$ |

## Discussion

The present study found significant effects of feeding frequency on the growth performance of golden pompano. Feeding four times a day has significantly improved fish growth. The fed 4TD group growth was significantly higher than that of the 1TD, 2TD, 3TD, 5TD and 6TD groups. These data suggest that a feeding frequency of 4TD might achieve optimal growth in the golden pompano.

The influence of optimal feeding frequency on fish growth has been examined in several species. Previous studies reported that the optimal feeding frequency was 3TD for *Limanda ferruginea* (Karen et al., 2002), the optimal feeding frequency was 8-times/d for *Pseudosciaena crocea* (Xie et al., 2011), and the optimal feeding frequency to enhance growth and boost immunity was 4TD for *Megalobrama amblycephala* (Li et al., 2014). The optimum feeding frequency seems to be dependent on fish species and size. The fish should have access to feed up to satiation for their optimum growth. However, lower and higher feeding frequencies had a negative influence on fish weight gain. Previous studies reported that low feeding frequency could not meet the nutrition and energy requirements of fish for growth and development due to decrease feed intake (Biswas et al., 2006). Repeated feeding throughout long periods of the day showed increase swimming activity of fish, resulting in energy expenditure and lower growth rate (Johansen and Jobling, 1998). It might be the reason that our high frequency fed fish had poor growth performance. Similar results where showed in *Centropristis striata* (Russo et al., 2017).

The quality of fish muscles can be expressed by the level of crude protein and crude fat. In the present study, feeding frequency has no significant effects on contents of moisture and crude ash. Similar results were found in other fish, such as *Paralichthys olivaceus* (Lee et al., 2000), *P. crocea* (Xie et al., 2011), *Salvelinus malma* (Guo et al., 2018). Our results showed that the feeding frequency had an effect on the crude fat and crude protein content, and the crude fat and crude protein content of the 4TD group were higher. Previous study showed that when fish are given low feeding frequency the used more energy to compete for limited food while avoiding cannibalism, which could accelerate the metabolism of lipid (Biswas et al., 2006). However, excessive food intake would induce an enhanced body lipid accumulation of fish, so more lipid was deposited in the fish body (Guo et al., 2018). However, under high frequency feeding, the content of crude fat and crude protein decreased compared with the suitable feeding frequency. Therefore, the crude fat and crude protein content has significantly increased with the increase of the feeding frequency.

The study also found that the hepatosomatic index increased and then decreased with the increase of feeding frequency, which might be related to liver fat accumulation. Liver fat metabolism could be reflected by serum related indicators, such as AST and ALT changes can be used to determine the health of the liver, liver function damage caused by high levels of AST and ALT. AST and ALT levels may increase in the serum when tissue damage and dysfunction occur (Canli and Canli, 2015). The release of AST and ALT from cells into the blood could be used to infer the extent to which the body's cells and tissues were damaged (Guo et al., 2018). In humans, the increase of ALT and AST is often associated with the extent and severity of cellular damage, and ALT and AST levels provide further information about severity of liver disease (Lin et al., 2010). The study found that ALT was highest in the 1TD and 6TD, indicated that low or high feeding frequency may cause liver damage or the function is impaired, the specific mechanism needs further study.

The blood of fish is closely related to its metabolism, nutrition and disease status. Blood component indicators are often used to evaluate the health of fish and are important physiological and pathological indicators. The metabolic state of lipids in aquatic animals can be reflected by the levels of total protein, triglycerides and cholesterol in the blood. Low-density lipoprotein and high-density lipoprotein levels reflect the body's cholesterol metabolism. At present, there are few studies on the physiological and biochemical indexes of fish serum in terms of feeding frequency (Li et al., 2014). We found that the frequency of feeding had certain influence on the biochemical and physiological indexes of golden pompano. The study found that serum total protein, total cholesterol, low-density lipoprotein, and high-density lipoprotein content varied depending on the feeding frequency, and were lowest at 4TD group. This was probably because the fish reached a satiety state at suitable feeding frequency, the exercise capacity was reduced, the energy consumption was reduced, the intake of nutrients and energy substances was increased, thus enhancing the body's own immune system.

In recent years, there has been less research on the relationship between the feeding frequency of fish and oxidative stress. The serum components of fish contain T-AOC, LZM, SOD, CAT and MDA and other immunologically active substances, which play an important role in enhancing the defense ability against external environmental stress (Munir et al, 2016). T-AOC is an indicator of antioxidant function (Sheikhzadeh et al, 2012). As an antioxidant enzyme, SOD can scavenge free radicals in the body and protect the animal from damage (den Hartog et al, 2003). In this experiment, the content of SOD increased and then decreased with the increase of feeding frequency, reaching the maximum in the 4TD group. MDA is a product of lipid peroxidation that accelerates cell and tissue damage (Nagasaka et al., 2004). Studies have reported that levels of MDA indicated higher peroxidation leading to cell degradation (Lüder et al., 2003). We have found that the content of MDA was highest in the 6TD group and was the lowest in the 1TD group. Thus, it seems that higher frequent activity or excessive feeding could reduce the antioxidant capacity and lead to the decline of immune function. LZM is an important component of the innate immune system of fish (Tafalla et al., 1999; Saurabh and Sahoo, 2008). We found that LZM activity in the 3TD and 4TD groups was significantly higher than that of the 1TD and 6TD group, which might indicate that a too low or too high feeding frequency can damage the liver function and lead to a decrease in enzyme activity.

In summary, the feeding frequency of the 4TD group could greatly improve the growth of golden pompano. Fish subjected to low feeding frequencies showed reduced growth and immunosuppression. High feeding frequencies not only produced poor growth, but may also cause oxidative damage. Overall, our finding showed that the optimal feeding frequency to enhance growth and boost immunity of the golden pompano during their juvenile stage is four times a day.

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